Abid A. Ansari · Sarvajeet Singh Gill
Ritu Gill · Guy R. Lanza · Lee Newman
*Editors*

# Phyto-remediation

## Management of Environmental Contaminants, Volume 2

Springer

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*Editors*
Abid Ali Ansari
Department of Biology
Faculty of Science
University of Tabuk
Tabuk, Saudi Arabia

Sarvajeet Singh Gill
Centre for Biotechnology
Maharshi Dayanand University
Rohtak, Haryana, India

Ritu Gill
Centre for Biotechnology
Maharshi Dayanand University
Rohtak, Haryana, India

Guy R. Lanza
College of Environmental Science and Forestry
State University of New York
Syracuse, NY, USA

Lee Newman
College of Environmental Science and Forestry
State University of New York
Syracuse, NY, USA

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# Preface

*If one way be better than another, that you may be sure is Nature's way.*

– Aristotle

Volume 2 of this two-volume series adds additional examples of the use of green plants and their associated microbial communities to remove, degrade, or stabilize inorganic and organic contaminants entering the air, water, and soil of a multitude of ecosystems. The chapters in Volume 2 provide additional examples that illustrate how phytoremediation applications can serve as one of several useful components in the overall management and control of contaminants using relatively low-cost solar-driven physiological/biochemical mechanisms common to most plants. Many of the phytoremediation applications provided in Volume 2 also have the added value of providing a remediation option that offers a minimum disruption to the ecosystem or habitat under repair.

Different forms of basic ecological restoration including phytoremediation have been used for centuries around the globe and reflect part of what the philosopher Immanuel Kant described as the need for people to consider the potential effects of their actions on the welfare of all of humankind for all time. Typically, an ecosystem restoration project aims to restore an impacted area to a state that is as close as possible to the conditions that existed prior to the disturbance. In the case of phytoremediation, one good way to achieve that goal involves a contaminant management process that assures a good match of the phytoremediation application to the type and concentration of contaminants and the critical site-specific characteristics of the area under remediation.

Volume 2 of this book series provides additional accounts of selected phytoremediation research projects and case histories from specific sites and/or laboratories in five continents around the world. Volume 2 provides a diverse global perspective and includes observations and data collected from multiple sites in nine countries in Africa, Asia, Australia, North and South America, and Europe. Organic and inorganic contaminants covered include petroleum hydrocarbons, heavy metals/metalloids, wastewater, and nutrients. Chapters in Volume 2 also discuss the use of different organisms to manage and treat contaminants in soil and water including mixed microbial communities, cyanobacteria, rhizobia, mycorrhiza, halophytes, and lichens.

All forms of ecosystem restoration including phytoremediation will have to be reexamined in the broad context of climate change. The editors and contributing authors hope that one result of publishing this book will be to provide a wide range of useful experimental data derived from global applications of phytoremediation. Hopefully, this book can also provide new insights into the advantages and disadvantages of using phytoremediation to manage the continuing threat of ecosystem degradation resulting from the interaction of contaminants and climate change.


Tabuk, Saudi Arabia — Abid Ali Ansari, M.Sc., Ph.D.
Rohtak, India — Sarvajeet Singh Gill, M.Sc., M.Phil., Ph.D.
Rohtak, India — Ritu Gill, Ph.D.
Syracuse, USA — Guy R. Lanza, Ph.D.
Syracuse, USA — Lee Newman, Ph.D.

# Contents

# Contributors

**Eric M. Adetutu, Ph.D.** School of Applied Sciences, RMIT University, Bundoora, Melbourne, VIC 3001, Australia

**Amrik Singh Ahluwalia, Ph.D.** Department of Botany, Panjab University, Chandigarh, India

**Eki T. Aisien B.Sc., P.G.D.E., M.Sc., Ph.D.** Department of Integrated Science, College of Education, Benin City, Edo, Nigeria

**Felix A. Aisien, B.Sc., P.G.ChE., M.Eng., Ph.D.** Department of Chemical Engineering, University of Benin, Benin City, Edo, Nigeria

**Elizabeth Temitope Alori, B.Agric., M.Sc., (Soil Science), Ph.D., (Soil Microbiology and Biochemistry)** Department of Crop and Soil Science, Landmark University, Omu Aran, Kwara State, Nigeria

**Abid Ali Ansari, Ph.D.** Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

**Zahid Khorshid Abbas, Ph.D.** Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

**Sümer Aras** Biotechnology Section, Department of Biology, Faculty of Science, University of Ankara, Tandogan, Ankara, Turkey

**Silvana Arreghini, Ph.D.** Cátedra de Química Analítica, Departamento de Recursos Naturales y Ambiente, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

**Carlos Andres Avila-Williams, B.Sc., Biologist.** Department of Biology, Universidad Del Valle, Cali, Valle del Cauca, Colombia

**Hamidi Abdul Aziz, Ph.D.** School of Civil Engineering, Engineering Campus, Universiti Sains Malaysia, Nibong Tebal 14300, Penang, Malaysia

Solid Waste Management Cluster, Universiti Sains Malaysia, 14300, Penang, Malaysia

**Andrew S. Ball, Ph.D.** School of Applied Sciences, RMIT University, Bundoora, VIC, Australia

**Gary S. Bañuelos** Key Laboratory of Agri-Food Safety of Anhui Province, School of Plant Protection, Anhui Agriculture University, Anhui, China

**Gabriel Basílico, Eng.** Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – Consejo Nacional de Investigaciones Científicas y Técnicas, CABA, Argentina

Universidad de Flores, CABA, Argentina

**Clarisse Brígido, Ph.D.** ICAAM-Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Evora, Portugal

**Laura de Cabo, Ph.D.** Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – Consejo Nacional de Investigaciones Científicas y Técnicas, CABA, Argentina

Universidad de Flores, CABA, Argentina

**Isabel Caçador, Ph.D.** MARE – Marine and Environmental Sciences Centre, Faculty of Sciences of the University of Lisbon, Lisbon, Portugal

**Demet Cansaran-Duman** Biotechnology Institute, Ankara University, Ankara, Tandogan, Turkey

**Araceli Amaya Chávez, Ph.D.** Departamento de Farmacia de la Facultad de Química, UAEMex, Paseo Tollocan esq. Paseo Colón, Toluca, Estado de México C.P., Mexico

**Jian Chen, Ph.D.** Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China

**Yi Chen, M.S.** Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, Nanjing, China

College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu, China

**Zueng-Sang Chen, Ph.D.** Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

**Bo-Ching Chen, Ph.D.** Department of Post-Modern Agriculture, MingDao University, Taipei, Taiwan

**Nazaré Couto, Ph.D.** CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

**Mudasir Irfan Dar, Ph.D. Student** Department of Botany, Aligarh Muslim University, Aligarh, UP, India

**Carlos Barrera Díaz, Ph.D.** Departamento de Química Ambiental, Facultad de Química, Universidad Autónoma del Estado de México Paseo Colón esq. Paseo Tollocan s/n Col. Residencial Colón, Toluca, Estado de México, Mexico

Centro Conjunto de Investigación en Química Sustentable UAEM – UNAM, Carretera Toluca-Atlacomulco, km 14.5, Unidad El Rosedal, Toluca, Estado de México C.P., México

**Bernardo Duarte** MARE – Marine and Environmental Sciences Centre, Faculty of Sciences of the University of Lisbon, Lisbon, Portugal

**Ana Faggi** Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – Consejo Nacional de Investigaciones Científicas y Técnicas, CABA, Argentina

Universidad de Flores, CABA, Argentina

**Shahla Faizan** Department of Botany, Environmental Physiology Laboratory, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

**Lucía Grijalbo Fernández** Department of Pharmaceutical and Health Sciences, Universidad San Pablo CEU, Madrid, Spain

**Mercedes Fernández-Pascual** CSIC, Crop Protection Department, Institute of Agricultural Sciences, Madrid, Spain

**Okoduwa I. Gabriel, B.Eng., M.Sc.** Chemical Engineering Department, University of Benin, Benin City, Edo, Nigeria

**Jose Antonio Lucas García** Department of Pharmaceutical and Health Sciences, Universidad San Pablo CEU, Madrid, Spain

**Shahin Gavanji, M.Sc.** Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran

**Ali Gholami, Ph.D.** Department of Soil Science, Khouzestan Science and Research Branch, Islamic Azad University, Ahvaz, Khouzestan, Iran

**Sarvajeet Singh Gill** Centre for Biotechnology, Maharshi Dayanand University Rohtak, Haryana, India

**Madhuri Girdhar, M.Tech.** Department of Biotechnology, Lovely Professional University, Jalandhar, Punjab, India

**Bernard R. Glick, Ph.D.** Department of Biology, University of Waterloo, Waterloo, ON, Canada

**Piotr Goliński** Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland

**Paula Guedes, M.Sc.** CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

**Fengxiang X. Han, Ph.D.** Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS, USA

**Indika Herath, M.Sc.** Chemical and Environmental Systems Modeling Research Group, Institute of Fundamental Studies, Kandy, Sri Lanka

**Alicia Fabrizio de Iorio, Ph.D.** Cátedra de Química Analítica, Departamento de Recursos Naturales y Ambiente, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

**Keshav Singh Jatav, M.Sc., Ph.D.** Department of Botany, Govt. Chhatrasal College Pichhore, Shivpuri, MP, India

**Anna Karczewska, Ph.D.** Institute of Soil Science and Environmental Protection, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

**Fareed Ahmad Khan, M.Phil., Ph.D.** Department of Botany, Aligarh Muslim University, Aligarh, UP, India

**Anil Kumar** National Institute of Immunology, New Delhi, India

**Hung-Yu Lai, Ph.D.** Department of Post-Modern Agriculture, MingDao University, Taipei, Taiwan

**Miao Li, Ph.D.** Key Laboratory of Agri-Food Safety of Anhui Province, School of Plant Protection, Anhui Agriculture University, Anhui, China

**Zhiqing Lin** Department of Biological Sciences & Environmental Sciences Program, Southern Illinois University Edwardsville, Edwardsville, Illinois, USA

**Ying Liu** Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Suzhou, Jiangsu, China

**Qaisar Mahmood, Ph.D.** Department of Environmental Sciences, COMSATS IIT, Abbottabad, Pakistan

**Francisco Javier Gutiérrez Mañero** Department of Pharmaceutical and Health Sciences, Universidad San Pablo CEU, Madrid, Spain

**Mohammad Miransari, Ph.D.** AbtinBerkeh Ltd., Company, Isfahan, Iran

**Nosheen Mirza, Ph.D.** Department of Environmental Sciences, COMSATS IIT, Abbottabad, Pakistan

**Mirosław Mleczek Ph.D.** Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland

**Andrzej Mocek** Department of Soil Science and Protection, Poznań University of Life Sciences, Poznań, Poland

**Anand Mohan, M.Sc., Ph.D.** Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India

**Amin Mojiri, Ph.D.** School of Civil Engineering, Universiti Sains Malaysia, Nibong Tebal, Penang, Malaysia

**Gabriela Roa Morales, Ph.D.** Departamento de Química Ambiental, Facultad de Química, Universidad Autónoma del Estado de México Paseo Colón esq. Paseo Tollocan s/n Col. Residencial Colón, Toluca, Estado de México, Mexico

Centro Conjunto de Investigación en Química Sustentable UAEM – UNAM, Carretera Toluca-Atlacomulco, km 14.5, Unidad El Rosedal, Toluca, Estado de México C.P., México

**Carlos Arturo Madera-Parra, Ph.D.** EIDENAR School, Engineering Faculty, Universidad del Valle, Cali, Valle del Cauca, Colombia

**Rubina Perveen, M.Phil., Ph.D. Student** Department of Botany, Environmental Physiology Laboratory, Aligarh Muslim University, Aligarh, UP, India

**Radha Prasanna, Ph.D.** Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

**Hasibur Rehman, Ph.D.** Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

**Farha Rehman, M.Phil., Ph.D.** Department of Botany, Aligarh Muslim University, Aligarh, UP, India

**Ana Lucia Rengifo-Gallego, B.Sc., Biological Sciences Ph.D. Student** Departament of Biology, Biology of Plants and Microorganism Research Group, Universidad del Valle, Cali, Valle del Cauca, Colombia

Biology Department, Biology of Plants and Microorganism Research Group, Universidad del Valle, Bogota, Cundinamarca, Colombia

**Nirmal Renuka, M.Phil., Ph.D. Student** Department of Botany, Panjab University, Chandigarh, India

**Alexandra B. Ribeiro, Ph.D.** CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

**Daniel Ascúntar Ríos, B.Sc., M.Sc.** EIDENAR School, Engineering Faculty, Universidad del Valle, Cali, Valle del Cauca, Colombia

**Shalini Saggu, Ph.D.** Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

**Enrique Javier Peña Salamanca, Ph.D.** Department of Biology, Universidad del Valle, Cali, Valle del Cauca, Colombia

**Anita Rani Santal, Ph.D.** Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, India

**Roberto Serafini, Ph.D.** Cátedra de Química Analítica, Departamento de Recursos Naturales y Ambiente, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina

**Shahida Shaheen, M.S., Ph.D. Student** Department of Environmental Sciences, COMSATS IIT, Abbottabad, Pakistan

**Esmaeil Shahsavari, Ph.D.** School of Applied Sciences, RMIT University, Bundoora, VIC, Australia

**Zhi-Qi Shi, Ph.D.** Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China

**N.P. Singh, Ph.D.** Centre for Biotechnology, Maharshi Dayanand University, Rohtak, Haryana, India

**Ram Pratap Singh, Ph.D.** Department of Botany, Govt. P.G. College, Morena, MP, India

**Anjuli Sood, Ph.D.** Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

**Yi Su, Ph.D.** College of Science, Technology, Engineering, Mathematics and Nursing, Texas A&M University-Texarkana's, Texarkana, TX, USA

**Ramlah Bt Mohd Tajuddin, Ph.D.** Faculty of Civil Engineering, Universiti Technology Mara (UiTM), Shah Alam, Selangor Darul Ehsan, Malaysia

**Norman Terry** Department of Plant and Microbial Biology, College of Natural Resources, UC Berkeley, CA

**Subrata Trivedi, M.Sc., M.Phil., Ph.D.** Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

**Meththika Vithanage, Ph.D.** Chemical and Environmental System Modeling Research Group, Institute of Fundamental Studies, Kandy, Sri Lanka

**Zhilin Wu** Key Laboratory of Agri-Food Safety of Anhui Province, School of Plant Protection, Anhui Agriculture University, Hefei Anhui, China

Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Jiangsu, China

**Xuebin Yin** Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Jiangsu, China

School of Earth and Space Sciences, University of Science and Technology of China, Hefei, China

**Linxi Yuan** Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Jiangsu, China

School of Earth and Space Sciences, University of Science and Technology of China, Hefei, China

**Dong-Mei Zhou, Ph.D.** Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

# Part I

# Phytoremediation Applications for Waste Water and Improved Water Quality

# 1 Improved Quality of Abattoir Wastewater Through Phytoremediation

Eki T. Aisien, Felix A. Aisien, and Okoduwa I. Gabriel

## 1.1 Introduction

Increasing population, industrialisation and urbanisation in Benin City, Nigeria are mainly responsible for the enormous discharge of various forms of human, animal and industrial wastewaters into nearby surface waters such as rivers, streams, lakes and ponds (Aisien et al. 2010a, b, c). This discharge contains large amount of different pollutants that have serious environmental and health hazard implications on humans, animals, plants and microorganisms in the environment and this usually leads to great environmental challenges (Aisien et al. 2009; Benka-Coker and Ojior 1995). A typical abattoir processes both red meat (beef, mutton and pork) and white meat (poultry). The abattoir wastewater contains dissolved pollutants, including blood and urine, and also high concentration of suspended solids, such as pieces of fat, grease, hair, feathers, flesh tissue, manure, grit and undigested feeds (Massé and Massé 2000; Tritt and Schuchardt 1992). These insoluble and slowly biodegradable components represented 50 % of the pollution load. Therefore, every effort should be made to maximise raw blood and fat collection and subsequent processing into blood meal, tallow or other value-added alternatives (Johns 1995; Mittal 2004).

The wastewater may also have pathogens, including *Salmonella* and *Shigella* bacteria, parasite eggs and amoebic cysts. Abattoir wastewater contains several million colony forming units (cfu) per 100 $cm^3$ of total coliform, faecal coliform, and *Streptococcus* groups of bacteria. The main organic pollution from abattoir wastewater is the slaughter animal's blood (Tritt and Schuchardt 1992; Osibajo and Adie 2007). Continuous discharge of untreated abattoir wastewater into nearby water bodies lead to increased algae growth; resulting in eutrophication; reduced aquatic plants and animals growth; increased water odour, foaming, colour, conductivity and temperature; and increased heavy metal toxicity. These all will contribute to the very poor water quality of the water bodies. Several physical, chemical and biological processes have been involved in the transformation and consumption of organic matter and plant nutrients within the wetland (Aisien et al. 2010a, b, c). The reuse of treated wastewater in aquaculture/agriculture practices is encouraged to minimise demand on freshwater resources.

Many researchers have applied various macrophytes such as water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes L.*), water spinach (*Ipomoea aquatica*), duckweed (Lemna spp.), bulrush (*Typha*), vetiver grass (*Chrysopogon zizanioides*), common reed (*Phragmites australis*), etc. and microalgae including *Chlorella vulgaris* for phytoremediation of different types of wastewater to achieve a better quality water for agricultural and domestic purposes. The macrophytes are cost-effective universally available aquatic plants and with their ability to survive adverse conditions and high colonisation rates, are excellent tools for studies of phytoremediation. However, one of the major problems in using microalgae for wastewater treatment is the difficulty of their recovery from the treated effluent, which can be address by employing immobilised microalgae (Sing-Lai et al. 2010; Hu et al. 2008; Lu et al. 2010; Dipu et al. 2011; Mkandawire and Dudel 2007; Lakshmana et al. 2008; Jing et al. 2002; Awuah et al. 2004). Also, it has been reported that the most important factor in the implementation of phytoremediation of contaminated water is the selection of appropriate plant that has a high uptake of nutrients and great capacity pollutants removal and grow well in polluted water (Mashauri et al. 2000; Baskar et al. 2009; Girija et al. 2011; Truong and Baker 1998; Fonkou et al. 2002). However, currently there seems to be neither sufficient measures nor facilities to treat abattoir wastewater in Benin City, Nigeria for environmental safety or to recover usable energy and material from abattoir by-products.

E.T. Aisien (✉)
Department of Integrated Science, College of Education Ekiadolor, Benin City, Edo State, Nigeria
e-mail: ekiaisien20@yahoo.com

F.A. Aisien • O.I. Gabriel
Department of Chemical Engineering, University of Benin, PMB 1154, Benin City, Edo State, Nigeria

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Therefore, in this study an integrated macrophytes and microalgae water treatment system was used to remediate abattoir wastewater in order to achieve high quality water, hence reducing the water quality indicators such as BOD, COD, DO, TDS, TSS, $NO_3^-$, $PO_4^{-3}$, coliform count, etc. below the values obtained by other researchers. As a result, the main objective of this study was to investigate the efficiency of phytoremediation of abattoir wastewater using integrated macrophytes and microalgae system and to ascertain which of the macrophytes (*Eichhornia crassipes*, *Ipomoea aquatica*) and microalgae (*Closterium turgidum, Chlorella vulgaris*) is the most effective in water quality enhancement of abattoir polluted water.

## 1.2 Materials and Methods

### 1.2.1 Material Collection and Preparation

Irorere Obazee abattoir in Evbuobanosa community, Benin City, Nigeria is located in 06º 20′ 06″ N latitude and 05º 37′ 38″ E longitude. It has four slaughter halls for cattle and goat. Fifty litres of abattoir wastewater was collected between 10.00 and 11.00 h with a clean sterilised plastic container from the point of discharge into the river. The wastewater was brought to the laboratory, mixed and allowed to settle for 3 h. The supernatant was then diluted with distilled water in 1–10 dilutions. This was to ensure that the concentration of the abattoir wastewater was favourable to the survival of the aquatic plants and algae.

The wastewater was transferred into a 30 L pre-sterilised aspirator bottle and allowed to flow from one stage to the other as shown in Fig. 1.1. This was a four-stage treatment system. Each stage has pre-sterilised 10 L open plastic tanks. Besides, the retention time for each stage was 7 days. Stages 1, 2, 3 and 4 contained fully grown 12 water hyacinth plants, algae, 12 water spinach plants and another 12 water hyacinth plants, respectively. These aquatic plants were grown in the wastewater exposed to sunlight. A 1.5 cm diameter plastic pipe with a control valve was used to facilitate the flow from one stage to another after the 7 days retention time. At the onset of the treatment process, the abattoir wastewater was allowed to flow into culture tank 1 (stage 1). After 7 days in culture tank 1, the partially treated wastewater was allowed to flow into the culture tank 2 (stage 2). This was achieved by opening the control valve connecting culture tank 1 to tank 2 at the end of the 7 days. This procedure continued until the treated wastewater finally flows into culture tank 4 (stage 4).

Water samples were collected from the aspirator bottle at the beginning of the treatment process and each culture tank at the end of the retention time of 7 days. They were analysed in triplicate for the physiochemical and bacteriological parameters using APHA 2005 standard method of analysis. The parameters include: soluble anion; chloride ($Cl^-$), nitrate ($NO^-_3$), phosphate ($PO^-_4$), sulphate ($SO_4^{-2}$), bicarbonate ($HCO^-_3$), heavy metals; Zinc (Zn), Iron (Fe), Lead (Pb), Cadmium (Cd);other parameters: total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total hardness, alkalinity, conductivity, turbidity,

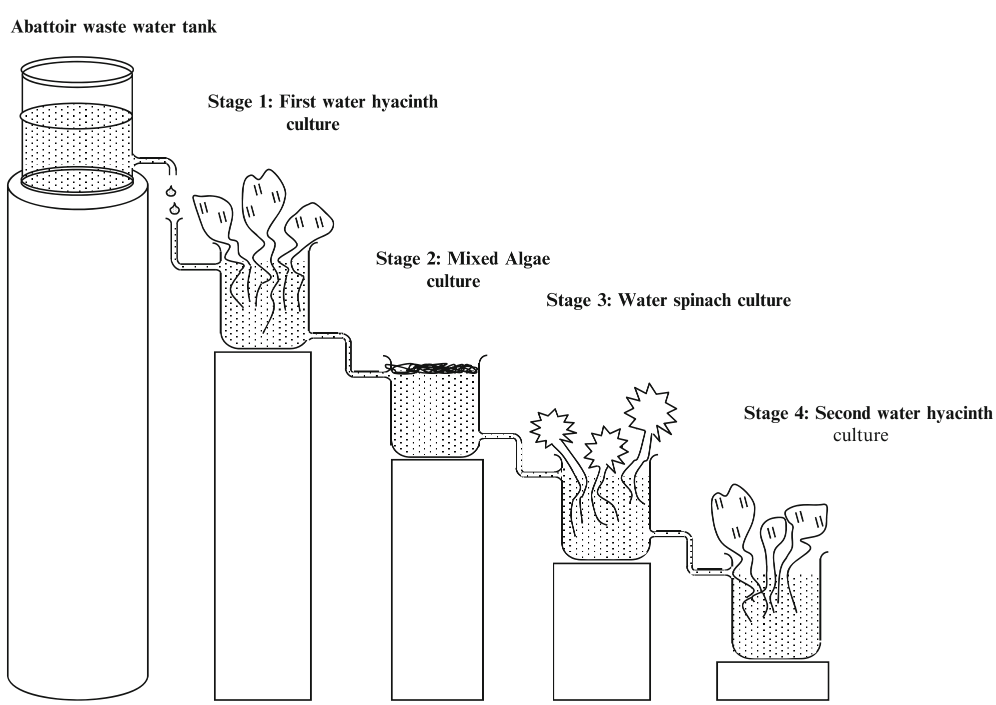


**Fig. 1.1** Schematic diagram of the laboratory setup for the phytoremediation of abattoir wastewater

temperature and pH. The metals concentrations were determined using atomic absorption spectrophotometer (Perking Elmer AA 100). The bacteriological parameters (Coliform count, *E. coli* count and streptococci count) were determined using presumptive and confirmative tests (APHA 2005).

### 1.2.2 Statistical Analysis

The statistical analyses were carried out using one way analysis of variance (ANOVA) of F-Test. The correlation between the different physiochemical and bacteriological parameters were evaluated by Pearson's coefficient at confidence intervals of 95 %.

## 1.3 Results and Discussion

The water quality values for the raw and treated abattoir wastewater are summarised in Table 1.1. The results showed that all the water quality indicators (BOD, COD, DO, TSS, coliform count, etc.) of the raw (untreated) abattoir wastewater were very high and exceeded the permissible limits of WHO/FEPA standards for discharge of wastewater into surface water. Therefore, it was quite obvious that treatment would be very necessary to improve the quality of the abattoir wastewater before it is discharged into the nearby surface water. This will minimise the addition of organic and inorganic matters, nutrients and microbial loads, thereby reducing the pollution level of the surface water and make it more useful for both domestic and industrial purposes.

The abattoir wastewater was treated in four stages macrophytes/microalgae phytoremediation processes. The treatment period for each stage was 7 days; hence the entire processes took 28 days. The summary of the results in Table 1.1 showed that for each stage of the phytoremediation treatment process, there were significant reduction of organic matters, nutrients and microbial loads of the abattoir wastewater. The water from the final or fourth stage showed that all the water quality indicators value were either within or below the permissible WHO/FEPA standards for the discharge of wastewater into surface water. The uptake and accumulation of pollutants (organic and inorganic nutrients, microorganisms) vary from stage to stage, plant to plant, plant to algae and also from pollutant to pollutant.

The temperature increased from 28 °C in the raw abattoir wastewater to 28.9 and 30 °C in stages 1 and 2,

**Table 1.1** Physiochemical and bacteriological properties of the raw and treated abattoir wastewater using aquatic plants and algae

| Parameters | Raw sample | First water hyacinth culture (stage 1) and removal (%) | Algae culture (stage 2) and removal (%) | Water spinach culture (stage 3) and removal (%) | Second water hyacinth culture (stage 4) and removal (%) | WHO/ FEPA limits |
|---|---|---|---|---|---|---|
| Temperature (°C) | 28.0 ± 0.7 | 28.9 ± 1.1 [a](3.1 %) | 30.0 ± 1.0 [a](6.67 %) | 29.0 ± 0.8 [a](3.4 %) | 28.6 ± 1.3 [a](2.1 %) | <40 °C |
| Turbidity (NTU) | 125.0 ± 2.2 | 9.75 ± 1.1 (92.2 %) | 36.3 ± 1.5 (71 %) | 6.5 ± 0.2 (94.8 %) | 4.0 ± 0.1 (96.8 %) | 5–10 |
| Conductivity (mS/cm) | 1,640 ± 22 | 600 ± 13.2 (63.4 %) | 492 ± 10.3 (70 %) | 310 ± 8.6 (81.1 %) | 175 ± 9 (89.3 %) | |
| Total suspended solid (mg/L) | 2,440 ± 33 | 602 ± 8.3 (75.3 %) | 969 ± 12 (60.3 %) | 385 ± 7.7 (84.2 %) | 146.2 ± 6 (94 %) | 600–1,000 |
| Total dissolved solid (mg/L) | 2,006 ± 21 | 474 ± 9.2 (76.4 %) | 305.4 ± 12 (84.8 %) | 152.5 ± 10 (92.4 %) | 84.5 ± 8.6 (95.8 %) | 200–500 |
| Bicarbonate $HCO^-_3$ (mg/L) | 431 ± 10.2 | 159 ± 7.3 (63.1 %) | 97.8 ± 6.4 (77.3 %) | 68.9 ± 4.2 (84 %) | 43.3 ± 3.5 (90 %) | |
| Chloride $Cl^-$ (mg/L) | 285.8 ± 13 | 142.9 ± 8 (50 %) | 81.5 ± 3.2 (71.4 %) | 57.2 ± 4 (80 %) | 38.6 ± 3.6 (86.5 %) | 350– |
| Sulphate $SO_4^{2-}$ (mg/L) | 345 ± 15.6 | 124.8 ± 7.2 (63.8 %) | 53.7 ± 4.5 (84.4 %) | 46.2 ± 8.5 (86.7 %) | 30.2 ± 5.1 (91.2 %) | 100–250 |
| Phosphate $PO_4^{2-}$ (mg/L) | 9.8 ± 0.9 | 5.14 ± 2.2 (47.6 %) | 4.62 ± 1.2 (52.9 %) | 3.39 ± 1.5 (65.4 %) | 2.82 ± 0.5 (71.2 %) | 5–10 |
| Nitrate $NO_3^-$ (mg/L) | 103.2 ± 0.7 | 43.2 ± 2. 3 (58.1 %) | 30.5 ± 1.6 (70.5 %) | 22.4 ± 0.8 (78.2 %) | 15.8 ± 1.2 (84.7 %) | 45–50 |
| Alkalinity (mg/L) | 222.5 ± 10 | 96.1 ± 8 (56.8 %) | 53.2 ± 4 (76.1 %) | 32.3 ± 5.3 (85.5 %) | 21.4 ± 3.6 (90.3 %) | |
| Total hardness (mg/L) | 265 ± 7.2 | 90.7 ± 4.2 (65.8 %) | 79.5 ± 3.9 (70 %) | 56.3 ± 4.2 (78.8 %) | 40.2 ± 3.2 (84.8 %) | 200–500 |
| pH | 9.0 ± 0.2 | 7.8 ± 0.1 (13.3 %) | 7.5 ± 0.15 (16.7 %) | 7.4 ± 0.2 (17.8 %) | 7.2 ± 0.12 (20 %) | 6.0–9.0 |
| Dissolved oxygen (mg/L) | 1.2 ± 0.04 | 4.1 ± 0.2 [a](70.7 %) | 4.7 ± 0.1 [a](74.5 %) | 5.8 ± 0.1 [a](79.3 %) | 6.3 ± 0.2 [a](81 %) | 4–5 |
| BOD (mg/L) | 621.3 ± 7.5 | 42.7 ± 1.5 (93.1 %) | 27.6 ± 0.9 (95.6 %) | 15.2 ± 1.3 (97.6 %) | 10.6 ± 0.3 (98.3 %) | 20–50 |
| COD (mg/L) | 1,244 ± 13.2 | 98 ± 4.7 (92.1 %) | 84 ± 5.8 (93.2 %) | 65 ± 4.2 (94.8 %) | 52 ± 3.5 (95.8 %) | 1,000 |
| Cadmium (mg/L) | 3.6 ± 0.2 | 0.2 ± 0.03 (94.4 %) | 0.04 ± 0.001 (98.9 %) | 0.01 ± 0.002 (99.7 %) | ND (100 %) | 0.01–<1.0 |
| Zinc (mg/L) | 2.63 ± 0.3 | 0.3 ± 0.001 (88.6 %) | 0.02 ± 0.001 (99.2 %) | ND (100 %) | ND (100 %) | <1.0–5 |
| Lead (mg/L) | 4.4 ± 0.03 | 0.25 ± 0.03 (94.3 %) | 0.04 ± 0.01 (99.0 %) | 0.01 ± 0.02 (99.7 %) | ND (100 %) | 0.01–0.05 |
| Iron (mg/L) | 5.7 ± 0.12 | 0.48 ± 0.05 (91.6 %) | 0.09 ± 0.001 (98.4 %) | 0.01 ± 0.001 (99.7 %) | ND (100 %) | 0.3–0.5 |
| Coliform count (MPN/100 mL) | 2,400 ± 30 | 38 ± 2.5 (98.4 %) | 2 ± 0.02 (99.9 %) | ND (100 %) | ND (100 %) | 0–<1 |
| *E. coli* count (MPN/100 mL) | 580 ± 15.2 | 12 ± 1.3 (97.9 %) | 1 ± 0.01 (99.8 %) | ND (100 %) | ND (100 %) | 0–<1 |
| *Streptococci* (MPN/100 mL) | 450 ± 12.3 | 15 ± 0.6 (96.7 %) | 1.6 ± 0.01 (99.7 %) | ND (100 %) | ND (100 %) | 0–<1 |

Mean ± standard error of mean (SEM) of water quality indicators and percentage reduction in wastewater
*ND* non detectable
[a]Percentage increase

respectively. It then decreased to 29 and 28.6 °C in stages 3 and 4, respectively. The efficiency of increased varied from 2.1 to 6.67 %. The slight increase will be as a result of increase microbial activities in the mixed algae culture (stage 2). However, the temperature values for the raw and treated abattoir wastewater from stage 1–4 were within the allowed limit of WHO/FEPA for the discharge of wastewater into surface water. These results conformed to those reported by Koottatep and Polprasert (1997) and Liao and Chang (2004).

The pH value of the abattoir wastewater was 9.0, which is an indication that the wastewater was alkaline. The pH of the abattoir wastewater decreases slightly as the water passes through the different stages of treatment using aquatic macrophytes and microalgae. The pH ranged from 9 to 7.8, 7.8 to 7.5, 7.5 to 7.4 and 7.4 to 7.2 for stages 1, 2, 3 and 4, respectively. Therefore, the percentage pH reduction ranged from 13.3 to 20 %. Similar results were reported by Aisien et al. 2010b; Snow and Ghaly 2008. The reduction in pH is due to absorption of nutrients or by simultaneous release of H+ ions with the uptake of metal ions present in the wastewater (Mahmood et al. 2005). The treated abattoir wastewater at the end of the fourth stage was almost neural and within the limit of WHO/FEPA standard for the discharge of wastewater into surface water.

Similar trends were observed for alkalinity with the efficiency of reduction ranged from 56.8 to 90.3 %. These results were in conformity with that reported by Aisien et al. 2009, Mishra et al. 2008. The conductivity is a proxy indicator of total dissolved solids, and therefore an indicator of the taste or salinity of the water. Table 1.1 showed that the conductivity of the abattoir wastewater decreased drastically in the water hyacinth culture in stage 1 and gradually in subsequent stages. Aisien et al. 2010c reported that the high conductivity indicates the presence of high concentration of dissolved salts as showed in Table 1.1. The efficiency of reduction ranged from 63.4 to 89.3 %. These reductions indicate the removal of salts the wastewater through plants uptake and root absorption (Ran et al. 2012; Valipour et al. 2011). The conductivity values for the different treatment stages were within the recommended WHO/FEPA standard.

The turbidity of the abattoir wastewater was quite high this can be associated with suspended matters, such as clay, silts, finely divided organic and inorganic matters, planktons and other microorganisms (Ignacio et al. 2010). The results from the various stages in Table 1.1 shows that it significantly decreased in stage 1 and greatly increased in stage 2 and then continual decreases in stages 3 and 4. The increase in stage 2 was as a result of the water coming in contact with a mixed algae culture (Alejandro et al. 2010). The efficiency of reduction of the wastewater turbidity ranged from 71 to 96.8 %. Apart from stage 2, the turbidity of the treated water was either lower or within the permissible limit of WHO/FEPA.

The dissolved oxygen (DO) increased from 2.2 to 4.1 mg/L, 4.1 to 4.7 mg/L, 4.7 to 5.8 mg/L and 5.8 to 6.3 mg/L for stages 1, 2, 3, and 4 treatments, respectively. The percentage increase ranged from 70.7 to 81 %. This can be explained as the presence of plants and algae reduces dissolved $CO_2$ during the period of photosynthetic activity and this lead to increase in DO of water (Awuah et al. 2004; Ignacio et al. 2010). Also, the increase in DO might be as result of increased biodegradation of the wastewater constituents (Aisien et al. 2009). The DO from each stage was within the favourable WHO/FEPA standard for wastewater discharge into river. Chapman (1997) reported that the standard for sustaining aquatic life was 5 mg/L and below this concentration the aquatic life is adversely affected.

The results of the soluble anions ($HCO_3^-$, $NO_3^-$, $SO_4^{2-}$, $Cl^-$ and $PO_4^{3-}$), heavy metals (Cd, Zn, Pb and Fe) and total hardness are represented in Table 1.1. It can be seen that the efficiency of reduction of the soluble anions, heavy metals and total hardness from stages 1 to 4 ranged from 47.6 to 91.2 %, 88.6 to 100 % and 65.8 to 84.8 %, respectively. The order of reduction by the macrophytes and microalgae was heavy metal > total hardness > soluble anions. At the last stage, the heavy metals were completely removed from the treated abattoir wastewater. The aquatic macrophytes and microalgae can be considered as a hyperaccumulators for trace metals such as Cd, Pb, Fe and Zn, hence their excellent removal efficiency. These results agreed with those reported by other researchers (Aisien et al. 2010c; Mishra and Tripathi 2008; Endut et al. 2011; Maine et al. 2001; Delgado et al. 1995; Soltan and Rashed 2003; Sato and Kondo 1981). Their concentrations were either below or within the WHO/FEFA standard for wastewater discharge into surface water.

The variations of other water quality indicators (BOD, COD, TSS and TDS) are shown in Table 1.1. The efficiency of reduction from stage 1 to 4 varied from 93.1 to 98.3 %, 92.1 to 95.8 %, 72.3 to 94 % and 76.4 to 95.8 % for BOD, COD, TSS and TDS, respectively. These results were in conformity to that reported by other researchers when aquatic plants was used to treat other wastewater such as textile mill, rubber factory water, grey water (Aisien et al. 2009; Gamage and Yapa 2001; Koottatep and Polprasert 1997; Kulatillake and Yapa 1984; Endut et al. 2011). Chapman (1997) reported that both BOD and COD are important water quality parameters and are very essential in water quality assessment. Also, Aisien et al. (2010a, b, c) stated that the more organic and inorganic matters in wastewater, the higher the BOD and COD. The high BOD in untreated abattoir wastewater shows that the water contains significant amount of biodegradable organics such as blood, urine undigested materials, animal tissues, and solid waste from the slaughter (Osibajo and Adie 2007). The results showed that in all the stages there was significant removal of organic matters and nutrients in the abattoir wastewater, hence the drastic reduction in BOD and COD even in stage 1 and subsequent stages. The reductions in pH

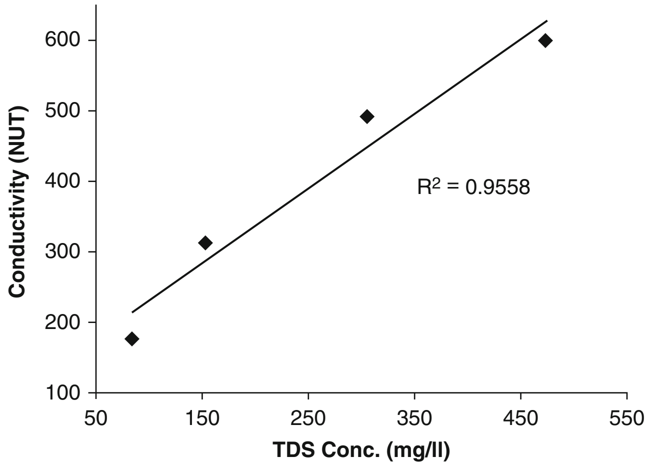


**Fig. 1.2** Variation of conductivity with TSS in abattoir wastewater

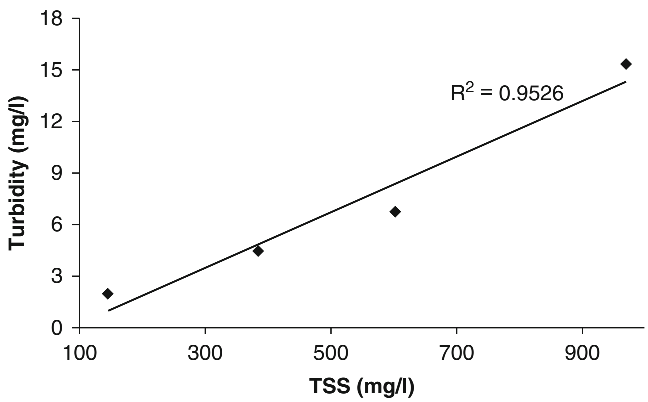


**Fig. 1.3** Variation of turbidity with TSS in abattoir wastewater

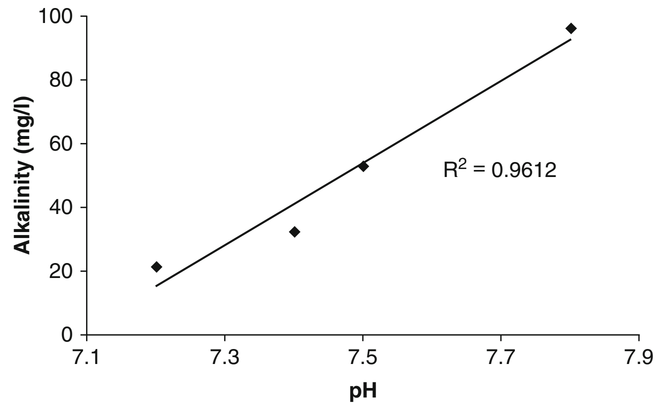


**Fig. 1.4** Variation of alkalinity with pH in abattoir wastewater

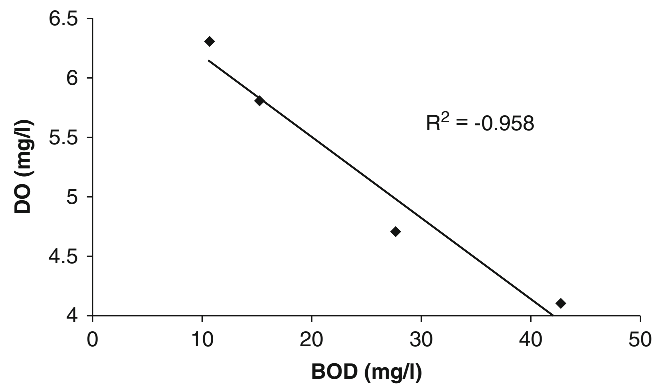


**Fig. 1.5** Variation of DO with BOD in abattoir wastewater

above encourage microbial action to degrade the inorganic and organic contaminants in the wastewater thereby causing reduction in BOD and COD (Aisien et al. 2009). Besides, the DO increases through the treatment period. The efficiency of increased ranged from 70.7 to 81 %. It has been reported that the presence of plants in the wastewater depletes dissolved carbon (IV) oxide during the period of photosynthetic activity and increase in DO of water, thus creates aerobic conditions in wastewater, which supports the aerobic bacterial activity to reduce BOD and COD. The water quality indicators (BOD, COD, TDS, TSS and DO) falls either below or within the WHO/FEPA permissible limits for discharge of wastewater into surface water.

The microbial contaminants which were express as Coliform count, *E. coli* and *Streptococci* were quite high as indicated in Table 1.1. These microorganisms will cause taste and odour problems when the untreated abattoir wastewater is released into the nearby surface water. It was observed that after the stage 2, the faecal coliform, *E. coli* and Streptococci were completely removed hence 100 % reduction. As a result, the treated wastewater met the zero level concentration of bacteria according to the WHO/FEPA standard for the discharge of wastewater into surface water. Similar results were reported previously by researchers (Aisien et al. 2010b; De-Busk and Reddy 1987; Dar et al. 2011). The efficiency of reduction of all the water quality indicators investigated using the water hyacinth (*Eichhornia crassipes*), water spinach (*Ipomoea aquatica*) and mixed algae (*Chlorella vulgaris and Closterium turgidum*) in the different treatment stages revealed the order of water hyacinth > water spinach > mixed algae.

Further evaluations were made to ascertain the actual relationship between some water quality indicators (conductivity, turbidity, TDS, TSS, BOD and COD). The linear regression results for the relationship between conductivity and TDS, turbidity and TSS, alkalinity and pH, and BOD and COD are represented in Figs. 1.2, 1.3, 1.4 and 1.5, respectively. The correlation coefficients revealed a positive linear correlation. This shows that as TDS increases the conductivity increases, and as TSS decreases the turbidity decreases. Also, as pH increases the alkalinity increases, and as BOD decreases the COD decreases. The correlation coefficients $R^2$ were 0.956, 0.953, 0.961 and 0.961 for relationship between conductivity and TDS, turbidity and TSS, alkalinity and pH and

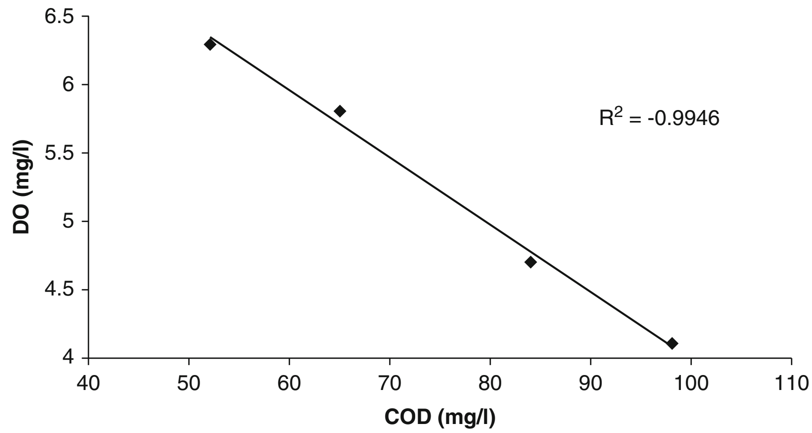


**Fig. 1.6** Variation of DO with COD in abattoir wastewater

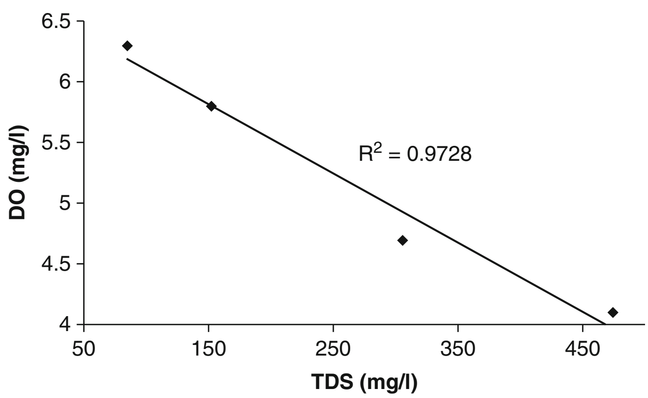


**Fig. 1.7** Variation of DO with TDS in abattoir wastewater

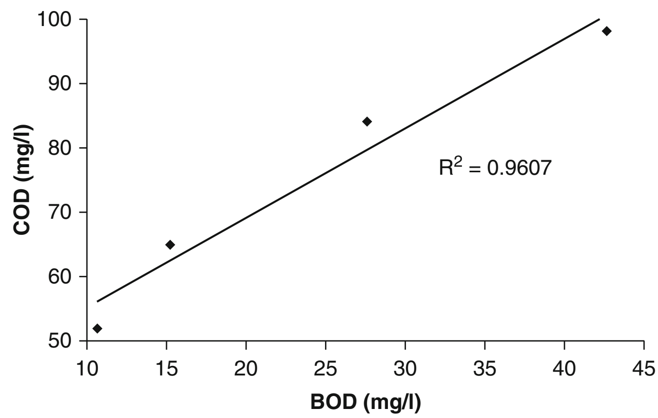


**Fig. 1.8** Variation of COD with BOD in abattoir wastewater

BOD and COD respectively. The very high correlation coefficients indicate a positive strong linear relationship between the water quality indicators of abattoir wastewater. It revealed that these indicators have common sources.

Figures 1.6, 1.7 and 1.8 show the regression results for the relationship between DO and BOD, DO and COD and DO and TDS, respectively. The results depict an inverse linear correlation. This indicates that as DO concentration increases the concentrations of TDS, BOD and COD decreases. The correlation coefficients $R^2$ were −0.958, −0.995 and −0.973 for relationships between DO and BOD, DO and COD, and DO and TDS, respectively. These correlation coefficients suggest similar sources.

The correlation coefficient of heavy metals in abattoir wastewater as indicated in Table 1.2 showed positive correlation and also supports the fact that they are from similar sources.

The Pearson's correlation matrix in Table 1.2 for DO, BOD, COD and TDS and Table 1.3 for heavy metals (Zn, Fe, Pb and Cd) for abattoir wastewater revealed linear and inverse linear correlation between different water quality indicators and different correlation coefficient which is either positive or negative.

**Table 1.2** Pearson's correlation matrix for pairs of water quality indicator in abattoir wastewater

| | DO | BOD | COD | TDS |
|---|---|---|---|---|
| DO | 1.00 | | | |
| BOD | −0.96 | 1.00 | | |
| COD | −0.99 | 0.96 | 1.00 | |
| TDS | −0.97 | 0.99 | 0.98 | 1.00 |

**Table 1.3** Pearson's correlation matrix for pairs of water quality indicator (heavy metals) in abattoir wastewater

| | Zn | Fe | Pb | Cd |
|---|---|---|---|---|
| Zn | 1.000 | | | |
| Fe | 0.987 | 1.000 | | |
| Pb | 0.993 | 0.998 | 1.000 | |
| Cd | 0.985 | 0.999 | 0.998 | 1.000 |

There was significant correlation regardless of which indicator was reduced. Also, it shows that regardless of which indicator reduced, the others were still significantly correlated. This implies that both indicators are not strongly driven by one particular indicator, but rather by the combination of all the water quality indicators.

## 1.4 Conclusions

The following conclusions can be drawn from this study:

The entire water quality parameters (indicators) levels of the abattoir wastewater released into the river was higher than the permissible limits of WHO/FEPA.

Hence, the river water is unsafe for both domestic and industrial usage.

The aquatic macrophytes (*Eichhornia crassipes*, *Ipomoea aquatica*) and the microalgae (*Closterium turgidum*, *Chlorella vulgaris*) significantly improved the abattoir wastewater quality by tremendously reducing all the water quality indicators.

The water quality indicators level were either within or below the WHO/FEPA recommended standard limit for the discharge of wastewater into surface water after virtually each treatment stage. Therefore, the macrophytes and microalgae treatment system can be used to treat abattoir wastewater for irrigation and other domestic and industrial purposes.

The treatment efficiency for all the macrophytes and microalgae applied were generally very good. The order of efficient reduction was water hyacinth > water spinach > mixed algae.

There was strong positive or negative correlation between the water quality indicators analysed, this indicates similar sources.

## References

Aisien FA, Oboh IO, Aisien ET (2009) Biotreatment of greywater. J Sci Indus Stud 7:5–8

Aisien ET, Gbegbaje-Das IIE, Aisien FA (2010a) Water quality assessment of river Ethiope in the Niger-Delta Coast of Nigeria. Elect J Environ Agr Food Chem 9:1739–1745

Aisien ET, Nwatah CV, Aisien FA (2010b) Biological treatment of landfill leachate from Benin City, Nigeria. Elect J Environ Agr Food Chem 9:1701–1705

Aisien FA, Faleye O, Aisien ET (2010c) Phytoremediation of heavy metals in aqueous solutions. Leonard J Sci 17:37–46

Alejandro R, Leopoldo GM, Tom S (2010) Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour Technol 101:58–64

APHA (2005) Standard methods for the examination of water and wastewater. 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC

Awuah E, Oppong-Peprah M, Lubberding HJ, Gijzen HJ (2004) Comparative performance studies of water lettuce, duckweed and algal-based stabilization ponds using low-strength sewage. J Toxicol Environ Health Part A 67:1727–1739

Baskar G, Deeptha VT, Rahman AA (2009) Treatment of wastewater from kitchen in an institution hostel mess using constructed wetland. Int J Recent Trends Engg 1:54–58

Benka-Coker MO, Ojior OO (1995) Effect of slaughterhouse wastes on the water quality of Ikpoba River, Nigeria. Bioresour Technol 52:5–12

Chapman D (1997) Water quality assessment. A guide to the use of biota, sediments and water in environmental monitoring, 2nd edn. EandFN Spon, London, UK

Dar SH, Kumawat DM, Singh N, Wani KA (2011) Sewage treatment potential of water hyacinth (*Eichhornia crassipes*). Res J Environ Sci 5:377–385

De-Busk TA, Reddy KR (1987) Wastewater treatment using aquatic macrophytes: contaminant removal processes and management strategies. In: Reddy KR, Smith WH (eds) Aquatic plants for water treatment and resource recovery. Mongolia publishing Inc., Orlando

Delgado M, Guardiola E, Bigeriego M (1995) Organic and inorganic nutrients removal from pig slurry by water hyacinth. J Environ Sci Health A 30:1423–1434

Dipu S, Kumar AA, Thanga VSG (2011) Phytoremediation of dairy effluent by constructed wetland technology. Environmentalist 31:263–278

Endut A, Jusoh N, Ali WB, Nik W (2011) Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. Desalinat Wat Treat 32:422–430

Fonkou T, Agendia P, Kengne I, Akoa A, Nya J (2002) Potentials of water lettuce (*Pistia stratiotes*) in domestic sewage treatment with macrophytic lagoon systems in Cameroon. In: Proceedings of international symposium on environmental pollution control and waste management, Tunis, pp 709–714

Gamage NS, Yapa PAJ (2001) Use of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in treatment systems for textile mill effluents—a case study. J Nat Sci Foundat Sri Lanka 29:15–28

Girija N, Pillai SS, Koshy M (2011) Potential of *Vetiver* for phytoremediation of waste in retting area. The Ecoscan 1:267–273

Hu MH, AoYS YXE, Li TQ (2008) Treating eutrophic water for nutrient reduction using an aquatic macrophyte (*Ipomoea aquatica* Forsskal) in a deep flow technique system. Agr Wat Manage 95:607–615

Ignacio G, Virginia AV, Saul B, Maria C, Roberto S, Pedro A, Eloy B, Raul MA (2010) Comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. Bioresour Technol 101:5150–5158

Jing SR, Lin YF, Wang TW, Lee DY (2002) Microcosm wetlands for wastewater treatment with different hydraulic loading rates and macrophytes. J Environ Qual 31:690–696

Johns MR (1995) Developments in wastewater treatment in the meat processing industry: a review. Bioresour Technol 54:203–216

Koottatep T, Polprasert C (1997) Role of plant uptake on nitrogen removal in constructed wetlands located in the tropics. Water Sci Technol 36:1–8

Kulatillake N, Yapa PAJ (1984) A study on the use of water hyacinth in rubber effluent treatment systems. In: Proceedings of the Malaysian chemical congress, Kuala Lumpur, Malaysia

Lakshmana PP, Jayashree S, Rathinamala J (2008) Application of vetiver for water and soil restoration. www.vetiver.org/TVN_INDIA_1stWORKSHOP_PROCEEDINGS/Chapter%202-3.pdf

Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquat Plant Manage 42:60–68

Lu Q, He ZL, Graetz DA, Stoffella PJ, Yang X (2010) Phytoremediation to remove nutrients and improve eutrophic stormwaters using water lettuce (*Pistia stratiotes L.*). Environ Sci Poll Res 17:84–96

Maine MA, Duarte MV, Sune NL (2001) Cadmium uptake by floating macrophytes. Water Res 35:2629–2634

Mahmood Q, Zheng P, Islam E, Hayat Y, Hassan MJ, Jilani G, Jin RC (2005) Lab scale studies on water hyacinth (*Eichhornia crassipes* Marts Solms) for biotreatment of textile wastewater. Caspian J Env Sci 3:83–88

Mkandawire M, Dudel EG (2007) Are *Lemna* spp. effective phytoremediation agents? Boremed Biodivers Bioavailab 1:56–71

Mashauri DA, Mulungu DMM, Abdulhussein BS (2000) Constructed wetland at the University of Dar Es Salaam. Wat Res 34:1135–1144

Massé DI, Massé L (2000) Characterization of wastewater from hog slaughterhouses in Eastern Canada and evaluation of their in-plant wastewater treatment systems. Agriculture and Agri-Food Canada, East, Lennoxville, QC, Canada

Mishra VK, Tripathi BD (2008) Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour Technol 99:7091–7097

Mishra VK, Upadhyay AR, Pandey SK, Tripathi BD (2008) Concentrations of heavy metals and aquatic macrophytes of Govind Ballabh Pant Sagar an anthropogenic lake affected by coal mining effluent. Environ Monit Assess 141:49–58

Mittal GS (2004) Characteristic of the effluent wastewater from abattoirs for land application. J Food Rev Int 20:229–256

Osibajo O, Adie GU (2007) Impact of effluent from Bodija abattoir on the physico-chemical parameters of Osinkaye stream in Ibadan City, Nigeria. Afr J Biotech 6:1806–1811

Ran Y, Qiping W, Xiwu L (2012) Constructed wetland in a compact rural domestic wastewater treatment system for nutrient removal. Environ Eng Sci 29:751–757

Sato M, Kondo S (1981) Biomass production of water hyacinth and its ability to remove inorganic minerals from water. Effect of the concentration of culture solution on the rates of plant growth and nutrient uptake. Jpn J Ecol 31:257–267

Sing-Lai L, Wan-Loy C, Siew-Moi P (2010) Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresour Technol 101: 7314–7322

Snow AM, Ghaly AE (2008) A comparative study of the purification of aquaculture wastewater using water hyacinth, water lettuce and parrot's feather. Am J Appl Sci 5:440–453

Soltan ME, Rashed MN (2003) Laboratory study on the survival of water hyacinth under several conditions of heavy metal concentrations. Adv Environ Res 7:82–91

Tritt WP, Schuchardt F (1992) Materials flow and possibilities of treating liquid and solids wastes from slaughterhouse in Germany. Bioresour Technol 41:235–245

Truong P, Baker D (1998) Vetiver grass system for environmental protection. Technical Bulletin no. 1998/1, Pacific Rim Vetiver Network, Office of the Royal Development Projects Board, Bangkok, Thailand

Valipour A, Raman VK, VS G (2011) Phytoremediation of domestic wastewater using *Eichhornia crassipes*. J Environ Sci Eng 53:183–190

# 2 Phytoremediation of Contaminated Waters to Improve Water Quality

Lucía Grijalbo Fernández, Mercedes Fernández-Pascual, Francisco Javier Gutiérrez Mañero, and José Antonio Lucas García

## Abbreviations

| | |
|---|---|
| BTEX | Benzene toluene, ethyl benzene, xylenes |
| COD | Chemical oxygen demand |
| $K_{ow}$ | Octanol–water partition coefficient |
| MWF | Metal working fluids |
| PAHs | Polycyclic aromatic hydrocarbon |
| PCB | Polychlorinated biphenyl |
| PGPB | Plant growth-promoting bacteria |
| PGPR | Plant growth-promoting rhizobacteria |
| STEM | Scanning transmision electron microscopy |
| TCE | Trichloroethylene |
| TEM | Transmision electron microscopy |
| TNT | 2,4,6-Trinitrotoluene |
| VOC | Volatile organic compounds |

## 2.1 Introduction

Water is our most important resource. We need water for various human activities such as the food supply, energetic resources, or industrial activities. Access to potable water has been problematic in the last decades (Lomborg 2001; The Millennium Development Goals Report 2008). Human activities are mainly responsible for global environmental change, especially since the Industrial Revolution, to the detriment of large parts of the world (Rockström et al. 2009).This global environmental change is directly related to climate change, which is responsible for around 20 % of the increment in the global water shortage (UNESCO 2009). It has been estimated that around 70 % of fresh water is used by agriculture (Baroni et al. 2007), 20 % by industry (refrigeration, transport, as a dissolvent for chemicals), and 10 % for domestic use (FAO 2003). Because of that, fresh water resources are reduced.

Rapid industrialization, urbanization, and population in the last few decades have added huge loads of pollutants to water resources (CPBC 2008). The main problem of water pollution is that it contains not only one single pollutant, so its contamination is very heterogeneous and there are really common hotspots of contamination (French et al. 2006). Industries have different processes and consume large volumes of water and chemicals causing changes in the composition and toxicity of the wastewater effluents. Some research has shown that 40 % of hazardous waste sites in the United States are thought to be cocontaminated by organic and inorganic pollutants (Sandrin and Maier 2003; Sandrin et al. 2000). The water pollution problem is very important, mainly in developing countries, due to industrial proliferation and modern agricultural technologies (Sood et al. 2012).

About two million tons of waste (industrial and agrochemical residues, human discharges) are spilled in the water courses every day, thus it has been estimated that the global residual water produced is around 1,500 km$^3$. It is known that 1 L of residual water can pollute 8 L of fresh water, so the global burden of pollution could be raised to 12,000 km$^3$ (UNESCO 2009).

Usually a combination of traditional techniques (chemical precipitation, ultrafiltration, chemical oxidation and reduction, electrochemical treatment, reverse osmosis, coagulation–flocculation, and ion exchange) would be used (e.g., Volesky 2001; Rai 2009). These remediation technologies have specific benefits and limitations but in general none of them is cost effective (Volesky 2001; Rai 2009). The main problem of these traditional techniques is that they need energy and this resource also pollutes the environment. However, other environmentally friendly techniques have been developed. Phytoremediation may provide a solution (Batty and Dolan 2013). Phytoremediation is emerging as an innovative tool, because plants are solar-driven and thus

L. Grijalbo Fernández (✉) • F.J. Gutiérrez Mañero
J.A. Lucas García
Department of Pharmaceutical & Health Sciences, Universidad San Pablo CEU, Madrid, Spain
e-mail: lgrijalbo@gmail.com

M. Fernández-Pascual
CSIC, Crop Protection Department, Institute of Agricultural Sciences, Madrid, Spain

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make this technology a cost-effective mode, with great potential to achieve a sustainable environment.

Various research has shown the ability of plants to remove pollutants. Since the 1970s several researchers including Boyd (1970), Stewart (1970), Wooten and Dodd (1976), and Conwell et al. (1977) have shown that aquatic plants can remove nutrients. Others such as Seidal (1976), Wolverton and McDonald (1976), and Wolverton and Mckown (1976) have shown plants' ability to remove organic pollutants from aquatic environments.

## 2.2 Phytoremediation

Phytoremediation is the use of plants and their associated microorganisms to remove, stabilize, and transform pollutants in the soil, water, and atmosphere (Batty and Dolan 2013). In this process natural or genetically engineered plants can be used with their ability to accumulate, degrade, or eliminate metals, pesticides, solvents, explosives, and crude oil and its derivatives (Flathman and Lanza 1998; Prasad and Freitas 2003).

There are a number of advantages in the phytoremediation strategy over traditional technologies. The most obvious is the significantly lower energy costs of between 50 and 90 % (Ensley 1997; Glass 1999) and their ecofriendly nature. There are also some limitations that can be overcome using plants with high biomass, faster growth rate, and ability to adapt to a wide range of environmental conditions. Other advantages and limitations of phytoremediation are compared in Table 2.1.

The main mechanisms involved in water phytoremediation are:

– *Phytoaccumulation*: Use of plants to remove contaminants from the site with its associated accumulation in leaf or root tissues.
– *Phytodegradation*: Involves a plant–microbe symbiotic relationship that degrades organic pollutants within the rhizosphere.
– *Phytovolatilization*: A process in which plants take up water–soluble contaminants via transpiration and convert them to a gaseous form that is released through the stomata of the plants.

*Phytofiltration or rhizofiltration*: The main phytoremediation process occurs through the roots of plants that are able to absorb, concentrate, and precipitate toxic metals and organic chemicals in polluted water. In this process, the rhizosphere is highly important, in that plants, microbes, and sediment act together as a biogeochemical filter that efficiently removes the wastewater pollutants (Hooda 2007).

Plants can be used as filters in constructed wetlands (Lytle et al. 1998; Horne 2000; Nwoko et al. 2004), for filtering large volumes of wastewater, or can be used in a hydroponic setup with a continuous air supply (Raskin et al. 1997). This is an indoor, contained setup and is relatively expensive to implement. It is most useful for bioremediation of small volumes of wastewater polluted with hazardous inorganics such as radionuclides (Negri and Hinchman 2000; Dushenkov and Kapulnik 2000).

Figure 2.1 shows a rhizofiltration mechanism in which plant roots have been rooted in esparto fibers and metal-working fluids constitute the toxic effluent (Grijalbo et al. 2013). In the rhizofiltration process plants need to be fixed to a support and it is necessary to have water recirculation to improve its efficiency. This process could not be used directly in aquatic effluents such as rivers or streams.

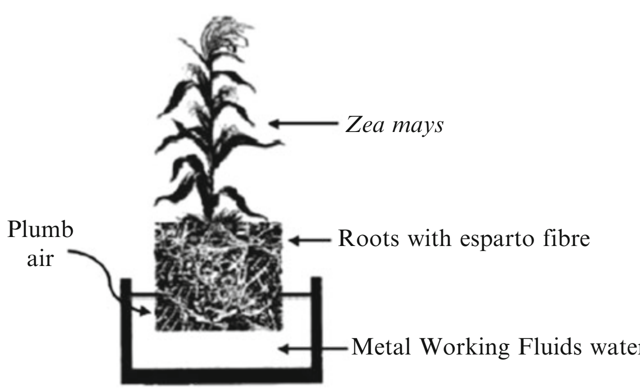


**Fig. 2.1** Rhizofiltration system. (from Grijalbo et al. 2013, reproduced with permission from the *Journal of Hazardous Materials*, 260: 220–230 © 2013 Elsevier B.V.)

**Table 2.1** Advantages and limitations of phytoremediation

| Advantages | Limitations |
|---|---|
| Cost- effective and eco-friendly technology as compared to traditional process both in situ and ex-situ | Process is effective with respect to the surface area covered and limited by the depth reached by the roots |
| It uses naturally inherent potential of naturally occurring plants and microbes to clean polluted sites. Help in preserving the natural state of environment | The response of plant and microbe varies under different growth conditions (i.e., climate, temperature, light intensity, altitude etc.) |
| It can be used to treat sites with more than one pollutant | Success of phytoremediation depends upon the tolerance plants used to treat pollutant. |
| After phytoremediation, the hyperaccumulating plants can be used for retrieval of the precious heavy metals as bio-ores | There exists a possibility of heavy metals re- entering the environment, because of their biodegradable nature |

From Sood et al. 2012, reproduced with permission from the *AMBIO*, 41: 122–137 © Royal Swedish Academy of Sciences 2011)

**Table 2.2** Summary of phytoremediated chemicals

| Type | Chemicals treated | Reference |
|---|---|---|
| Phytoaccumulation/extraction | Cd, Cr, Pb, Zn, radionuclides BTEX, pentachlorophenol, short chained aliphatic compounds | Horne (2000); Blaylock and Huang (2000) |
| Phytodegradation/transformation | Nitrobenzene, nitroethane, nitrotoluene, atrazine, chlorinated solvent for example DDT, chloroform, etc.) | Schnoor et al. (1995); Jacobson et al. (2003) |
| Phytostabilization | Heavymetals in ponds, phenols and chlorinated solvents | McCutcheon and Schnoor (2003); Newman et al. (1997) |
| Phytostimulation | Polycyclicaromatic hydrocarbon, BTEX, PCB, tetrachloroethane | Hutchinson et al. (2003); Olson et al. (2003) |
| Phytovolatilization | Chlorinated solvent, Hg, Se | Hutchinson et al. (2003); Olson et al. (2003) |
| Phytofiltration | Heavymetals, organics and radionuclides. Plant nutrients | Horne (2000); Nwoko et al. (2004) |

From Nwoko 2009, reproduced with permission from the *African Journal of Biotechnology*, 9: 6010–6016 © 2010 Academic Journals
BTEX = benzene, toluene, ethyl benzene, xylenes; PCB = Polychlorinated biphenyl

The plants used in this process should have fast growing dense roots, be tolerant to the pollutant(s), use precipitation, absorption, or adsorption methods to remove the pollutant(s), and be able to maintain this ability for a long period of time (Marin et al. 2005).

Table 2.2 presents a summary of phytoremediated chemicals and some research where they have been used.

## 2.3 Phytoremediation of Inorganic Pollutants in Water

Inorganic contaminants come from natural processes such as volcanic eruptions and continental dusts. They are mineral based and have a wide range of anthropogenic sources within the environment including extraction of ores, industrial processing of many kinds, gas generation, landfill, and transport (e.g., Batty et al. 2010; Liu et al. 2009; Thums et al. 2008). The majority of these pollutants cannot be degraded into simple compounds, so they cannot be fully removed from the environment, but instead they can be transported or transformed into less toxic forms. Among various water inorganic pollutants, heavy metals are of major concern because of their persistent and bio accumulative nature (Lokeshwari and Chandrappa 2007; Chang et al. 2009; Yadav et al. 2009). Heavy metals have a high atomic weight and around five times more density than water. They are highly toxic and cause ill effects at very low concentrations. Their origin in aquatic environments is either natural, by slow leaching from soil/rock to water, or through anthropogenic sources, such as the discharge of untreated effluent (waste water; Sood et al. 2012).

Plants used in phytoremediation can transform, stabilize, or accumulate inorganic matter. They need to be tolerant to high concentrations of inorganic compounds or avoid uptake of the pollutants (Batty and Dolan 2013).

Pollutants are taken up by biological processes in which different membrane transporters interact (plant nutrients or other compounds chemically similar to nutrients that allow pollutants to pass inadvertently into root cells, for example, arsenate that is taken up by phosphate transporters, selenate by sulphate transporters; Abedin et al. 2002). It is important to know that phytoaccumulation is saturable, according to Michaelis Menten kinetics, because it depends on a discrete number of membrane proteins (Marschner 1995).

### 2.3.1 Phytovolatilization

Plants are able to remove inorganic pollutants through volatilization, which is a process that completely removes the pollutant from the site as a gas, without the need for plant harvesting and disposal.

One example is the Se volatilizers. Some aquatic species, such as rice, rabbit foot grass, Azolla, and pickle weed are the foremost plants with the ability to volatilize this compound (Hansen et al. 1998; Lin et al. 2000; Pilon-Smits et al. 1999; Zayed et al. 2000). Volatilization of Se involves assimilation of inorganic Se into the organic seleno amino acids selenocysteine (SeCys) and selenomethionine (SeMet). The latter can be methylated to form dimethylselenide (DMSe), which is volatile (Terry et al. 2000).

The phytovolatilization process could be improved using plant species with high transpiration rates, which can overexpress enzymes such as cystathionine-V-synthase that mediates S/Se volatilization (Van Huysen et al. 2003) and by transferring the gene for Se volatilization from hyperaccumulators to nonaccumulators (Le Due et al. 2004).

### 2.3.2 Hyperaccumulator Plants

Hyperaccumulator plants play an important role in inorganic phytoremediation. In contrast with other species/genotypes, these organisms tolerate extremely high concentrations of bioavailable metal (Baker 1981; Baker and Whiting 2002).The shoot/root or leaf/root quotient for metal concentration is >1 indicating preferential partitioning of metals to the shoot.

Baker et al. (2000) suggested that there are around 400 species from 45 plant families that are hyperaccumulators. Such plants can accumulate As, Cu, Co, Cd, Mn, Ni, Se, Pb, or Zn up to levels that are 100–1,000 times those normally accumulated by plants grown under the same conditions (Brooks 1998; Baker et al. 2000; Ma et al 2001).

Table 2.3 lists some important hyperaccumulators including the recently discovered ones.

The use of these organisms is limited, because they are usually slow growing and produce low biomass. To take advantage of the hyperaccumulation ability and to make it a commercially viable technology, genetic engineering may be used by introducing the relevant genes into a higher biomass producing non accumulators (Hooda 2007).

High quantities of small organic molecules that can operate as metal-binding ligands appear in hyperaccumulator roots, contributing to metal detoxification by buffering cytosolic metal concentrations. In plants, the principal classes of metal chelators include phytochelatins, metallothionins, organic acids, and amino acids (Shah and Nongkynrih, 2007).

One interesting hyperaccumulation process occurs in heavy metal. This involves several steps, such as the transport of the heavy metal across the plasma membrane, translocation of the heavy metal, detoxification, and sequestration at the cellular and whole plant levels (Shah and Nongkynrih 2007; Rascio and Navari-Izzo 2011). Several plant metal transporters identified so far include ZIP1-4, ZNT1, IRT1, COPT1, Nramp (natural-resistance–associated macrophage protein), AtVramp1/3/4, and LCT1 on the plasma membrane–cytosol interface; ZAT, CDF (cation diffusion facilitator), ABC type, AtMRP, HMT1, CAX2 seen in vacuoles, and RAN1 seen in Golgi bodies.

Finally, it is important to note that living and dead biomass could take part in the phytoaccumulation process. One example is the aquatic macrophytes that are used to remove heavy metal from aquatic effluents (Umali et al. 2006; Rai 2008; Mashkani and Ghazvini 2009; Mishra et al. 2009). Depending on the type of biomass we can distinguish:

– *Biosorption*: It is a passive pollutant uptake. It uses dead/inactive biological material or material derived from biological sources.
– *Bioaccumulation*: This consists of the uptake of heavy metals using living cells. The problem with this biomass is that toxic effluents affect biological processes, so it may not be a viable option in waters that are extremely polluted. Plants are able to tolerate different toxic concentrations, up to a saturation level (Eccles 1995); when it is exceeded, the plant metabolism is interrupted, and the organism dies.

Some differences between both methods are summarized in Table 2.4.

**Table 2.3** Several metal hyperaccumulator species with respective metal accumulated

| S.No. | Plant species | Metal | References |
|---|---|---|---|
| 1. | *Thlaspi caerulescens* | Zn, Cd | Reeves and Brooks (1983); Baker and Walker (1990) |
| 2. | *Ipomea alpine* | Cu | Baker and Walker (1990) |
| 3. | *Sebertia acuminata* | Ni | Jaffre et al. (1976) |
| 4. | *Haumaniastrum robertii* | Co | Brooks (1977) |
| 5. | *Astragalus racemosus* | Se | Beath et al. (2002) |
| 6. | *Arabidopsis thaliana* | Zn, Cu, Pb, Mn, P | Lasat (2002) |
| 7. | *Thlaspi goesingens* | Ni | Kramer et al. (2000) |
| 8. | *Brassica oleracea* | Cd | Salt et al. (1995) |
| 9. | *Arabidospsis halleri* | Zn, Cd | Reeves and Baker (2000); Cosio et al. (2004) |
| 10. | *Sonchus asper* | Pb, Zn | Yanqun et al. (2005) |
| 11. | *Corydails pterygopetala* | Zn, Cd | Yanqun et al. (2005) |
| 12. | *Alyssum bertolonii* | Ni | Li et al. (2003); Chaney et al. (2000) |
| 13. | *Astragalus bisulcatus* | Se | Vallini et al. (2005) |
| 14. | *Stackhousia tryonii* | Ni | Bhatia et al. (2005) |
| 15. | *Hemidesmus indicus* | Pb | Chandrasekhar et al. (2005) |
| 16. | *Salsola Kali* | Cd | De la Rosa et al. (2004) |
| 17. | *Sedum alfredii* | Pb, Zn | Li et al. (2005) |
| 18. | *Pteris Vittata* | As | Ma et al. (2001); Zhang et al. (2004); Tu and Ma (2005) |
| 19. | *Helianthus anus* | Cd, Cr, Ni | Turgut et al. (2004) |

From Hooda 2007, reproduced with permission from the *Journal of Environmental Biology*, 28: 367–376 ©Triveni Enterprises, Lucknow (India)

**Table 2.4** Differences between bioaccumulation and biosorption

| Characters | Bioaccumulation | Biosoption |
|---|---|---|
| Biomass type | Living | Dead |
| Commercial applicability | Relatively less applicable because living material require additions of nutrients and other inputs | More applicable |
| Cost | Usually high | Low |
| Maintenance/storage | External energy is required to maintained culture in active growth phase | Low maintenance required and easy to store |
| Selectivity | Better | Less effective than bioaccumulation which can be improved by modification/processing of biomass |
| Sensitivity | Nutrient dependent | Nutrient independent |
| Temperature | Severely affect the process | Does not affect the process because biomass is dead |
| Metal location | Inter and intracellular | Extracellular |
| Degree of uptake | Active process | Passive process |
| Rate of uptake | Slower | Very fast |
| Desorption | Not possible | Possible |
| Regeneration and use | Since metal is intracellularly accumulated, the chances are very limited | High possibility of biosorbent regeneration, with possible reuse for a number of cycles |

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### 2.3.3 Research on Phytoremediation of Inorganic Pollutants

Inorganics that can be phytoremediated include macronutrients such as nitrates and phosphates (Horne 2000; Nwoko et al. 2004) and plant trace elements such as Cr, Ni, Zn, Mn, Mo, Fe, and Cu (Lytle et al. 1998), non essential elements such as Hg, Se, Cd, Pb, V, and W (Horne 2000; Nwoko and Egunjobi 2002; Okeke et al. 2004), and radioactive isotopes such as $^{238}U$, $^{137}Cs$, and $^{90}Sr$ (Dushenkov 2000).

Constructed wetlands have been used for many inorganics including metals, selenium (Se), nitrates, phosphates, and cyanide (Horne 2000). Sunflower or *Brassica juncea* roots and some aquatic plants including *Eichorniacrassipes*, *Hydrocotile umbellate*, *Lemna minor,* and *Azollapinnata* could accumulate heavy metals (Dushenkov et al. 1995; Dushenkov and Kapulnik 2000). Other plant tissues are used in phytofiltration processes, such as *Hemidesmusindicus* bark inmobilized in a column to remove Pb from aqueous solutions.

Phytoextraction is mainly used for metals and toxic inorganics (Se, Pb As; Blaylock and Huang 2000). Plants accumulate these metals in their tissues which are subsequently harvested. The harvested parts can be used for non food purposes (wood, cardboard) or reduced to ash and disposed of in a landfill.

In phytoremediation of inorganic pollutants aquatic macrophytes are commonly used. In contrast to terrestrial plants, these organisms have faster growth, larger biomass production, relatively higher capability of pollutant uptake, and better purification effects due to direct contact with contaminated water. Another important advantage is that they interact with the structural and functional aspects of aquatic ecosystems, improving water quality by regulating oxygen balance and the nutrient cycle, and accumulating heavy metals (Srivastava et al. 2008; Dhote and Dixit 2009).

Different researchers have shown the potential of aquatic macrophytes for heavy metal removal, such as (Brook and Robinson (1998), Cheng (2003), Prasad and Freitas(2003), Suresh and Ravishankar (2004), Srivastava et al. (2008)), Dhir et al. (2009), Dhote and Dixit (2009), Marques et al. (2009), and Rai (2009). These plants can be used in the treatment of industrial effluents and sewage waste water (Mkandawire et al. 2004; Arora et al. 2006; Upadhyay et al. 2007; Mishra et al. 2009; Rai 2010). Aquatic macrophytes have the ability to concentrate heavy metals that is much higher in the roots of these plants (Mishra et al. 2009; Paiva et al. 2009; Mufarrege et al. 2010).

Among macrophytes, Azolla should be mentioned as a nitrogen-fixing *pteridophyte* that could be an excellent candidate for the removal, disposal, and recovery of heavy metals from wastewater systems (Bennicelli et al. 2004; Arora et al. 2006; Umali et al. 2006; Upadhyay et al. 2007; Rai 2008; Mashkani and Ghazvini 2009). This organism also has the ability to hyperaccumulate a variety of pollutants such as radionuclides, dyes, pesticides, and the like from aquatic ecosystems along with other macrophytes (Padmesh et al. 2006; Rai and Tripathi 2009; Mashkani and Ghazvini 2009; Sood et al. 2011).

## 2.4 Phytoremediation of Organic Pollutants in Water

Organic contaminants are carbon based and can be released as a result of a wide range of activities such as gas works (Cofield et al. 2008; Luthy et al. 1997), timber treatment

(Mills et al. 2006), and coal processing (Cooke and Dennis 1983). The types of substances released are also extremely variable and include trichloroethylene, strazene, 2, 4,6-trinitrotoluene (TNT), gasoline, polycyclic aromatic hydrocarbon (PAHs), methyl tert-butyl ether, and polychlorinatedphenols (Batty and Dolan 2013).

The main problem in the phytoremediation process is that organic compounds have a wide range of chemical compositions and structures. If the compounds are in a structure that is available for the organisms (plants and/or microbes) the phytoremediation process will be successful (Batty and Dolan 2013). With some organic pollutants, plants are able to degrade them in the root zone; with others they can up take, degrade, sequestrate, or volatilize them. In most cases, plants are able to mineralize organic pollutants completely into relatively non toxic constituents, such as $CO_2$, nitrate, chlorine, and ammonia (Cunningham et al. 1996).

Plants take up substances into their roots through a combination of active and passive transport such as carrier proteins (Marschner 2002). In some cases, these transporters are not able to take up the compounds because of their anthropogenic origin. In these cases, they are usually taken up by. Hydrophobic compounds could enter the plant through the hemicellulose of the cell wall and lipid bilayer of the plant membranes (Cherian and Oliviera 2005). Organics with a log $K_{ow}$ (octanol–water partition coefficient) between 0.5 and 3 are sufficiently hydrophobic to move through the lipid bilayer of membranes but if they have a log $K_{ow}<0.5$ they cannot pass through membranes (Pilon-Smits 2005).

The most important factor influencing the phytoremediation of persistent organic pollutants is bioavailability (Mohan et al. 2006; Reid et al. 2000). This problem could be reduced by agitation and mixing (in bioreactor systems), and/or by the addition of surfactants (Mohan et al. 2006), therefore it is important to use the rhizofiltration system to improve bioavailability.

### 2.4.1 Phytodegradation or Rhizofiltration

In the phytoremediation process the plant selected has great influence, thus the rate of degradation can be maximized (Batty and Dolan 2013). One of the most important mechanisms is phytodegradation. In an aquatic environment, this is known as rhizofiltration.

In this process plant interaction is improved with the activity of the rhizospheric microorganisms that contribute to the degradation of the organic compounds. This interaction initially allows the dissipation of organic contaminants (Liste and Prutz 2006; Rezek et al. 2008). This plant–microbial relationship has a critical influence on phytoremediation: on the one hand, the microbial population degrades the pollutants; however, plants support their growth by the release of plant exudates including organic acids and enzymes (Macek et al. 2000) or by the release of degradative enzymes into the rhizosphere (Schnoor et al. 1995). Plant exudates and allelopathic chemicals released by plants in response to pathogen activity could act as analogues or co metabolites of organic pollutants. They seem to be the origin of the detoxifying microbial communities in the rhizosphere (Siciliano and Germida 1998), because it seems that these compounds induce catabolic enzymes in degrader organisms initiating the rhizodegradation of pollutants with a similar structure to the compounds exudated. This is important especially where microorganisms cannot utilize the pollutant as a sole carbon source (Hyman et al. 1995).

### 2.4.2 Research on Phytoremediation of Organic Pollutants

Phytodegradation works well for organics that are mobile in plants such as herbicides, TNT, and trichloroethylene (TCE). Phytovolatilization can be used for volatile organic compounds (VOC; Winnike-McMilliam et al. 2003) such as TCE (Hansen et al. 1998). Much research has demonstrated the effectiveness of the rhizofiltration system. Constructed wetlands have been used for organics such as explosives and herbicides (Schnoor et al. 1995; Jacobson et al. 2003). An interesting rhizofiltration research (Lucas García et al. 2013) was made with a maize–esparto system (Lucas García et al. 2011). This system was used to rhizoremediate an industrial effluent that contained metal-working fluids, an operationally exhausted synthetic fluid, used as a coolant and lubricant in large-scale continuous metal-working processes. The research has shown that the system is able to reduce COD (Chemical oxygen demand) and pH (ANOVA, $P < 0.05$) significantly, with both parameters below the regulatory limits as specified by the regional law10/1993 on industrial waste discharges into urban sanitary sewer systems (city of Madrid, Spain). A quantitative analysis was also done which showed that total hydrocarbons in the non phytoremediated MWFs were decreased significantly (ANOVA, $P < 0.05$) after the phytoremediation process.

Lucas García et al. (2013) also studied the final toxicity of the phytoremediated water. For this they used two systems: the photosynthetic efficiency of maize plants using PAM chlorophyll fluorescence and the study of the inhibition of the bioluminescence of the recombinant self-luminescent cyanobacterium Anabaena 4337, an ecologically relevant organism for aquatic environments. The first one demonstrated that the initial non phytoremediated MWFs were quite toxic to the maize plants, showing a significant reduction in the photosynthetic efficiency of the plants; however, no significant (ANOVA, $P<0.05$) differences were observed in the main chlorophyll fluorescence parameters in plants treated with the phytoremediated MWFs with respect to the control plants indicating that the phytoremediation process significantly decreased the toxicity associated with the initial non phytoremediated MWF. The second assay also showed that the initial MWFs were extremely toxic to the cyanobacterium with an EC50 of 0.083; toxicity was also indicated by the high dilution factor necessary to reach this EC50 value. However, in all cases, phytoremediation produced a significant (ANOVA, $P<0.05$) reduction of the MWF toxicity as evidenced by the increase in the $EC_{50}$ values and decrease in calculated dilution factors.

## 2.5 Phytoremediation of Co contaminated Sites

Most places have mixed pollution, with organic and inorganic compounds. This is known as a cocontaminated site. In these cases, phytoremediation is affected by the interaction of the substances, modifying the form and availability of the pollutants. For example, in a place contaminated with metals and organics, the interaction affects and even increases the metal bioavailability; this has been demonstrated by (Rosner and Aumercier (1990). Sandrin and Maier's (2003)) shows that depending on the metal concentration, the biodegradation of organic matter by microorganisms could be inhibited. Also, the presence of both pollutants could affect plant–microbe interaction and therefore phytoremediation success. Inorganic compounds (metals) decrease the microbial biomass (Brookes and McGrath 1984) or shift the community structure (Gray and Smith 2005). On the other hand, the presence of organic pollutants may also affect plant growth. To avoid these problems the engineered organisms could be used to obtain plants and bacteria with a higher ability to degrade or tolerate these co contaminated plants (Batty and Dolan 2013).One example is *Helianthus anus* (Dushenkov et al. 1995) and *Chenopodium amaranticolor* (Eapen et al. 2003) to rhizofiltration of uranium.

(Cherian and Oliviera (2005)) suggested that the use of transgenic plants may be a possible avenue for remediation of co contaminated sites. However, this area requires more research and field trials before constituting a realistic alternative.

## 2.6 Bioaugmentation

The interaction between plants and microorganisms improves phytoremediation. To increment the number of microorganisms a bioaugmentation process could be done, consisting of the inoculation of different microorganisms. The best inoculation is the one that uses bacteria which beneficially affect plant growth, known as plant growth-promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (Glick et al. 1999).

One benefit of these bacteria is that they are able to produce chemical substances that can change environmental conditions or are able to protect plants from disease (Uppal et al. 2008) by decreasing or preventing some of the negative effects of phytopathogenic organisms (Glick 2003). Some bacteria release biosulfactants (rhamnolipids) that make hydrophobic pollutants more water soluble (Volkering et al. 1998). Plants could release organic acids that can solubilize previously unavailable nutrients such as phosphorus (Cakmakciet al., 2006) or contain lipophilic compounds that increase pollutant water solubility or enhance biosulfactant-producing bacterial populations (Siciliano and Germida 1998).

However, researchers such as Nie et al. (2002) have shown that under high contaminant levels, the growth of PGPB can be severely inhibited and that plant growth was inhibited and any positive action cancelled out (Lucy et al. 2004).

One interesting research work related to bioaugmentation was done with a phytoremediation system with maize–esparto (Lucas García et al. 2011) inoculated with different strains (*Enterobacter* spJF690924, the yeast *Rhodotoruladairenensis* AF444501, and a consortium made with *Pseudomonas* sp., two *Acinetobacterjohnsonii* strains, and *Sphingobiumxenophagum*with the GenBank numbers JF937328, JF937329, JF937331, and JF937337, respectively). This system has shown that the non inoculated phytoremediation system has a higher reduction in COD, except when the consortium was inoculated. This review aims to discover the damage originated in physiological parameters. The strains inoculated, particularly the consortium, improve some of these measured parameters, thus it seems that the inoculation with bacteria can protect the plants against these harmful effects.

## 2.7 Pollutant Phytotoxicity

As has been shown, pollutants can be remediated in plants by different biophysical and biochemical processes (adsorption, transport, hyperaccumulation, and/or transformation and mineralization). First, elemental pollutants enter the plant through the normal nutrient uptake mechanism of the plant. Plants are protected against the toxicity effect by the degradation of toxic organics and the sequestration of inorganic pollutants in vacuoles. Another protection is the over expression of plant existing genes or transgenic expression of bacterial genes (Nwoko 2009). In spite of these protective processes, the pollutants produce high negative effects on the plants' biophysiological processes (photosynthesis, pigments, ultra-structural cell composition).

### 2.7.1 Inorganic Pollutant Phytotoxicity

First, we look at inorganic phytotoxicity. In this phytoremediation process, the pollutants are not degraded, but are sequestrated instead. One example is the heavy metals introduced into the aquatic system that are known to pose high levels of toxicity to aquatic organisms and human beings (Sánchez-Chardi et al. 2009; Siwela et al. 2009). These pollutants could cause DNA damage and carcinogenic effects in animals and humans due to their mutagenic ability (Knasmulier et al. 1998; Baudouin et al. 2002). When plants are grown in inorganic polluted water, different morphological, physiological/biochemical, and ultra-structural alteration can be shown in aquatic organisms. This damage could be used as a biomonitoring tool for the assessment of metal pollution in aquatic ecosystems (Zhou et al. 2008).

Various research has shown that exposure of heavy metals suppressed the vegetative growth and sporulation in different species of *Azolla* (Arora et al. 2004, 2006). Arora et al. (2006) observed that under Cr pollution *A. filiculoides* was most successful, producing 72 % of control biomass. The application of Cd, Cr, Mo, and Mn at concentrations of 3, 6, 5, and 10 mg/ml, respectively, significantly decreased the sporulation frequency and number of sporocarps per plant in *A. microphylla* and *A. caroliniana* (Kar and Singh 2003).

Only a few researchers (Sarkar and Jana 1986; Shi et al. 2003; Dai et al. 2006) have studied the influence of heavy metals in biochemical parameters: pigments, photosynthesis, and activities of oxidative enzymes. Sarkar and Jana (1986) observed that the treatment of *A. pinnata* with As, Pb, Cu, Cd, and Cr (2 and 5 mg L–1 each), decreased Hill activity, chlorophyll content, protein and dry weight, and increased tissue permeability with respect to the control. Shi et al. (2003) showed that an increase in concentration of Hg and Cd resulted in a drop in the chlorophyll content, mainly chlorophylls a and b. The damage observed in the chlorophyll process related to heavy metals can be a useful physiological tool to assess early changes in the photosynthetic performance of *Azolla* (Sánchez-Viveros et al. 2010).

The accumulation of heavy metals is known to result in various types of damage at the ultra-structural level (Sela et al. 1988, 1990; Shi et al. 2003; Gaumat et al. 2008); for example, Cd accumulation engenders some small dark grains with a high content of cadmium, phosphate, and calcium along the epidermal cells (Sela et al. 1990). The increase of heavy metal (Hg and Cd) concentration and incubation time in *A. imbricata* showed a higher. The results showed swelling of the chloroplast, disruption, and disappearance of the chloroplast membrane, and disintegration of chloroplasts; swelling of cristae of mitochondria, deformation and vacuolization of mitochondria; condensation of chromatin in the nucleus, dispersion of the nucleolus, and disruption of the nuclear membrane (Shi et al. 2003).

### 2.7.2 Organic Pollutant Phytotoxicity

One interesting review that shows the damage originated by organic pollutants was done by Grijalbo et al. (2013). They created a phytoremediation system with maize–esparto (Lucas García et al. 2011) inoculated with *Pseudomonas fluorescens*HM486749.TEM (Transmision Electron Microscopy) and STEM (Scanning Transmision Electron Microscopy) has been used to determine the effects of MWF water and inoculation with Aur6 on the cell structure of leaves and roots. The leaf cell structure of controls without MWF water shows a well-organized ultra-structure. See Figs. 2.2a, b and 2.3a, b, in which are shown undegraded chloroplasts (Fig. 2.2a), and in such case grana interconnected with intergrana (Fig. 2.3b). Also shown is a well-developed nucleus with nucleoli and condensed chromatin materials (Fig. 2.2a).

In plants growing in spent MWFs, early senescence symptoms were shown, either in inoculated or non inoculated plants, such as thylakoid swelling and a lamellar separation (Figs. 2.2c, d and 2.3c, e). It is interesting to see that in these treatments, the chloroplasts are beginning to degrade at the ends, being elongated (Fig. 2.2c, e) and the chloroplast membrane suffers degradation even becoming broken in some chloroplast (Figs. 2.2f and 2.3f). The mitochondria and the vesicles also show senescence symptoms (Figs. 2.2f and 2.3c, d, f).

The LTSEM shows the presence of a vacuole with plenty of water and solutes, with numerous chloroplasts in controls (Fig. 2.4a, b). However, plants in the presence of MWFs change the leaves' hydric content and it seems that the chloroplast number is reduced (Fig. 2.4c, d, g, h).

It is interesting to emphasize that inoculation with the strain (i.e., a PGPR) might protect the plant against the MWF effects, as shown, for example, in (Fig. 2.4c, d, g, h) where it seems to be a higher chloroplast number than that existing in non inoculated ones.

This research also shows that the organic pollutants of MWFs degrade cell roots' ultra-structure, with the appearance of extremely degenerative organelles such as vesicles or mitochondria. However, the vascular bundles have less deformation and xyleme and floeme could be distinguished even in MWF water treatment (Fig. 2.5b, d). In MWF water with Aur6 treatments (Fig. 2.5d) cuticle deterioration is observed, creating an irregular structure instead of an oval one such as in other treatments (Fig. 2.5a, b, c).

## 2.8 Conclusion

Phytoremediation is an emergent technique that has beneficial effects on wastewater treatment. It has been proven that this process could be used either with organic or inorganic pollutants. It is important to follow up the investigation of these strategies in order to know the processes that improve them and the phytotoxicity originated.

Finally, it is important to emphasize that phytoremediation gives an ecological vision that is environmentally

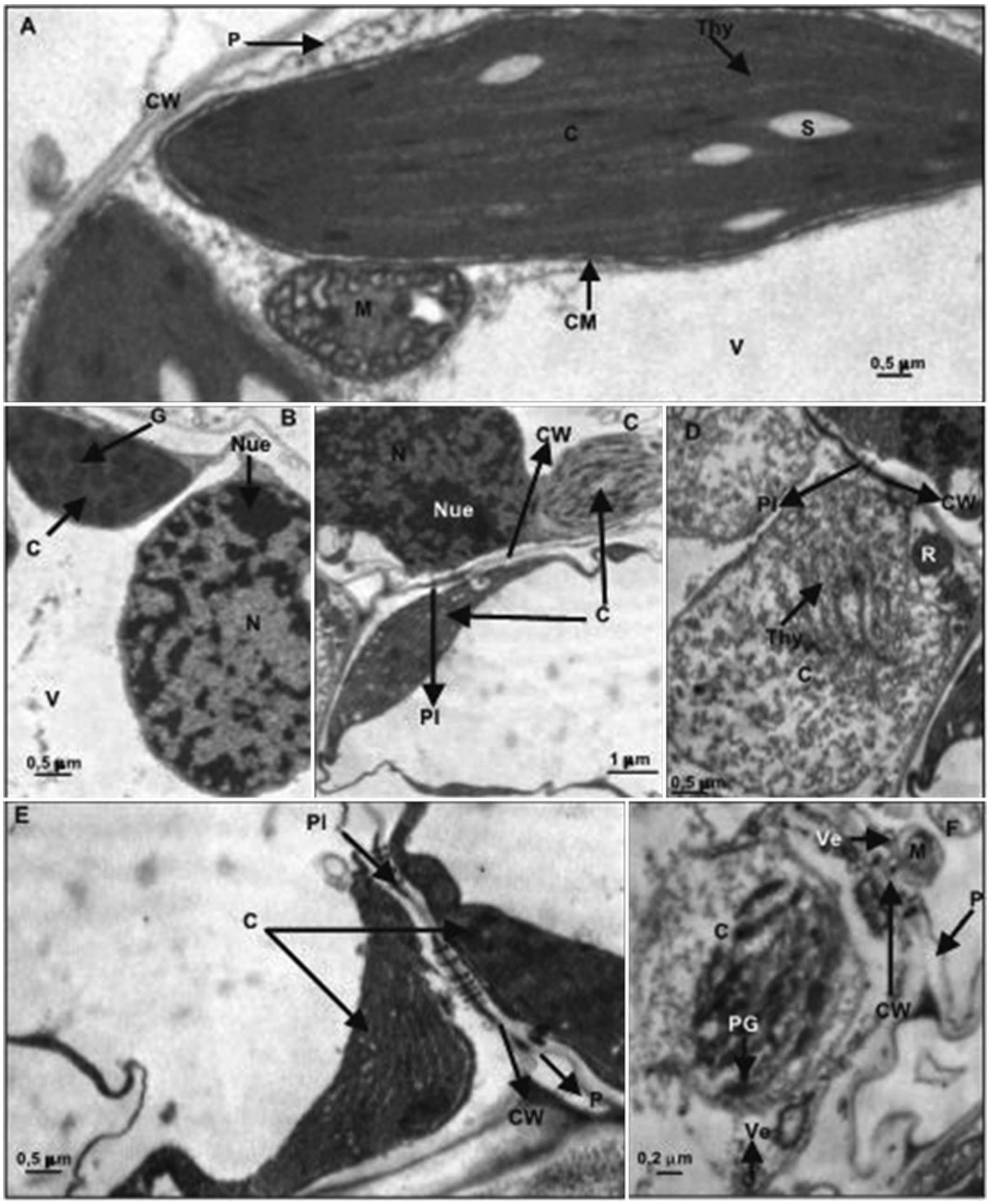


**Fig. 2.2** Electron transmision micrographs of leaf mesophyll cells of 20-day–old corn plants non inoculated with Aur6 and grown for 5 days with and without MWFS water. Treatments: tap water without Aur6 (**a, b**) and MWFS water without Aur6 (**c**, **d**, **e, f**). Starch granules (S), Chloroplast (C), Granum (G), Plasma membrane (P), Chloroplast membrane (CM), Mitochondria (M), Nucleus (N), Nucleolus (Nue), Cell wall (CW), Plasmodesme (Pl), Plastoglobuli (PG), Ribosome (R), Thylakoid (Thy), Vacuole (V), and Vesicle (Ve). Magnification bars correspond to 1,000 mm (**c**), 500 mm (**a**, **b**, **d**, **e**), and 200 mm (**g**). (from Grijalbo et al. 2013, reproduced with permission from the *Journal of Hazardous Materials*, 260: 220–230 © 2013 Elsevier B.V.)

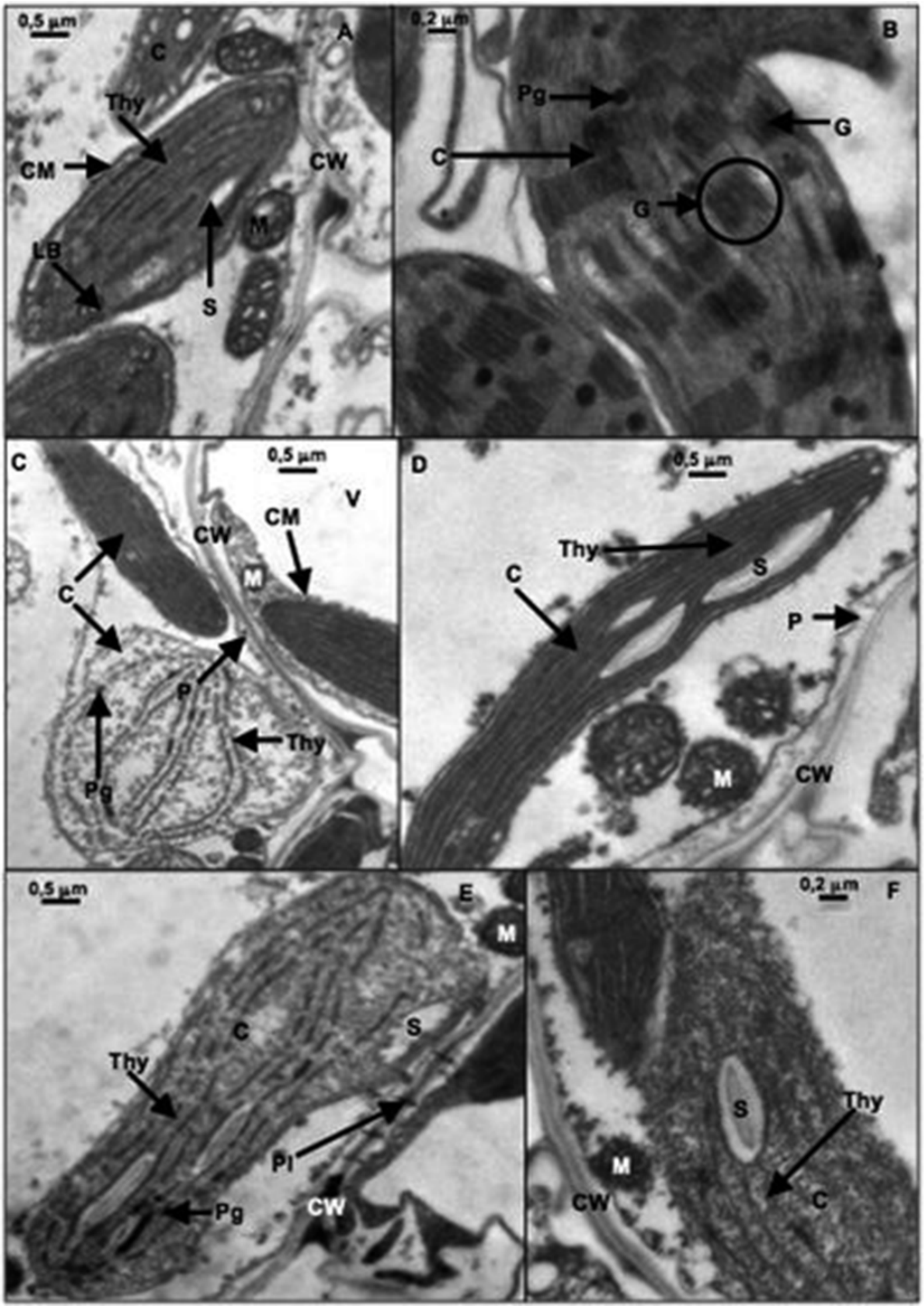


**Fig. 2.3** Electron transmision micrographs of leaf mesophyll cells of 20-day–old corn plants inoculated with Aur6 and grown for 5 days with and without MWFS water. Treatments: tap water with Aur6 (**a, b**) and MWFS water with Aur6 (**c, d, e, f**). Starch (S), Chloroplast (C), Granum (G), Lipid bodies (LB), Chloroplast membrane (CM), Plasma membrane (P), Mitochondria (M), Cell wall (CW), Plasmodesme (Pl), Plastoglobuli (Pg), Thylakoid (Thy), and Vacuole (V). Magnification bars correspond to 500 mm (**a**, **c**, **d**, **e**) and 200 mm (**g**). (from Grijalbo et al. 2013 reproduced with permission from the *Journal of Hazardous Materials*, 260: 220–230 © 2013 Elsevier B.V.)

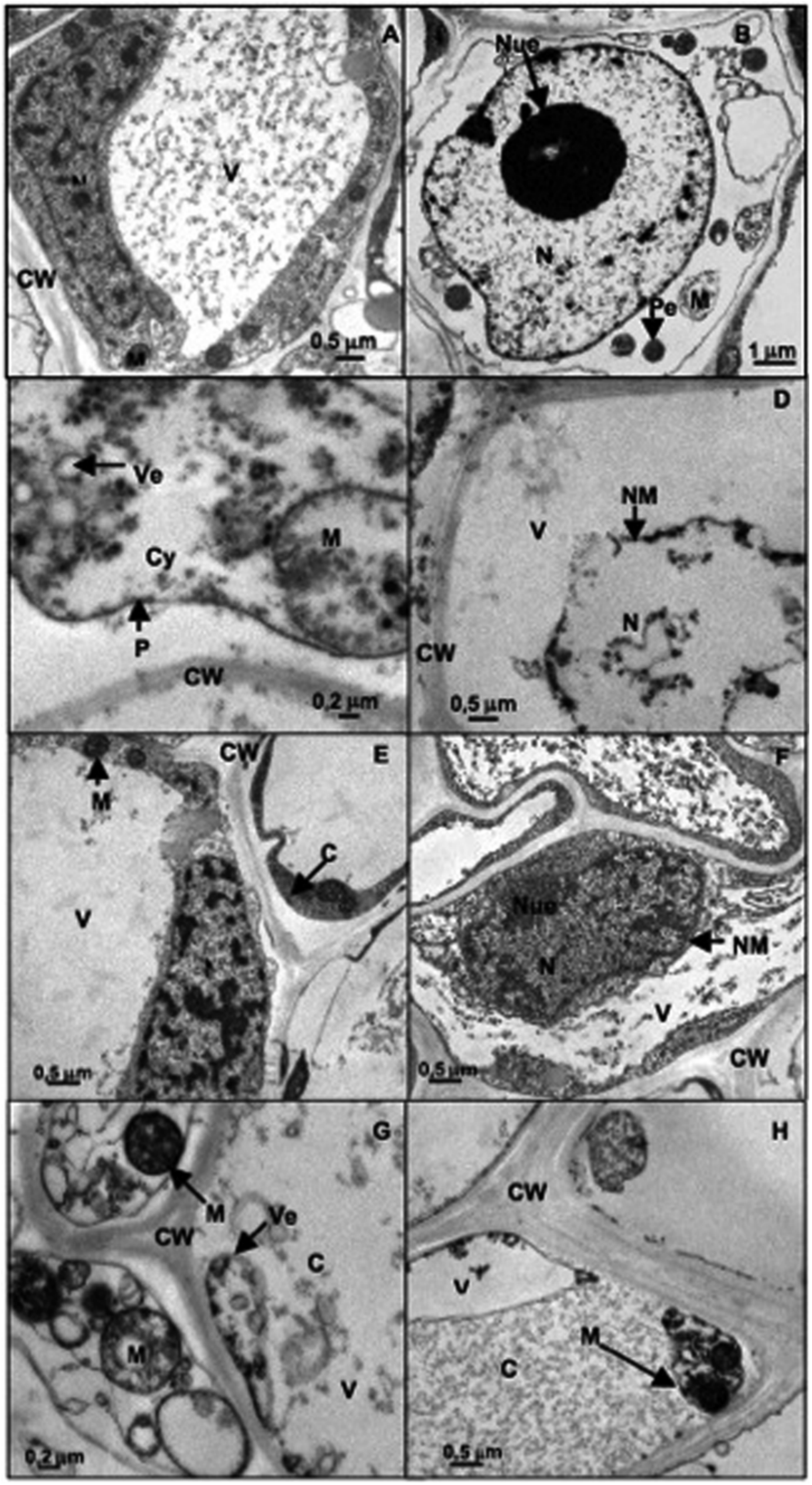


**Fig. 2.4** LTSEM micrographs of leaf mesophyll cells of 20-day–old corn plants inoculated and non inoculated with Aur6 and grown for 5 days with and without MWFS water. Treatments: tap water without Aur6 (**a, b**); MWFS water without Aur6 (**c, d**), tap water with Aur6 (**e, f**), and MWFS water with Aur6 (**g, h**). Bundle sheath cells (BSCs), Chloroplast (C), vascular bundles (VB), Nucleus (Nue), Cell wall (CW), Tonoplast (T), and Vacuole (V). Magnification bars correspond to 200 mm (**a**, **c**, **e**, **g**), 100 mm (**b**, **d**, **h**), and 50 mm (**f**). (from Grijalbo et al. 2013, reproduced with permission from the *Journal of Hazardous Materials*, 260: 220–230 © 2013 Elsevier B.V.)

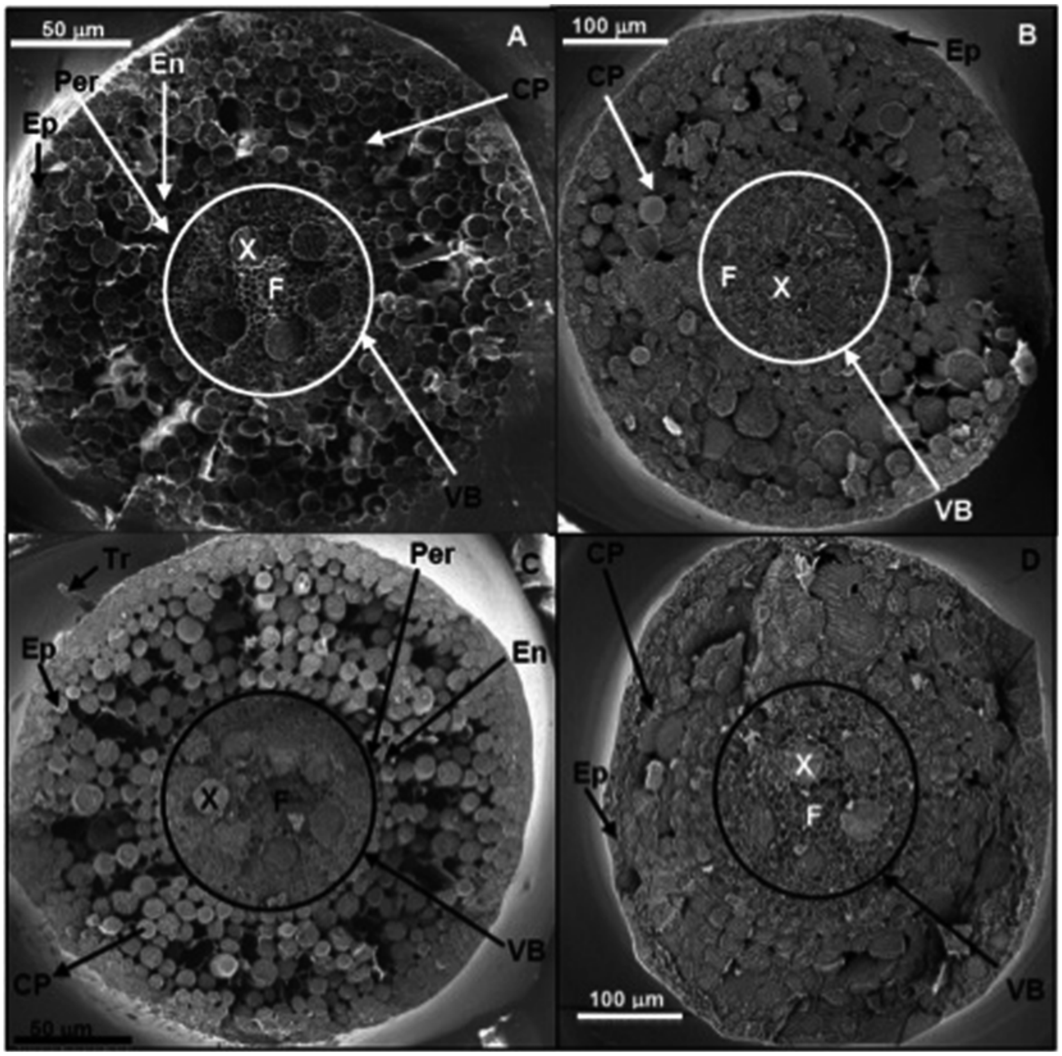


**Fig. 2.5** LTSEM micrographs of roots corn from different treatments: tap water without Aur6 (**a**); MWFS water without Aur6 (**b**), tap water with Aur6 (**c**) and MWFS water with Aur6 (**d**). Epidermis (Ep), Endodermis (En), Floeme (F), Vascular bundles (VB), Cortical Parenchyma (CP), Pericycle (Per), Tricome (Tr), and Xileme (X). Magnification bars correspond to 1,000 mm (**b**, **d**) and 500 mm (**a**, **c**). (from Grijalbo et al. 2013, reproduced with permission from the *Journal of Hazardous Materials*, 260: 220–230 © 2013 Elsevier B.V.)

friendly and with better aesthetic appeal than other physical means of remediation.

## References


Abedin MJ, Feldmann J, Medarg AA (2002) Uptake kinetics of arsenic species in rice plants. Plant Physiol 128:1120–1128

Arora A, Sood A, Singh PK (2004) Hyperaccumulation of cadmium and nickel by Azolla species. Indian J Plant Physiol 3:302–304

Arora A, Saxena S, Sharma DK (2006) Tolerance and phytoaccumulation of chromium by three Azolla species. World J Microbiol Biotechnol 22:7–100

Baker AJM (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, pp 155–177

Baker AJM, Whiting SN (2002) In search of the Holy Grail: a further step in understanding hyperaccumulation? New Phytol 155:1–4

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 85–107

Baroni L, Cenci L, Tettamanti M, Berati M (2007) Evaluating the environmental impact of various dietary patterns combined with different food production systems. Eur J Clin Nutr 61:279–286

Batty LC, Auladell M, Sadler JP (2010) The impacts of metalliferous drainageon aquatic communities in streams and rivers. In: Batty LC, Hallberg KB (eds) Ecology of industrial pollution. Cambridge University Press, Cambridge

Batty LC, Dolan C (2013) The potential use of phytoremediation for sites with mixed organic and inorganic contamination. Crit Rev Environ Sci Technol 43:217–259

Baudouin C, Charveron M, Tarroux R, Gall Y (2002) Environmental pollutants and skin cancer. Cell Biol Toxicol 18:341–348

Beath OA, Eppsom HF, Gilbert GS (2002) Selenium distribution in and seasonal variation of vegetation type occurring on seleniferous soils. J Am Pharm Assoc 26:394–405

Bennicelli R, Stezpniewska Z, Banach A et al (2004) The ability of *Azollacaroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. Chemosphere 55:141–146

Bhatia NP, Walsh KB, Baker AJ (2005) Detection and quantification of ligands involved in nickel detoxification in a herbaceous Ni hyperaccumulator*Stackhousiatryonii* Bailey. J Exp Bot 56:1343–1349

Blaylock MJ, Huang JW (2000) Phytoextraction of metals: in phytoremediation of toxic metals. In: Raskin I, Ensley BD (eds) Using plants to clean up the environment. Wiley, New York, pp 53–70

Boyd CE (1970) Vascular aquatic plants for mineral nutrient removal from pollutant water. Econ Bot 24:95–103

Brook RR, Robinson BH (1998) Aquatic phytoremediation by accumulator plants. In: Brook RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CABI International, Wallingford, pp 203–226

Brookes PC, McGrath SP (1984) Effects of metal toxicity on the size of the soil microbial biomass. J Soil Sci 35:341–346

Brooks RR (1977) Copperand cobalt uptake by *Haumaniastrum species*. Plant Soil 48:541–544

Brooks RR (1998) Plants that hyperaccumulate heavy metals. CAB Intl, Wallingford

Cakmakci R, Donmez F, Aydin A, Sahin F (2006) Growth promotion of plants by plant growth-promoting rhizobacteria under green hause and two different field soil conditions. Soil Biol Biochem 38:1482–1487

Chandrasekhar K, Kamala CT, Chary NS, Balaram V, Garcia G (2005) Potential of *Hemidesmus indiens* for phytoextraction of lead from industrially contaminated soils. Chemosphere 58(4):507–514

Chaney RL, Li YM, Brown SL, Homer FA, Malik M (2000) Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems; approaches and progress. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 129–158

Chang JS, Yoon IH, Kim K-W (2009) Heavy metal and arsenic accumulating fern species as potential ecological indicators in As-contaminated abandoned mines. Ecol Indic 9:1275–1279

Cheng S (2003) Heavy metals in plants and phytoremediation. Environ Sci Pollut Res Int 10:335–340

Cherian S, Oliviera MM (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390

Cofield N, Banks MK, Schwab AP (2008) Lability of polycyclic aromatichydrocarbons in the rhizosphere. Chemosphere 70:1644–1652

Conwell DA Jr, Zoltek J, Patrinely CD, Furman TS, Kim JI (1977) Nutrient removal by water hyacinths. J Water Poll Control Fed 49:57–65

Cooke M, Dennis AJ (1983) Polycyclic aromatic hydrocarbons: formation, metabolism and measurement. Battelle, Columbus

Cosio C, Martinoia E, Keller C (2004) Hyperaccumulation of cadmium and zinc in *Thlaspica erulescens* and *Arabidopsis halleri* at the leaf cellular level. Plant Physiol 134(2):716–725

Cunningham SD, Anderson TA, Schwab P, Hsu FC (1996) Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56:55–114

Dai LP, Xiong ZT, Huang Y, Li MJ (2006) Cadmium induced changes in pigments, total phenolics, and phenylalanine ammonia-lyase activity in fronds of *Azollaimbricata*. Environ Toxicol 21:505–512

De la Rosa G, Peralta Videa JR, Montes M et al (2004) Cadmium uptake and translocation in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species; ICP/OES and XAS studies. Chemosphere 55(9):1159–1168

Dhir B, Sharmila P, Saradhi PP (2009) Potential of aquatic macrophytes for removing contaminants from the environment. Crit Rev Environ Sci Technol 39:754–781

Dhote S, Dixit S (2009) Water quality improvement through macrophytes- a review. Environ Monit Assess 152:149–153

Dushenkov S (2000) Trends in phytoremediation of radionuclides. Plant Soil 249:167–175

Dushenkov S, Kapulnik Y (2000) Phytofiltration of metals. In: Raskin I, Ensley DD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, EEUU, pp 89–106

Dushenkov V, Nanda Kumar PBA, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239–1245

Eapen S, Suseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003) Potential for rhizofiltration of uranium using hairy roots cultures of Brassica junicea and Chenopodium amaranticolor. Environ Res 91:127–133

Eccles H (1995) Removal of heavy metal from effluent streams-why select a biological process. Int Biodeterior Biodegradation 1–3:5–16

Ensley BD (1997) Phytoremediation is the most cost effective approach for many sites. Paper presented at IBC phytoremediation conference, Seattle, WA, 18–19 June

Food and Agriculture Organization of the United Nations (FAO) (2003) No hay crisis mundial de agua, pero muchos países en vías de desarrollo tendrán que hacer frente a la escasez de recursos hídricos. En http://www.fao.org/spanish/newsroom/news/2003/15254-es.html 12 de marzo de 2013

Flathman PE, Lanza GR (1998) Phytoremediation: current views on an emerging green technology. J Soil Contam 7:415–432

French CJ, Dickinson NM, Putwain PD (2006) Woody biomass phytoremediation of contaminated brownfield land. Environ Pollut 141:387–395

Gaumat S, Mishra K, Rai UN, Baipal U (2008) Ultramorphological variation in *Azollapinnata* R. Br. Under single and mixed metal treatment with lead and iron. Phytomorphology 58:111–116

Glass DJ (1999) U. S. and international markets for phytoremediation 1999–2000. D. Glass Associates, Needham, MA

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacteria signaling processes. Soil Biol Biochem 37:395–412

Grijalbo L, Fernandez-Pascual M, García-Seco D, Gutierrez-Mañero FJ, Lucas JA (2013) Spent metal working fluids produced alterations on photosynthetic parameters and cell-ultrastructure of leaves and roots of maize plants. J Hazard Mater 260:220–230

Hansen D, Duda PJ, Zayed A, Terry N (1998) Selenium removal by constructed wetlands: role of biological volatilization. Environ Sci Technol 32:591–597

Hooda V (2007) Review paper: phytoremediation of toxic metals from soil and waste water. J Environ Biol 28(2):367–376

Horne AJ (2000) Phytoremediation by constructed wetlands. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 13–40

Hutchinson SL, Schwab AP, Banks MK (2003) Biodegradation of petroleum hydrocarbons in the rhizosphere. In: McCutcheon S, Schnoor J (eds) Phytoremediation: transformation and control of contaminants. Wiley, Hoboken, NJ, pp 355–386

Hyman MR, Russell SA, Ely RL, Williamson KJ, Arp DJ (1995) Inhibition, inactivation, and recovery of ammonia oxidizing activity in co-metabolism of trichloroethylene by *Nitrosomonas europaea*. Appl Environ Microbiol 61:1480–1487

Jacobson ME, Chiang SY, Gueriguian L, Weshtholm LR, Pierson J (2003) Transformation kinetics of trinitrotoluene conversion in aquatic plants. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley, New York, pp 409–427

Jaffre T, Brooks RR, Lee J, Reeves RD (1976) *Sebertia acuminata*: a nickel accumulating plant from New Caledonia. Science 193:579–580

Kar PP, Singh DP (2003) Effect of some heavy metals on sporulation and growth of *Azolla caroliniana* and *Azolla microphylla*. Asian J Microbiol Biotechnol Environ Sci 5:105–114

Knasmulier S, Gottmann E, Steinkellner H et al (1998) Detection of genotoxic effects of heavy metal contaminated soils with plant bioassays. Mutat Res 420:37–48

Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non accumulator *Thlaspi species*. Plant Physiol 122:1343–1353

Lasat MM (2002) Phytoextraction of toxic metals. J Environ Qual 31:109–120

Le Due DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF (2004) Overexpression of selenocysteinemethyltransferase in *Arabidopsis* and *Indian mustard* increases selenium tolerance and accumulation. Plant Physiol 135:377–383

Li YM, Chaney R, Brewer E, Roseberg R, Angle SJ (2003) Development of a technology for commercial phytoextraction of nickel; economic and technical considerations. Plant Soil 249:107–115

Li TQ, Yang XE, Jin XF et al (2005) Root responses and metal accumulation in two contrasting ecotypes of *Sedum alfredii* Hance under lead and zinc toxic stress. J Environ Sci Health A Tox Hazard Subst Environ Eng 40(5):1081–1096

Lin ZQ, Schemenauer RS, Cervinka V et al (2000) Selenium volatilization from a soil plant system for the remediation of contaminated water and soil in the San Joaquin Valley. J Environ Qual 29:1048–1056

Liste HH, Prutz I (2006) Plant performance, dioxygenase-expressing rhizosphere bacteria and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere 62:1411–1420

Liu H, Chen L-P, Ai YW et al (2009) Heavy metal contamination in soil alongside mountain railway in Sichuan, China. Environ Monit Assess 152:25–33

Lokeshwari H, Chandrappa GT (2007) Effects of heavy metal contamination from anthropogenic sources on Dasarahalli tank, India. Lakes Reserv Res Manag 12:121–128

Lomborg B (2001) The skeptical environmentalist. Cambridge University Press. pp 0-521–01068-3:22

Lucas García JA, Grijalbo L, Ramos B, Gutierrez Mañero FJ (2011) Procedimiento para la reducción de la DQO de efluentes líquidos aceitososmediante fitorremediación con maíz-esparto; su aplicación al tratamiento detaladrinas agotadas, Patent No. 2 350 433. Spanish Office of Patents

Lucas García JA, Grijalbo L, Ramos B, Fernández-Piñas F, Rodea-Palomares I, Gutierrez-Mañero FJ (2013) Combined phytoremediation of metal-working fluids with maize plantsinoculated with different microorganisms and toxicity assessmentof the phytoremediated waste. Chemosphere 90:2654–2661

Lucy M, Reed E, Glick BR (2004) Application of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1–125

Luthy RG, Aiken GR, Brusseau ML et al (1997) Sequestration of hydrophobic organic contaminants by geosorbents. Environ Sci Technol 31:3341

Lytle CM, Lytle FW, Yang N et al (1998) Reduction of Cr (VI) to Cr (III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32:3087–3093

Ma LQ, Komar KM, Tu C (2001) A fern that accumulates arsenic. Nature 409:579

Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18:23–34

Marín I, De la Fuenta V, Rufo L, Sanz JL, Amils R (2005) Biotecnología y medioambiente. Edit Ephemera Capitulo 9:156

Marques APGC, Rangel AOSS, Castro PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654

Marschner H (1995) Mineral nutrition of higher plants. Academic, San Diego, p 889

Marschner H (2002) Mineral nutrition of higher plants, 2nd edn. Academic, London

Mashkani SG, Ghazvini PTM (2009) Biotechnological potential of *Azollafiliculoides* for biosorption of Cs and Sr: application of micro-PIXE for measurement of biosorption. Bioresour Technol 100:1915–1921

McCutcheon SC, Schnoor JL (eds) (2003) Phytoremediation: transformation and control of contaminants. Wiley, Hoboken, NJ

Mills T, Arnold B, Sivakumaran S et al (2006) Phytoremediation and long-term site management of soil contaminated with pentachlorophenol (PCP) and heavy metals. J Environ Manage 79:232–241

Mishra VK, Tripathi BD, Kim KH (2009) Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. J Hazard Mater 172:749–754

Mkandawire M, Taubert B, Dudel EG (2004) Capacity of *Lemnagibba* L. (Duckweed) for uranium and arsenic phytoremediation in mine tailing waters. Int J Phytoremediation 6:347–362

Mohan SV, Kisa T, Ohkuma T, Kanaly RA, Shimizu Y (2006) Bioremediation technologies for treatment of PAH contaminated soil and strategies to enhance process efficiency. Rev Environ Sci Biotechnol 5:347–374

Mufarrege MM, Hadad HA, Maine MA (2010) Response of *Pistiastratiotes* to heavy metals (Cr, Ni, and Zn) and phosphorous. Arch Environ Contam Toxicol 58:53–61

Negri MC, Hinchman RR (2000) The use of plants for the treatment ofradionuclides. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals using plants to cleanup the environment. Wiley, New York, pp 107–132

Newman LA, Strand SE, Choe N, Duffy J, Ekuan G, Ruszaj M, Shurtleff BB, Wilmoth J, Heilman P, Gordon MP (1997) Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ Sci Technol

Nie L, Shah S, Burd GI et al (2002) Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium Enterobacter cloacae CAL2. Plant Physiol Biochem 40:355–361

Nwoko CO (2009) Review: trends in phytoremediation of toxic elemental and organic pollutants. Afr J Biotechnol 9(37):6010–6016

Nwoko CO, Egunjobi JK (2002) Lead Contamination of Soil and Vegetation in an abandoned battery factory site in Ibadan. Nig J Sustain Agric Environ 4(1):91–96

Nwoko CO, Okeke PN, Ac-Chukwuocha N (2004) Preliminary studies on nutrient removal potential of selected aquatic plants. J Discov Innovat Afr Acad Sci 16(3):133–136

Olson PE, Reardon KF, Pilon-Smits EAH (2003) Ecology of rhizosphere bioremediation. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley, New York, NY, pp 317–354

Okeke PN, Benny BM, Ezurike U, Nwoko CO (2004) Lead and Zinc contamination of soils and crops in an industrial waste dumpsite in Aba, Nigeria. Environmental Analar 10:1219–1230

Padmesh TVN, Vijayaraghavan K, Sekaran G, Velan M (2006) Application of *Azolla rongpong* on biosorption of acid red 88, acid green 3, acid orange 7 and acid blue 15 from synthetic solutions. Chem Eng J 122:55–63

Paiva BL, de Oliveira JG, Azevedo RA et al (2009) Ecophysiological responses of water hyacinth exposed to $Cr^{3+}$ and $Cr^{6+}$. Environ Exp Bot 65:403–409
Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 56:15–39
Pilon-Smits EAH, de Souza MP, Hong G, Amini A, Bravo RC (1999) Selenium volatilization and accumulation by twenty aquatic plant species. J Environ Qual 28:1011–1017
Central Pollution Control Board (CPCB) (2008) Status of water quality in India 2007. CPCB, New Delhi
Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants—biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:285–321
Rai PK (2008) Phytoremediation of Hg and Cd from industrial effluent using an aquatic free floating macrophyte*Azollapinnata*. Int J Phytoremediation 10:430–439
Rai PK (2009) Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Crit Rev Env Sci Technol 39:697–753
Rai PK (2010) Heavy metal pollution in lentic ecosystem of subtropical industrial region and its phytoremediation. Int J Phytoremediation 12:226–242
Rai PK, Tripathi BD (2009) Comparative assessment of *Azolla pinnata* and *Vallisneri aspiralis* in Hg removal from G.B. Pant Sagar of Singrauli Industrial region, India. Environ Monit Assess 148:75–84
Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181
Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals; using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
Reeves RD, Baker AJM (2000) Phytoremediation of toxic metals. Wiley, New York, pp 93–229
Reeves RD, Brooks RR (1983) European species of *Thlaspi* L (Cruciferae) as indicators of nickel and zinc. J Geochem Explor 18:275–283
Reid BJ, Jones KC, Semple KT (2000) Bioavailability of persistent pollutants in soils and sediments—a perspective on mechanisms, consequences and assessment. Environ Pollut 108:103–112
Rezek J, Wiesche CID, Mackova M et al (2008) The effect of ryegrass (Loliumperenne) on decrease of PAH content in long term contaminated soil. Chemosphere 70:1603–1608
Rockström J et al (2009) A safe operating space for humanity. Nature 461:472–475
Rosner JL, Aumercier M (1990) Potentiation by salicylate and salicyl alcohol of cadmium toxicity and accumulation in *Escherichia coli*. Appl Environ Microbiol 34:2402–2406
Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in *Indian mustard*. Plant Physiol 109:1427–1433
Sánchez-Chardi A, Peñarroja-Matutano C, Borrás M, Nadal J (2009) Bioaccumulation of metals and effects of a landfill in small mammals Part III: structural alterations. Environ Res 109:960–967
Sánchez-Viveros G, González D, Alacon A, Ferrera-Cerrato R (2010) Copper effects on photosynthetic activity and membrane leakage of *Azolla filiculoides* and *A. caroliniana*. Int J Agr Biol 12:365–368
Sandrin TR, Maier RM (2003) Impacts of metals on the biodegradation of organic pollutants. Environ Health Perspect 111:1093–1101
Sandrin TR, Chech AM, Maier RM (2000) A rhamnolipidbiosurfactant reduces cadmium toxicity during naphthalene biodegradation. Appl Environ Microbiol 66:4585–4588
Sarkar A, Jana S (1986) Heavy metal pollutant tolerance of *Azolla pinnata*. Water Air Soil Pollut 27:15–18
Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreria LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–323
Seidal K (1976) Macrophytes and water purification. In: Tourbier J, Pierson RW (eds) Biological control for water pollution. Pennsylvania University Press, Pennsylvania, pp 109–121
Sela M, Tel-Or E, Eberhardt F, Huttermann A (1988) Localization and toxic effects of cadmium, copper, and uranium in *Azolla*. Plant Physiol 88:30–36
Sela M, Fritz E, Huttermann A, Tel-Or E (1990) Studies on cadmium localization in the water fern *Azolla*. Physiol Plantarum 79:547–553
Shah K, Nongkynrih JM (2007) Metal hyperaccumulation and bioremediation. Biol Plantarum 51:618–634
Shi GX, Xu QS, Xie KB et al (2003) Physiology and ultrastructure of *Azollaimbricata* as affected by $H^{2+}$ and $Cd^{2+}$ toxicity. Acta Botanica Sinica 45:437–444
Siciliano SD, Germida JJ (1998) Bacterial inoculants of forage grasses enhance degradation of 2-chlorobenzoic acid in soil. Environ Toxicol Chem 16:1098–1104
Siwela AH, Nyathi CB, Naik YS (2009) Metal accumulation and antioxidant enzyme activity in C. gariepinus, Catfish, and O. mossambicus, tilapia, collected from lower Mguza and Wright Dams, Zimbabwe. Bull Environ Contam Toxicol 83:648–651
Sood A, Pabbi S, Uniyal PL (2011) Effect of paraquat on lipid peroxidation and antioxidant enzymes in aquatic fern *Azolla microphylla* Kual. Russ J Plant Physiol 58:667–673
Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, *Azolla*. Ambio 41:122–137
Srivastava J, Gupta A, Chandra H (2008) Managing water quality with aquatic macrophytes. Rev Environ Sci Biotechnol 7:255–266
Stewart KK (1970) Nutrient removal potential of various aquatic plants. Hyacinth Control J 8:34–35
Suresh B, Ravishankar GA (2004) Phytoremediation: a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24:97–124
Terry N, Zayed AD, de Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Physiol Plant Mol Biol 51:401–432
The Millennium Development Goals Report (2008) United Nations
Thums CR, Farago ME, Thornton I (2008) Bioavailability of trace metals in brownfield soils in an urban area in the UK. Environ Geochem Health 30:549–563
Tu C, Ma LQ (2005) Effects of arsenic on concentration and distribution of nutrients in the trends of the arsenic hyperaculator *Pteris vittala* L. Environ Pollut 135(2):333–340
Turgut C, Katie Pepe M, Cutright TJ (2004) The effect of EDTA and citric acid on phytoremediation of Cd, Cr and Ni from soil using *Helianthus annuus*. Environ Pollut 131(1):147–154
Umali LJ, Duncan JR, Burgess JE (2006) Performance of dead *Azolla filiculoides* biomass in biosorption of Au from wastewater. Biotechnol Lett 28:45–49
United Nations Educational, Scientific and Cultural Organization (UNESCO) (2009) Water for People, Water for Life Executive Summary of the UN World Water Development Report
Upadhyay AR, Mishra VK, Pandey SK, Tripathi BD (2007) Biofiltration of secondary treated municipal wastewater in a tropical city. Ecol Eng 30:9–15
Uppal AK, El Hardami A, Adam LR et al (2008) Biological control of potato *Verticillium* wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. Biol Contr 44:90–100
Vallini G, Di Gregorio S, Lampis S (2005) Rhizosphere induced selenium precipitation for possible applications in phytoremediation of Se polluted effluents. Z Naturforsch C 60(3–4):349–356
Van Huysen T, Abdel Ghany S, Hale KL et al (2003) Overexpression of cystathionine synthase enhancesselenium volatilization in *Brassica juncea*. Planta 218:71–78
Volesky B (2001) Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy 59:203–216
Volkering F, Breure AM, Rulkens WH (1998) Microbiological aspects of sulfactant use for biological soil remediation. Biodegradation 8:401–417

Winnike-McMilliam SK, Zhang Q, Davis LC, Erickson LE, Schnoor JL (2003) Phytoremediation of methyl tertiary-butyl ether. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley, New York, pp 805–825

Wolverton BC, McDonald RC (1976) Don't waste waterweeds. New Sci 71:318–320

Wolverton BC, Mckown MM (1976) *Water hyacinth* for removal of phenols from polluted waters. Aquat Bot 30:29–37

Wooten JW, Dodd DJ (1976) Growth of *water hyacinth* in treated sewage effluent. Econ Bot 30:29–37

Yadav SK, Juwarkar AA, Kumar GP et al (2009) Bioaccumulation and phyto-translocation of arsenic, chromium and zinc by Jatrophacurcas L.: impact of dairy sludge and biofertilizer. Bioresour Technol 100:4616–4622

Yanqun Z, Yuan L, Jianjun C et al (2005) Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. Environ Int 31(5):755–762

Zayed A, Pilon Smits E, de Souza M, Lin ZO, Terry N (2000) Remediation of selenium polluted soils and waters by phytovolatilization. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 61–83

Zhang W, Cai Y, Downum KR, Ma LO (2004) Arsenic complexes in the arsenic hyperaccumulator *Pterisvittala*, Chinese brake fern. J Chromatogr 1043(2):249–254

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606:135–150

# On-Site and Full-Scale Applications of Phytoremediation to Repair Aquatic Ecosystems with Metal Excess

3

Laura de Cabo, Roberto Serafini, Silvana Arreghini, and Alicia Fabrizio de Iorio

## 3.1 Introduction

Aquatic ecosystems perform numerous valuable environmental functions. They recycle nutrients, purify water, attenuate floods, augment and maintain streamflow, recharge ground water, and provide habitat for wildlife. Also, these ecosystems provide sources of hydroelectric power, water for municipal, industrial, and agricultural purposes, and sites for people recreation. However, rapid population increases—accompanied by intensified industrial, commercial, and residential development—have led to the pollution of surface waters by metals, fertilizers, insecticides, motor oil, toxic landfill leachates, feedlot waste, industrial and municipal sewage effluents. At the same time, water consumption has also increased, thus reducing the flows available for the dilution of wastes (Committee on Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy; National Research Council 1992). Metals are a group of contaminants of great environmental importance. While many authors support the idea of a segregation of pollutants based on their chemical characteristics (organic or inorganic), its resistance to degradation (biodegradable, persistent or recalcitrant) or its similarities with preexisting biomolecules (biogenic or xenobiotics), others take into account their mechanism of toxic action. Thus, in the environment many metals generate reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide ($H_2O_2$), and in particular hydroxyl radical ($^{\cdot}OH$) (Aravind and Prasad 2004). In Haber–Weiss cycle (Eqs. 3.1 and 3.2) and Fenton reaction (Eq. 3.2) metals with multiple oxidation states like iron, lead, copper, and chromium catalyze $^{\cdot}OH$ synthesis from $H_2O_2$ and $O_2^{-\cdot}$.

$$Me^{n+1} + O_2^{-\cdot} \leftrightarrow Me^{n+} + O_2 \tag{3.1}$$

$$Me^{n+} + H_2O_2 \leftrightarrow Me^{n+1} + {}^{\cdot}OH + OH^{-} \tag{3.2}$$

ROS accomplish a key role regulating growth and development of the biota, thus a change in its delicate balance can lead to necrosis of cells, tissues and eventually death of organisms. Since ROS have the ability to oxidize the four groups of cellular macromolecules (proteins, lipids, polysaccharides, and nucleic acids), and while the degradation of a protein with enzymatic activity can lead to the loss of a metabolic pathway, or a lipid peroxidation can result in damage to the membranes responsible for energy metabolism or structural integrity, DNA damage can also generate both carcinogenic and teratogenic effects on organisms (Leonard et al. 2004).

On the other hand, greater knowledge about the dynamics of metals and their potential effects on the environment questions the "dilution paradigm" as a sustainable remediation strategy. This paradigm shift has been based on ethical and preservation issues, but is also the result of understanding that biogeochemical conditions determine metal mobility, potential dispersion within or between different environmental compartments, and mechanisms of toxic action.

Besides in a similar way to certain soluble organic contaminants, some organometallic compounds such as methylmercury have a high tendency to biomagnify in the food chain and can exert a devastating teratogenic action although initial levels of metal discharges are low (Tadiso et al. 2011; Harmelin-Vivien et al. 2012).

Inland water-bodies are complex systems that have a profound influence on the characteristics and toxicity of contaminants. It is generally considered that aerobic or anaerobic degradation of organic compounds is the only effective way of removal, since produced mineral compounds can be integrated into the cycles of elements. However, the degradation process

L. de Cabo, Ph.D. (✉)
Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – Consejo Nacional de Investigaciones Científicas y Técnicas, Av. Ángel Gallardo 470, CABA, Argentina

Universidad de Flores, Camacuá 245, CABA, Argentina
e-mail: ldecabo@macn.gov.ar

R. Serafini, Ph.D. • S. Arreghini, Ph.D. • A.F. de Iorio, Ph.D.
Cátedra de Química Analítica, Departamento de Recursos Naturales y Ambiente, Facultad de Agronomía-Universidad de Buenos Aires, Av. San Martin 4453 (1417), CABA, Argentina

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mainly mediated by microorganisms has the particularity of altering the physicochemical conditions of the environment such as pH and redox potential, generating chemical species able to precipitate metallic ions (Eqs. 3.4 and 3.5), or organic and inorganic ligands that can produce coordination compounds in which heavy metals act as Lewis acids.

$$2\{CH_2O\} + SO_4^{2-} + H^+ \leftrightarrow 2CO_2 + HS^- + 2H_2O \tag{3.4}$$

$$Pb^{2+} + HS^- \leftrightarrow PbS(s) + H^+ \tag{3.5}$$

Moreover, endogenous or exogenous primary and secondary minerals such as clays behave like chemically reactive surfaces providing high densities of binding sites to retain metals through the formation of outer or inner sphere complexes.

Since a proper understanding of contaminant–environment interactions in aquatic ecosystems requires considering abiotic and biotic factors, it is essential to analyze processes mediated by organisms.

Aquatic plants that have been studied for the remediation of soil and water contaminated by metals include floating species, such as *Salvinia herzogii* (Maine et al. 2004), water hyacinth (*Eichhornia crassipes*) (Mishra et al. 2008), duckweed (including *Lemna polyrrhiza* L., *Lemna minor*, and *Spirodela polyrrhiza* W. Koch) (John et al. 2008; Mishra and Tripathi 2008), mosquito fern (*Azolla pinnata* R. Brown) (Mishra et al. 2008), and water lettuce (*Pistia stratiotes*) (Maine et al. 2004; Mishra et al. 2008), emergent plants such as common cattail (*Typha latifolia*) (Das and Maiti 2008), giant bulrush (*Schoenoplectus californicus*) (Arreghini et al. 2001; Chiodi Boudet et al. 2011), common reed (*Phragmites australis*) (Peruzzi et al. 2011), and submerged plants, such as pondweed (*Potamogeton pectinatus* or *Potamogeton crispus*) (Badr and Fawzy 2008; Mishra et al. 2008), hydrilla (*Hydrilla verticillata*) (Bunluesin et al. 2004; Mishra et al. 2008), and coontail (*Ceratophyllum demersum* L.) (Badr and Fawzy 2008; Bunluesin et al. 2004). Metal–plant interaction depends on metal bioavailability and plant structure involved in absorption. In the case of emergent plants and numerous floating species the preferential uptake structure is the root, where the accumulated metal translocates to other morphological structures, while in the case of submersed plants the root could play an auxiliary role of fixing and the uptake occurs through all the epidermis of the plant. Uptake and mobility of metals in the plant are different for different types and species of macrophytes, also depending on the metal involved (Deng et al. 2004) and its concentration.

While the basic premise for starting any mitigation project is eliminating the source of contamination, this approach is necessary but insufficient. Rivers and their floodplains are so intimately linked that they should be understood, managed, and restored as integral parts of a single ecosystem. The interception of point and nonpoint sources of contamination is necessary to improve ecosystem attribute. However, the costs of treating nonpoint sources by engineered systems are high. Man-made and natural wetlands have been successful, in some cases, in retaining suspended matter in water flowing through them. Also, the plants remove phosphorus, coliform bacteria, biochemical oxygen demand; and dissolved organic carbon. In situ methods are defined as destruction or transformation of the contaminant, immobilization to reduce bioavailability, and separation of the contaminant from the bulk soil (Reed et al. 1992). In situ techniques are better than the ex-situ techniques due to their low cost and reduced impact on the ecosystem. Wetlands are also highly effective in reducing stream loads of metals.

The aim of this chapter is to discuss several applications of phytoremediation at full scale and on-site for metal excess in aquatic ecosystems using several macrophytes.

## 3.2 Plants as Promoters of a Better Environment

Preservation of the littoral zone in contaminated environments is crucial, since it contributes in different ways to metal stabilization in sediment. Thus, macrophytes could be a powerful tool in treatment of aquatic ecosystems receiving industrial effluents, municipal wastewater, or agricultural runoff (Rai 2009). The long-term stability that the plants provide in terms of preventing metals from leaving the site means that this technology is often termed phytostabilization. Phytostabilization has a wide application in metal-contaminated vegetated sites.

## 3.3 Geophysical and Biochemical Processes of Stabilization

Unvegetated sediments tend to show little aggregation due to the weakening effect of water on the bonds holding sediment particles (Reddy et al. 2000). This makes them more susceptible to weathering, by increasing the contact surface between the solid fraction and the solution phase, and facilitates also their transport through erosional agents. Sedimentation processes combined with oxidation, sorption, and precipitation reactions are effective at removing metals from the system (Bednar et al. 2013).

Natural and anthropogenic events can generate local base levels that decrease the current velocity of rivers and favor sedimentation of the suspended material. Moreover, aquatic vegetation increases resistance to flow and significantly affects sediment transport. In the low-load basin, aquatic plants such as cattail (*Typha* sp.) has a substantial and significant effect on transport capacity since it increase sediment deposition (Brueske and Barrett 1994). Also, riparian vegetation plays an important role in mitigation and prevention against hydromorphological hazards (flooding, floodplain erosion, protection of river infrastructures), if correctly managed (Kothyari et al. 2009; Cavedon 2012).

The vegetated marsh zones are associated with finer grain particles and exhibit higher metal concentrations in contaminated environment in comparison to the bare mudflats (Daskalakis and O'Connor 1995; Zhang et al. 2001). However, in some cases where there is direct input of contaminants, no clear relationship between the proportion of fine-grained particles and metal concentration has been established (Hosono et al. 2011). Natural events like tidal currents, winds, and storms, and human activities as dredging result in major sediment disturbances, leading to changes in chemical properties that stimulate the mobilization of contaminants from sediment and sediment pore water into the water column (Zhang et al. 2001; Eggleton and Thomas 2004).

Biochemical processes that lead to the formation of a solid phase may involve diverse and complex mechanisms. Chemical precipitation is determined primarily by solubility product of salts (Kps), by the concentration of ions forming the precipitate, by the pH and redox potential of the water solution, by the presence of complexing agents and by the ionic strength of the medium. However, the formation of a solid phase requires supersaturated solutions in a stable media rich in precipitation nuclei. Accumulation of organic matter and microorganisms in sediments can provide a high density of nuclei which serves for growth of the precipitates. Both the subsequent aggregation of nuclei in larger particles, leading to decreased surface/volume relationships, and rearrangement of atoms in the crystal lattice of the solid diminish solubility of salts. In this way, organic matter degradation may stimulate the chemical precipitation and consequent stabilization of metals in the solid phase.

Suspended material includes a colloidal fraction constituted by amorphous or crystalline solids of small radius, humic substances, and cellular macromolecules of high molecular weight (Konhauser et al. 2002). Therefore, colloids include a heterogeneous group of compounds characterized by high surface/volume ratio and high density of electric charge. Vegetation release substantial amounts of colloidal material to the water body during its life cycle. These compounds can interact with metals through adsorption phenomena and generation of outer sphere complexes, complexation and generation of inner sphere complexes or eventually through surface precipitation processes (Suteerapataranon et al. 2006). While colloids are stable in aqueous solution and tend to remain in suspension, changes in environmental conditions such as the increase of ionic strength can lead to flocculation and coagulation processes that remove from the solution both colloids as "associated" metals (Suteerapataranon et al. 2006; Ren et al. 2010).

Sediments accumulated by described physical and biochemical processes serve as a substrate for the growth of aquatic vegetation. Emergent plants of the coastal area with high productivity produce a net enrichment of sediments with organic matter (Lenssen et al. 1999). In this way, riparian vegetation contributes to sediment stabilization decreasing risk of contamination of the water column. This enrichment with organic matter is often overlooked by many authors, partly due to the short time span out of most studies—too short for the initial organic matter to be consumed in the chemical processes of removing metals from water (Marchand et al. 2010).

In wetland sediments the organic matter accumulation generates a high oxygen demand. Aerobic microorganisms are replaced by anaerobic ones that use alternative electron acceptors in the process ($NO_3^-$, $MnO_4^-$, $Fe^{3+}$, $SO_4^{2-}$, $CO_2$), depending on both their relative abundance in the environment and, in the situation when multiple acceptors are present, on the energy yield of redox reactions (Konhauser et al. 2002). Unlike aerobic degradation, where the process can be performed by a single type of organism, anaerobic degradation often requires a consortium of interdependent microorganisms. In this way, and depending on the rate of settleable organic material, net accumulation of reduced forms of sulfur and organic matter represent a significant reserve of binding sites for metals. While some metals such as copper, chromium, and lead have high affinity for the organic matter/sulfides fraction and thus can be immobilized, other metals such as cadmium and zinc are predominantly associated with the exchangeable fraction and iron and manganese oxides, respectively and are comparatively more bioavailable (Tessier et al. 1979). Several authors argue that the less mobile fractions of sediment tend to get rich in metals as time passes from contamination event. Thus although metal shows a greater affinity for a more mobile fraction as exchangeable, the "aging" that occurs in sediment generates a redistribution to thermodynamically more stable phase (McLaren and Clucas 2001; Halim et al. 2003; Evangelou et al. 2007). Peruzzi et al. (2011) have reported that in wetlands vegetated with *Phragmites australis*, built for municipal wastewater treatment, the less available metal-organic fraction increased over time for Cu, Cr, Pb, Ni, Cd, and Zn, thus indicating their lesser availability for plant uptake. Since bioavailability and bioaccumulation of metals in an aquatic ecosystem is mainly dependent on its partitioning behavior or its binding strength to sediment (Eggleton and Thomas 2004) then the input of high quantity of organic matter from aquatic macrophytes as a solid phase with high metal sorption capacity is decisive. Dissolved or weakly adsorbed contaminants are more bioavailable to aquatic biota compared to more structurally complex mineral-bound contaminants (Calmano et al. 1993; Eggleton and Thomas 2004).

In ex situ remediation processes, changes in physicochemical conditions of the environment can lead to a redistribution of metals from “aged” fractions of sediments to more mobile ones, increasing the environmental risk (Rodriguez Salemi et al. 2010).

## 3.4 Tolerant Plants Favor the Settling of Other Species

Metal contamination is a stress factor limiting plant growth and development, which leads to decreased levels of organic matter and nutrients of sediments and negatively affects the establishment of plant. Numerous studies show the recovery of contaminated habitats through revegetation with tolerant species, so implanted areas with wetland plants show an increase in the organic matter content of sediments, a consequent improvement in infiltration and fertility in general, an increase in the N, P, and K available and an improve in water quality (Deng et al. 2004; Mitsch et al. 2005; Lei and Duan 2008; Lottermoser and Ashley 2011; Wang et al. 2012). Although restoration efficiency mainly depends on species selected, colonization by pioneer plants could promote the development of aquatic communities that lead to stabilization of metals and prevent the transfer of environmentally significant contaminants into food-chains.

Metallophytes are species that have evolved biological mechanisms to resist, tolerate, or thrive on toxic metalliferous soils, and are typically endemic of these habitats. Metallophytes are the optimal choice for in situ restoration of closed mines, for the rehabilitation of metal-contaminated land (reclamation and rehabilitation) and to provide knowledge for development of environmental technologies such as phytoextraction of metals from soils (Whiting et al. 2004).

Wetland plants growing in contaminated sediments present characteristic patterns of accumulation and translocation of metals, thus findings on terrestrial plants cannot be directly extrapolated. Besides the use of native species in remediation strategies, not always considered in scientific or technical papers and essential to preserve biological diversity, require detailed studies for each natural ecosystem.

## 3.5 Metal Accumulation in Plants as an Avoidance Strategy

### 3.5.1 Emergent Macrophytes

Although high levels of metals reported in wetland sediments, their low bioavailability and limited transfer into the water column determine that macrophytes of the littoral zone typically exhibit relatively low concentrations of these contaminants. Whereas it was observed that in the same environment submerged and floating plants exhibit higher concentrations of metals in their organs than emergent ones, there are no reported hyperaccumulators in wetland habitats (Cardwell et al. 2002). Thus, despite its great development in biomass, the amount of metals accumulated by emerging plants represents only a small fraction of the total content in sediments, so they cannot be considered as a metal sink (Lee and Scholz 2007; Marchand et al. 2010).

Although it is recognized that the particular properties of wetland sediments (organic matter content, pH, redox potential, levels of P and N) influence the bioavailability of metals, there are contrasting observations regarding the role played by each environmental factor.

Organic matter content of sediment can be negatively correlated with chromium uptake by the roots, as this metal can form inner-sphere complexes with organic matter and oxides making it less available to plants (Iorio et al. 2007; Valea 2011). Moreover, association of metals with amino groups ($-NH_2$) of humic compounds (Bargiela 2009) favors solubilization and increases their bioavailability.

McGrath (1995) and Vicari Mellis et al. (2004) argue that Ni sorption to sediment is maximum with high pH and high content of organic matter, so acidic pH and negative redox potential found in highly contaminated sites could facilitate solubilization of essential and nonessential metals and uptake by plants. Armstrong (1967) reports that oxidation of immediate root environment promote the uptake of essential elements and prevent the internalization of toxic components. Metals mobilized from the reduced sediments can precipitate with Fe and Mn (oxy)hydroxides on the root surface (“root plaque”) (Gries et al. 1990), resulting in high rhizoconcentrations on a small scale or even in elevated concentrations in bulk sediments from a vegetated marsh compared to non-vegetated sediments (Teuchies et al. 2013).

Some authors emphasize the antagonistic relationship between P and metals since chemical reactions in the rhizosphere produce highly insoluble precipitates (Kabata-Pendias 2011; Olsen 1991; Deng et al. 2004), although others report increases in bioavailability in the presence of soluble P (Kidd et al. 2007). Therefore, depending on the nature of the phosphorus compounds and the heavy metal species, metal bioavailability can be favored or disadvantaged (Bolan et al. 2003).

Lambert et al. (1979) and Taiz and Zeiger (2002) state that mycorrhizal associations produce a remarkable increase in P, Zn, and Cu uptake by terrestrial plants, yet not in common symbiosis studies with aquatic plants exposed to high levels of nonessential metals. Another factor than can affect the accumulation of metals in wetland plants is the presence of microbial symbionts such as rhizosphere bacteria. Mycorrhizae provide an interface between the roots and the soil increasing the absorptive surface area and are effective at assimilating metals that may be present at toxic concentrations in the soil (Meharg and Cairney 2000). However, Khan et al. (2000) suggested that they play a protective role, restricting the uptake of metals by plants by immobilizing the metals in the fungal tissue.

Wetlands are complex ecosystems, so availability and uptake of metals by emergent plants is the result of interactions between biotic and abiotic compartments that create conditions for immobilization or release of contaminants.

Plant metal uptake can be measured through different indexes. One of the most used is bioconcentration factor, BCF, obtained as metal concentration ratio of plant root to sediment. There is great variation among the reported BCF values for the different species of emergent macrophytes and heavy metals, especially related to its essentiality (Tables 3.1 and 3.2). Thus, the species indicated in Table 3.2 shows similar behavior in the uptake of Zn and Cu. For example, *Schoenoplectus californicus*, *Scirpus sylvaticus*, *Cyperus esculentum*, *Cyperus alternifolius*, *Sagittaria montevidensis*, *Equisetum arvense*, *Hydrocotyle americana*, and *Phragmites australis* have always BCF values close to or less than unity over a wide range of sediment concentrations, from 28.5 to 5,400 μg Zn/g and from 5 to 2,100 μg Cu/g. *Cyperus eragrostis* and *Equisetum fluviatile* have high BCF values (close to 4 for Zn, and between 7 and 16 for Cu) at levels of these metals that do not exceed the LEL (lowest effect level) level in sediment. *Schoenoplectus validus*, *Typha domingensis*, *Typha orientalis*, *Typha latifolia*, and *Eleocharis equisetina* present high values of BCF at Zn or Cu sediment levels between LEL and SEL (severe effect level). For Ni, almost all species reported showed BCF values near or less than 1 especially at sediment levels above LEL, except *T. domingensis* and *T. latifolia* whose BCF values are between 2 and 10 at levels of Ni slightly above LEL (Table 3.2). For nonessential metals Pb, Cd, and Cr the BCF values reported are generally lower than those for essential ones. *Cyperus*

**Table 3.1** List of emergent and floating macrophyte species grown in natural and contaminated environments with their scientific name, common name, authors, and location of different studies

| Code | Species name | Common name | Study | Authors | Location |
|---|---|---|---|---|---|
| Emergent plants | | | | | |
| *Sch cal* | *Schoenoplectus californicus* | Giant bulrush | 1 | Chiodi Boudet et al. (2011) | South America |
| | | | 2 | Valea (2011) | South America |
| *Sch val* | *Schoenoplectus validus* | River bulrush | 3 | Cardwell et al. (2002) | Oceania |
| *Sci syl* | *Scirpus sylvaticus* | Sylvan bulrush | 4 | Hozhina et al. (2001) | Europe |
| *Cyp esc* | *Cyperus esculentum* | Yellow nutsedge | 5 | Yoon et al. (2006) | North America |
| *Cyp era* | *Cyperus eragrostis* | Tall flatsedge | 3 | | |
| *Cyp alt* | *Cyperus alternifolius* | Umbrella sedge | 6 | Yang et al. (2010) | Asia |
| *Ele equ* | *Eleocharis equisetina* | | 7 | Lottermoser and Ashley (2011) | Oceania |
| *Typ dom* | *Typha domingensis* | Southern cattail | 8 | Maine et al. (2006) | South America |
| | | | 3 | | |
| | | | 9 | Kamel (2013) | Africa |
| | | | 13 | Bonanno (2013) | Europe |
| *Typ lat* | *Typha latifolia* | Broadleaf cattail | 4 | | |
| | | | 11 | Klink et al. (2013) | Europe |
| | | | 12 | Sasmaz et al. (2008) | Africa |
| *Typ ori* | *Typha orientalis* | Cumbungi cattail | 3 | | |
| *Phr aus* | *Phragmites australis* | Common reed | 10 | Sawidis et al. (1995) | Europe |
| | | | 9 | | |
| | | | 13 | Bonanno (2013) | Europe |
| *Hyd ame* | *Hydrocotyle americana* | American pennywort | 5 | | |
| *Sag mon* | *Sagittaria montevidensis* | Giant arrowhead | 2 | | |
| *Equ arv* | *Equisetum arvense* | Field horsetail | 5 | | |
| *Equ flu* | *Equisetum fluviatile* | Fluvial horsetail | 4 | | |
| Floating plants | | | | | |
| *E crass* | *Eichhornia crassipes* | Water hyacinth | 8 | | |
| | | | 14 | Kumar et al. (2012) | Asia |
| | | | 9 | | |
| | | | 15 | Agunbiade et al. (2009) | Africa |
| | | | 16 | Kumar et al. (2008) | Asia |
| *L gibb* | *Lemna gibba* | Duckweed | 9 | | |
| *M min* | *Marsilea minuta* | Dwarf waterclover | 14 | | |
| *S mol* | *Salvinia molesta* | Kariba weed | 17 | Ashraf et al. (2011) | Asia |

**Table 3.2** Metal sediment concentration (μg/g); Bioconcentration factor (BCF) and translocation factor (FT) of different emergent macrophytes

| | | Zn | | | Cu | | | Pb | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species name | Study[a] | Sed | BCF | TF | Sed | BCF | TF | Sed | BCF | TF |
| *Sch cal* | 1 | 28.5 | 1.22 | 0.66[b] | 9.2 | 0.97 | 0.61[b] | – | – | |
| | 1 | 28.5 | 0.58 | 1.69[b] | 13.5 | 0.88 | 0.22[b] | – | – | |
| | 1 | 54.5 | 0.65 | 0.46[b] | 21.9 | 0.55 | 0.14[b] | – | – | |
| | 2 | 95 | 0.48[b] | 0.26[b] | 23 | 0.32[b] | 0.26[b] | – | – | |
| | 2 | 99 | 0.77[b] | 0.22[b] | 28 | 0.50[b] | 0.41[b] | – | – | |
| | 2 | 404 | 0.36[b] | 0.20[b] | 143 | 0.15[b] | 0.29[b] | – | – | |
| *Sch val* | 3 | 514 | 3.05 | 0.03[c] | 38.3 | 2.00 | 0.04[c] | 72.5 | 1.74 | 0.03[c] |
| *Sci syl* | 4 | 40 | 1.00 | 2.10 | 15 | 0.80 | 0.83 | 25 | 0.92 | – |
| | 4 | 5,400 | 0.03 | 2.73 | 2,100 | 0.02 | 0.57 | 3,000 | 0.01 | 0.84 |
| *Cyp esc* | 5 | 195 | 0.83 | 1.02 | 20 | 0.80 | 0.63 | 90 | 0.31 | 0.64 |
| | 5 | 200 | 0.86 | 0.47 | 21 | 0.48 | 2.80 | 143 | 0.11 | 1.64 |
| | 5 | 572 | 0.45 | 1.12 | 300 | 0.50 | 0.13 | 1,451 | 0.29 | 0.06 |
| *Cyp era* | 3 | 93.4 | 2.14 | – | 17.6 | 1.68 | – | 12.9 | 2.84 | – |
| | 3 | 128 | 4.55 | – | 36.6 | 7.19 | – | 77.2 | 1.63 | – |
| *Cyp alt* | 6 | 62 | 0.71 | 0.68 | – | – | | 29 | 0.20 | – |
| | 6 | 580 | 0.27 | 0.24 | – | – | | 711 | 0.25 | 0.01 |
| *Ele equ* | 7 | 174 | 2.37 | 0.67 | 84 | 2.67 | 0.32 | 59 | 0.49 | 0.09 |
| | 7 | 282 | 1.21 | 0.67 | 331 | 2.12 | 0.10 | 222 | 0.21 | 0.11 |
| *Typ dom* | 8 | 60 | 2.15 | 0.14[c] | – | – | | – | – | |
| | 3 | 93.4 | 3.81 | 0.06[c] | 17.6 | 4.65 | 0.04[c] | 12.9 | 1.64 | 0.07[c] |
| | 3 | 128 | 3.88 | 0.17[c] | 36.6 | 3.48 | 0.12[c] | 72.5 | 1.81 | 0.03[c] |
| | 13 | 294 | 0.29 | 0.27[c] | 115 | 0.14 | 0.15[c] | 64.2 | 0.12 | 0.09[c] |
| | 3 | 514 | 2.00 | 0.07[c] | 38.3 | 1.40 | 0.16[c] | 77.2 | 2.61 | 0.01[c] |
| | 9 | 1,201 | 0.30[b] | 0.60[c] | 52.7 | 1.00[b] | 1.00[c] | 12.2 | 0.77[b] | 0.87[c] |
| *Typ lat* | 4 | 40 | 0.78 | 1.65 | 15 | 1.33 | 0.24 | 25 | 0.8 | |
| | 11 | 62.2 | 6.00 | 0.10[d] | 4.98 | 1.73 | 0.43[d] | 10.0 | 1.21 | 0.34[d] |
| | 12 | 70.0 | 4.80 | 0.69[c] | 45.0 | 1.27 | 0.61[c] | 10.0 | 1.51 | 0.56[c] |
| | 4 | 2,700 | 0.16 | 0.08 | 1,600 | 0.02 | 0.18 | 900 | 0.05 | |
| | 4 | 5,400 | 0.09 | 0.41 | 2,100 | 0.04 | 0.23 | 3,000 | 0.03 | |
| | 4 | 7,800 | 0.06 | 0.09 | 2,200 | 0.26 | 0.17 | 4,900 | 0.49 | |
| | 4 | 8,000 | 0.18 | 0.29 | 2,600 | 0.05 | 0.26 | 5,400 | 0.02 | |
| | 4 | 19,000 | 0.23 | 0.08 | 2,600 | 0.06 | 0.19 | 5,400 | 0.11 | |
| *Typ ori* | 3 | 29.7 | 0.45 | 1.52[c] | 5.1 | 0.80 | 0.58[c] | 14.9 | 0.01 | 0.35[c] |
| *Phr aus* | 10 | 70 | 1.55[c] | 0.15[c] | 20.3 | 1.56[c] | 1.08[c] | 16.3 | 0.15[c] | 0.08[c] |
| | 9 | 1,201 | 0.06[b] | 4.84[c] | 52.7 | 0.92[b] | 0.91[c] | 12.8 | 1.49[b] | 0.70[c] |
| | 13 | 294 | 0.44 | 0.19[c] | 115 | 0.14 | 0.40[c] | 64.2 | 0.15 | 0.12[c] |
| *Hyd ame* | 5 | 572 | 0.47 | 0.19 | 26 | 0.81 | 0.76 | 1,451 | 0.07 | 0.08 |
| | 5 | 720 | 0.09 | 0.58 | 300 | 0.11 | 0.41 | – | – | |
| *Sag mon* | 2 | 241 | 0.57[b] | 0.53[b] | 87 | 0.46[b] | 0.31[b] | – | – | |
| | 2 | 1,138 | 0.45[b] | 0.24[b] | 365 | 0.25[b] | 0.46[b] | – | – | |
| *Equ arv* | 5 | 2,200 | 0.11 | 0.65 | 990 | 0.11 | 0.21 | 4,100 | 0.07 | 0.13 |
| *Equ flu* | 4 | 40 | 16.8 | 0.04 | 15 | 16.7 | 0.16 | 25 | 4.00 | 0.01 |
| | 4 | 19,000 | 0.25 | 0.25 | 2,600 | 0.22 | 0.12 | 5,400 | 0.04 | 0.12 |
| ISQG | | 123 | | | 35.7 | | | 35 | | |
| LEL | | 129 | | | 13 | | | 19 | | |
| SEL | | 1,300 | | | 85 | | | 167 | | |

*ISQG* Canadian interim sediment quality guideline (μg/g) from CCME (2002), *LEL* lowest effect level (μg/g), *SEL* severe effect level (μg/g) (de Deckere et al. 2011)
[a]Study number from Table 3.1
[b]BCF and TF values from corresponding studies, no calculated
[c]TF obtained from ratio metal concentration leaf/root
[d]TF obtained from ratio metal concentration low part of leaf/root

*esculentum*, *C. alternifolius*, *S. sylvaticus*, *E. equisetina*, *T. orientalis*, *E. arvense*, *H. americana*, and *P. australis* have always BCF values of Pb below unity over a wide range of concentrations in the sediment (from 10 to 4,100 µg Pb/g). Although *C. eragrostis*, *E. fluviatile*, *S. validus*, *T. domingensis*, and *T. latifolia* show similar behavior for Pb to that of Zn and Cu, its BCF values are well below of the others.

The BCF values for Cr and Cd reported were below 0.7 in a broad range of sediment concentrations (from 26 to 881 µg Cr/g, and from 0.22 to 84 µg Cd/g), with some exceptions; *T. domingensis* and *T. latifolia* have chromium BCF values near to 5 at sediment levels below or near LEL (Maine et al. 2006; Klink et al. 2013), and *T. domingensis*, *T. orientalis*, *T. latifolia, E. fluviatile*, *E. equisetina*, and *S. sylvaticus* show high values for Cd (up to 21) at levels in sediment lower than LEL. *Phragmites australis* and *S. validus* have cadmium BCF values of 2.4 and 3.8 respectively at levels in sediment between LEL and SEL.

Plants have developed a defense system to deal with metals and can resist environmental toxic concentrations, implementing avoidance or tolerance strategies (Levitt 1980). Avoidance includes a set of mechanisms that prevent the access of metals to sites of toxic action, thereby limiting its adverse effects on plant metabolism (i.e., immobilization in the rhizosphere by organic chelators, cell wall retention, endocytosis of metal transporters to prevent uptake). Low rates of metal accumulation estimated from the BCF ratio could be explained by the action of plant avoidance strategies in an environment that also behaves as a strong metal immobilizer. On the other hand, when plants are unable to completely prevent metal uptake, mechanisms to limit their toxic action are implemented. The efficiency of this response will determine the tolerance of the species or ecotype studied to toxic metal concentrations. Tolerance strategies can be divided in three major groups: those oriented to neutralize nonessential metals (i.e., molecular chaperons that prevent substitution of essential metals by nonessential ones in metalloenzymes); those directed to neutralize the adverse effects produced by some metals (i.e., enzymatic and non-enzymatic response to reactive oxygen species generated by metals with redox activity) and those directed to retain metals in extra-cytoplasmic compartments such as the vacuole or the cell wall (Clemens et al. 2002; Carroll et al. 2004; Hall 2002; Shanker et al. 2005; Krämer et al. 2007). In emergent aquatic plants the uptake of metals occurs primarily through the root, so it is expected that tolerance strategies involve an accumulation of contaminants in belowground biomass thus preventing transport to photosynthetic structures.

It is essential to understand the distribution of the metal adsorbed onto the surface in relation to the metal accumulated inside the cell, in order to understand the predominant removal mechanisms and to make decisions of the viability of the recovery of the adsorbed metals (Olguín and Sánchez-Galván 2012). The primary cell wall is composed of an amorphous matrix of polysaccharides (hemicellulose and pectin), plus a small amount of structural proteins that bind cellulose fibers with varying degree of crystallization by covalent and non-covalent bonds (Taiz and Zeiger 2002; Caffall and Mohnen 2009).

Polysaccharides play a significant role in the immobilization of metals, particularly those containing high amount of carboxyl groups are able to bind divalent and trivalent metal ions. These groups have a high metal binding capacity by forming inner sphere complexes through the inclusion of divalent and trivalent metals in place of calcium ions, which mostly pectins stabilize (Krzesłowska 2011).

Several authors (Khotimchenko et al. 2007; Colzi et al. 2011; Krzesłowska 2011) suggest that both acetylation and methylation of the carboxyl groups of pectins diminish its affinity for heavy metals, favoring and increasing their toxicity, while others suggest that the methylation level would not be constant but may vary response to the metal abundance in the environment, this being a characteristic of the tolerant species or ecotypes (Krzesłowska 2011).

Emergent macrophytes generally show metal accumulation in belowground biomass as tolerance strategy. Metal mobility within the plant measured by the translocation factor (TF, ratio of metal concentration leaf/root) differs among metals and plant species, generally following a decreasing order Ni > Cr > Cd = Zn = Cu > Pb (from the median of the data reported in Table 3.2). Cardwell et al. (2002) indicated that *T. domingensis* translocates essential metals such as zinc and copper much more easily than nonessential such as lead and cadmium. Despite this, generally the concentrations of metals at roots are greater than in the aerial parts (Cheng et al. 2002).

All species reported in Table 3.2 showed TF values near or less than 1 for all metals, and generally lower than the respective values of BCF, except for Zn in *S. sylvaticus* and *P. australis* whose TF value are 2.8 and 4.8 respectively (Hozhina et al. 2001; Kamel 2013) at elevated sediment levels, although in both cases BCF values were very low (less than 0.05). Metal translocation into shoots appears to be very restricted in all wetland plants so that harvesting plants will not be an effective source of metal removal in a wetland system. However, in the view of toxicology, this could be a desirable property, as metals would not pass into the food chain via herbivores, and thus avoid potential risk to the environment (Deng et al. 2004).

### 3.5.2 Submersed Macrophytes

Rooted submersed plants have a great importance owing to the fact that their roots, rhizomes, and stolon can facilitate the colonization of bacteria, algae and other microorganisms that help in phytoremediation process. Submersed aquatic macrophytes have got ability to extract metals from the sediments via their root systems and directly from the surrounding water. Plant uptake of trace elements by leaves of submersed macrophytes becomes more important when the trace element concentrations in the surrounding environment are high (Guilizzoni 1991).

Most studies were conducted in laboratory or greenhouse settings using metal-enriched nutrient solutions (Bunluesin et al. 2004; John et al. 2008; Maine et al. 2004; Mishra and Tripathi 2008). Results from these studies were usually very impressive with high metal uptake or accumulation (Mishra and Tripathi 2008). However, it may be entirely different when these aquatic plants are applied to field condition such as lakes, reservoirs, and estuaries where both metals and nutrients are of much lower concentrations and other environmental factors are far less favorable. On the other hand, the performance of aquatic plants in natural water bodies is more meaningful as degradation of natural aquatic ecosystem is a worldwide concern and yet conventional physical or chemical treatments are not cost-effective due to the nature of non-point source pollution. Investigations have been conducted in natural water bodies such as lakes (Kamel 2013; Badr and Fawzy 2008; Vardanyan and Ingole 2006), rivers (Borisova et al. 2014), reservoirs (Mishra et al. 2008; Molisani et al. 2006), estuaries (Almeida et al. 2006), and stormwater in detention plants (Lu 2009).

Borisova et al. (2014) examined the uptake of five metals (Cu, Fe, Ni, Zn, and Mn) in *Ceratophyllum demersum* L. (hornwort) and *Potamogeton alpinus* Balb. (pondweed) from Iset' river, Ural region, Russia. Differential accumulation pattern was noted for metals. Higher amounts of metals were accumulated in *C. demersum* compared to *P. alpinus*. Also it was shown that in leaves of *C. demersum* there were high amount of total phosphorus, nitrogen, organics acids, and ash.

Kamel (2013) assessed heavy metal concentration in water and sediment of a polluted lake in Egypt along with two native submersed macrophytes *Ceratophyllum demersum* and *Myriophyllum spicatum*. The mean values of the six investigated metals in the selected aquatic macrophytes were Zn > Pb > Cu > Ni > Co > Cd. Regarding the mean values of the metal concentrations among the submersed plants it showed the following pattern *Ceratophyllum demersum* > *Myriophyllum spicatum*. The BCF (bioconcentration factor) value for each individual heavy metal was *Ceratophyllum demersum* > *Myriophyllum spicatum*. The highest BCF values were estimated for Zn in *C. demersum* (903.3). Translocation factors were low for both species.

Another submersed plant, *Vallisneria spiralis*, was promising for chromium accumulation in different parts of plant from chromium containing solutions and tannery effluents. *Vallisneria spiralis* also tolerates high chromium concentration, thus the plant resulted highly suitable for phytoremediation of chromium-polluted wastewater (Vajpayee et al. 2001).

*Potamogeton pectinatus* is a submersed aquatic plant that can survive in metal-polluted lakes and can accumulate significant amounts of cadmium (266.3 μg/g). However, the adverse effect of cadmium was reported on loss in photosynthetic pigment. *P. pectinatus* showed cadmium tolerance through increased levels of cysteine, non-protein thiol, and carotenoids and an increase of protein content (Rai et al. 2003).

Lead concentrations in plant tissue were found to be 1,621 and 1,327 times those in the external solution for *C. demersum* and *C. caroliniana*, respectively (Fonkou et al. 2005).

*Ceratophyllum demersum* and *Hydrilla verticillata* showed high cadmium accumulation (7,381 and 7,942 mg Cd/Kg) and a high BCF (>2,500) at 1 mg/L. There was significant growth decrease in both species with increasing metal concentration. But cadmium was less toxic to *H. verticillata* and its cadmium accumulation capacity was higher (Bunluesin et al. 2004).

Mazej and Germ (2009) determined trace elements in sediment of Velenjsko Jezero Lake and their concentrations were found to be above the European background concentration. Given their low concentrations in the water of lake they concluded that the trace elements found in above-ground parts is predominantly the result of their translocation from roots to stems and leaves. Entirely submersed species (*Najas marina* and *Potamogeton lucens*) have been shown to accumulate relatively large amounts of trace elements than the studied floating species. Essential metals as Zn and Cu appear to be more readily translocated from roots to shoots than other elements in submersed and floating plants. Cr accumulation in roots and translocation from roots to shoots were very low. Kähkönen et al. (1997) affirmed that there is usually no mobility of Cr from roots to shoots and leaves due to barriers or lack of transport mechanisms. The concentration of Pb was the lowest of all the trace elements in roots probably due to its strong binding to organic matter and other components and to the roots. Also, Pb mobility was low. Translocation factors from roots to stem or leaves were very low in *N. marina* (0.05), and *P. lucens* (0.1).

With regard to the uptake ability of submersed plants, *C. demersum* seems to be a promising species for remediation of sediments contaminated with metals mainly essentials metals. Denny and Wilkins (1987) observed that tendency to use shoots as sites of metal uptake instead of roots increases with progression towards submergence and simplicity of shoot structure. However, translocation factors were low and metal uptake by leaves from the surrounding water is negligible in submersed plants because element concentrations in water are generally low. Most of the metals in the aquatic phase pass to bottom sediments in natural or artificial water bodies and become particulate, complexed, or chelated complex practically not bioavailable.

### 3.5.3 Floating Macrophytes

Different plant species have different allocation patterns of metals. With the purpose of evaluating strategies for the remediation, we selected four species of floating macrophytes studied in natural or artificial contaminated water bodies. Metal concentrations in water, roots and leaves of selected floating macrophytes, metal bioconcentration factor (BCF), and translocation factor (TF) are presented in Tables 3.1 and 3.3.

*Eichhornia crassipes* (water hyacinth) has been listed as most troublesome weed in aquatic system. It is a floating aquatic plant, found abundantly throughout the year in very large amounts. It originated in tropical South America, but has become naturalized in many warm areas of the world: Central America, North America (California and southern states), Africa, Asia, and Australia. It is one of the most commonly used plants in constructed wetlands because of its fast growth rate and large uptake of nutrients and contaminants. While there are numerous references relating to the capabilities of heavy metal removal by *E. crassipes* in laboratory experiments (e.g.: Mishra and Tripathi 2008; Maine et al. 2001), this is not true for on-site and full-scale applications. There are also limited data on the capacity of *E. crassipes* to remediate a broad spectrum of metals particularly the highly toxic ones. *Eichhornia crassipes* absorbs and translocates essentials metals Cu, Ni, and Zn (Table 3.3) and nonessentials metals Cr, Pb, and Cd (Table 3.3). However, water hyacinth generally locates the elements into the roots which imply that the plant has a high capacity to absorb the metals and reveals its ability to serve as rhizofiltration plant in phytoremediation technology. The concentrations of essentials metals (Zn, Cu, and Ni) in

**Table 3.3** Metal water concentration (mg/L); metal root and leaf concentration (mg/kg); Bioconcentration factor (BCF) and translocation factor (TF) of different macrophytes

| Species name | *E. crass* | | | | | | *L. gibb* | *M. min* | *S. mol* | Normal range in plants[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Study[b] | | 8 | 14 | 9 | 15 | 16 | 16 | 14 | 19 | |
| Zn | Water | ND | – | 340 | 3.28 | 1,600 | 340 | – | – | |
| | Root | 24 | – | 604.5 | 131.88 | $7.09 \times 10^5$ | 252.4 | – | 128.31 | 1–400 |
| | Leaf | 15 | – | 492 | 223 | – | 93 | – | 223 | |
| | BCF | – | – | 1.78 | 40 | 443 | 0.74 | – | – | |
| | TF | 0.62 | – | 0.81 | 1.69 | – | 0.36 | – | 1.73 | |
| Ni | Water | 0.017 | 0.080 | – | 0.062 | 10.13 | – | 0.080 | – | |
| | Root | 42 | 16.5 | 48.7 | 0.72 | $2.8 \times 10^4$ | 14.31 | 5.5 | – | 0.89–2.04 |
| | Leaf | 21 | 8.5 | 40.4 | 1.41 | – | 2.4 | 1 | – | |
| | BCF | 2,471 | 206 | – | 11.6 | 2,764 | – | 69 | – | |
| | TF | 0.5 | 0.51 | 0.83 | 1.96 | – | 0.16 | 0.18 | – | |
| Cu | Water | – | 0.260 | – | 0.044 | 19.670 | – | 0.260 | – | |
| | Root | - | 4.25 | 22.5 | 31.40 | $4.4 \times 10^4$ | 16.8 | 2.3 | 680.91 | 7.53–8.44 |
| | Leaf | – | 2.8 | 14.5 | 56.58 | – | 8.2 | 0.8 | 91.40 | |
| | BCF | – | 16.3 | – | 714 | 2,237 | – | 8.8 | – | |
| | TF | – | 0.66 | 0.64 | 1.80 | – | 0.49 | 3.83 | 0.13 | |
| Cr | Water | 0.022 | 4.670 | – | 1.330 | – | – | 4.670 | – | |
| | Roots | 78 | 16.85 | – | 5.05 | – | – | 0.2 | – | |
| | Leaf | 9 | 28 | – | 10.12 | – | – | 10 | – | |
| | BCF | 3,546 | 3.6 | – | 3.8 | – | – | 0.04 | – | |
| | TF | 0.11 | 1.67 | – | 2 | – | 0.49 | 3.83 | – | |
| Pb | Water | – | 0.04 | 18.27 | 0.018 | 6.11 | 18.27 | 0.04 | – | |
| | Roots | – | 2.8 | 16.8 | 0.39 | 9,800 | 52.1 | 2.4 | 162.72 | 0.2–2.0 |
| | Leaf | – | 1.4 | 11.3 | 0.65 | – | 13.5 | 1 | 367 | |
| | BCF | – | 70.0 | 0.9 | 21.4 | 1,604 | 2.9 | 60.0 | | |
| | TF | – | 0.53 | 0.67 | 1.67 | – | 0.26 | 0.42 | 2.25 | |
| Cd | Water | – | – | 20.9 | 0.01 | 0.74 | 20.9 | – | – | |
| | Root | – | – | 0.8 | 0.19 | 790 | 0.62 | – | – | 0.1–2.4 |
| | Leaf | – | – | 0.52 | 0.50 | – | 0.16 | – | – | |
| | BCF | – | – | 0.04 | 17.3 | 1,067 | 0.030 | – | – | |
| | TF | – | – | 0.65 | 2.63 | – | 0.26 | – | – | |

[a]Kabata-Pendias (2011)
[b]Study number from Table 3.1

*E. crassipes* (Kumar et al. 2008) were higher than standard normal ranges for plants, defined by Kabata-Pendias (2011) associated with high levels of metals detected in water reservoir.

The relatively low leaf metal contents indicated the presence of a prevention mechanism to inhibit uptake until drastic conditions. However, Agunbiade et al. (2009) (Table 3.3) detected translocation factor above 1 for essentials and non-essential metals in coastal water at optimum removal conditions (pH: 5.5–6.5; salinity below 2 % and dissolved oxygen above 6 mg/L). Water hyacinth plants had high bioconcentration factor with low water concentrations of the six elements. Also, water hyacinth has a high tolerance to toxic contaminants. This shows that water hyacinth can be a promising candidate to remove heavy metals. *E. crassipes* could be eaten by humans and animals. Then, harvesting of biomass must be considered in restoration or mitigation plans for specific contaminated sites.

Duckweed commonly refers to a group of floating, flowering plants of the family Lemnaceae. It is fast-growing and adapts easily to various aquatic conditions. The different species (*Lemna*, *Spirodela*, *Wolffia*, and *Wolfiella*) are worldwide distributed in wetlands, ponds, and some effluent lagoons. The plants can grow at temperature ranging from 5 to 35 °C with optimum growth between 20 and 31 °C and across a wide range of pH (3.5–10.5) (Cayuela et al. 2007). Wetlands and ponds are the most common sites to find duckweed. The capacity of aquatic plant such as duckweed (*Lemna* sp.) to remove toxic metals from water are well documented through laboratory experiences (e.g., Sharma and Gaur 1995; Tripathi and Chandra 1991, Lahive et al. 2011). *Spirodela intermedia* W. Koch (duckweed) and Lemna minor L. (duckweed) present a high growth rate and have been used for the removal of Cd, Cr, and Pb from water column (Maine et al. 2001; Cardwell et al. 2002).

Kamel (2013) examined ability of *Lemna gibba* to remove cadmium, copper, nickel, lead, and zinc in a contaminated lake in Egypt. The species was tolerant to high metal concentrations, although copper, nickel, and lead concentrations in roots were higher than normal range in plants according Kabata-Pendias (2011). In general, concentration factors were low. Like the rest of the macrophytes chosen, its translocation factors were also low.

Kumar et al. (2012) assessed accumulation potential in native macrophytes growing naturally in a drain receiving tannery effluent. *Marsilea minuta* accumulated lead from the water mainly in roots. Lead concentration in roots was higher than normal concentration for plants (Kabata-Pendias 2011). Nickel showed the same behavior. BCF and TF were elevated for copper, associated with low metal concentrations in the tissue, below the normal range for plants. Cr accumulation from the environment was low. It is known that in many plant species the mobility of Cr is low due to the fact that there are barriers or lack of transport mechanism suitable for Cr transport from roots to shoots (Kähkönen et al. 1997). In *Spirodela polyrhiza*, the Cr presence decreases growth rate inhibiting photosynthesis (Appenroth et al. 2001). However, chromium translocation factor in *M. minuta* was higher than 1.

*Salvinia molesta* is an aquatic fern, native to south-eastern Brazil and is widely distributed in tropical and subtropical areas. It has a fast growing rate, and is tolerant to pollution, favorable properties in species to be used in phytoremediation. *Salvinia minima* (Olguín et al. 2002 and Casares 2013) can accumulate chromium, lead, cadmium, copper, and zinc in bioassays performed in laboratory conditions. Ashraf et al. (2011) investigated polluted soils that surround mining slag pile and the potential remediation ability of *S. molesta* among others native macrophytes growing in the area. Concentrations of copper and lead in pseudoroots and leaves were higher than defined toxic levels for plants (Deng et al. 2004). Also, the plants translocated lead and zinc. *S. molesta* can tolerate the adverse environmental conditions, colonize the waterbodies in tailings areas and accumulate toxic metals.

According to Baker and Brooks (1989) and Srivastava et al. (2006), a plant can be considered as hyperaccumulator when the metal concentration in the shoots (stems or leaves) is 10,000 µg/g for Zn; above 1,000 µg/g dry mass for As, Pb, Cu, Ni, and Co and 100 µg/g for Cd. Then, the selected plants cannot be considered hyperaccumulators in natural or artificial environments. Also, translocation (Baker and Brooks 1989) and bioconcentration factors (Weiss et al. 2006) should be higher than 1. In most of study cases, these factors were lower than 1. BCF were recorded only greater than 1 in the case of extremely high or low water metal concentrations (Kumar et al. 2008 and Maine et al. 2006) (Table 3.3). The differences in TF indicate the preferential accumulation–uptake pattern of metals. TF was typically lower than 1 in all selected study cases (Table 3.3). However, in Agunbiade et al. (2009) and Ashraf et al. (2011), *E. crassipes* and *S. molesta* effectively transported metals from root to shoot ($TF > 1$) due to efficient metal transporter system and probably sequestration of metals in vacuoles and apoplast (Lasat et al. 1998). Also, Agunbiade et al. (2009) metal concentrations in plants respect to water concentrations were low ($BCF < 1$), revealing an excluder strategy to transport of metals from abiotic environment to macrophytes. Both roots and aboveground biomass have a kind of natural controlling mechanism regarding the quantity of metals taken from the environment.

The general trend shows that the root tissues accumulate significantly greater concentrations of metals than shoots, indicating plant availability of metals as well as its limited mobility once inside the plant. The exclusion of metals from aboveground tissues has been suggested as a metal tolerant strategy in many plants (Deng et al. 2004). This strategy allows plant photosynthetic machine preservation.

Metal tolerant strategy is widely evolved and exists in wetland plant species when they grow in metal-contaminated areas. As Deng et al. (2004) said for wetland emergent plants that can colonize heavily metal-polluted areas, the floating plants can tolerate metals mainly by their metal exclusion ability. However, the higher-than-toxic level of metal concentrations in leaves indicates that internal detoxification metal tolerance mechanisms might also exist.

Therefore floating macrophytes might have developed internal exclusion strategies whereby the toxic metal is prevented from damaging the cell. Also, studies on metal compartmentalization in floating macrophyte bioassays (Casares 2013; Mouvet and Claveri 1999; Vazquez et al. 1999) showed that the removal by adsorption extracellular compartment was higher than intracellular accumulation. Plant species with a high capacity for removing heavy metals in solution have cell walls or membranes with abundant carboxyl, sulfhydryl, amino, and phosphate groups and a large specific surface enabling greater metal biosorption (Bates et al. 1982).

## 3.6 Decomposition of Plant Biomass and Release of Metals into the Environment

Wetland plants behave as transient reservoirs of nutrients, metals, and metalloids, so litter decomposition represents a pathway for the release of contaminants from plant biomass into water bodies (Eid et al. 2012; Schaller et al. 2011). During the primary stage of the decomposition process, microorganisms and exudates form a heterotrophic biofilm on plant litter (Kominkova et al. 2000) where bacteria and fungi can accumulate high amounts of contaminants. Molecular oxygen is usually considered as a primary electron acceptor in organic matter degradation. However, since dissolution of gases in water is a relatively slow process from a kinetic perspective and also considering that in metal-contaminated sediments, biofilms and organic matter content may limit oxygen diffusion. Oxygen depletion leads to changes in the decomposers communities, from aerobic to anaerobic and then less efficient microorganisms.

Organic matter accumulation in sediments will be favored by high sedimentation rates and high proportion of organic compounds in the settleable material. In the early stages of decomposition process, the labile organic compounds with low C:N:P ratios are preferentially degraded. At the advanced stages of decomposition, the residual compounds (waxes, polyphenols) with high C:N:P ratios are degraded at lower rates. Several authors argue that the sink/source behavior of plant litter depends on the metal content and the plant accumulation organ. Thus, plants which translocate metals to photosynthetic structures, will produce litter with high levels of pollutants and in this case the more appropriate management strategy would be harvesting, in order to phytoextract metals. However, as stated previously, metal hyperaccumulation and high rates of translocation are not widespread among aquatic macrophytes in contaminated environments. Therefore, if accumulation exists probably involve to belowground biomass. In situ decomposition in anaerobic habitats will contribute to form litter with high tendency to immobilize metals. Thus, at the same time that metals are released during the decomposition by mineralization, generating new binding sites could immobilize them again.

## 3.7 Concluding Remarks

With regard to the metal uptake capacity of aquatic plants at full scale and on-site treatments, concentration factors found to be low and the exclusion strategy seems to be more widespread among aquatic plants. This strategy was shared with other plants tolerant to stress by toxic excess. Both bioconcentration factors such as translocation were lower than those calculated in bioassays conducted under laboratory conditions. There have been no hyperaccumulators among the studied plants, but there are accumulator species under certain conditions of pH, dissolved oxygen, and metal concentrations. Furthermore, translocation factors were in most cases less than 1. However, regarding the capacity of accumulation of metals in the standing crop, the floating plants are most effective, followed by submersed species and then emergent species. The metal concentrations in leaves of various floating species were higher than tolerable levels for other macrophytes. Therefore floating macrophytes might have developed internal exclusion strategies whereby the toxic metal is prevented from damaging the cell. Also, removal by adsorption extracellular compartment was higher than intracellular accumulation in floating plants.

The application of phytoremediation at full scale and on-site for metal excess in aquatic ecosystems using several macrophytes is limited mainly to the immobilization of toxics in the sediments and rhizosphere-root system. The low translocation to the aboveground tissues main advantage is to avoid the dispersion of pollutants into the food chain. Moreover, in situ decomposition of the macrophytes used in phytoremediation is a valid strategy since the contribution of detritus favors the input of organic matter in sediment and in turn the complexation of metal ions in the bottom of water bodies contaminated. Since floating plants are those that can occasionally translocate, harvesting is advisable in these cases. On the other hand, the emergent plants are more effective for phytostabilization. In situ decomposition in anaerobic habitats will contribute to form litter with high tendency to immobilize metals. The metals released during the decomposition could be further retained by the new binding sites generated in bottom sediment.

The use of native species in remediation projects, not always considered in scientific or technical papers is essential to preserve biological diversity and, requires more detailed studies for each natural ecosystem.

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## References


Agunbiade FO, Olu-Owolabi BI, Adebowale KO (2009) Short communication: phytoremediation potential of Eichhornia crassipes in metal-contaminated coastal water. Bioresour Technol 100: 4521–4526

Almeida CMR, Mucha AP, Vasconcelos MTSD (2006) Comparison of the role of the sea club-rush *Scirpus maritimus* and the sea rush *Juncus maritimus* in terms of concentration, speciation and bioaccumulation of metals in the estuarine sediment. Environ Pollut 142:151–159

Appenroth K, Stöckel J, Srivastava A, Strasser R (2001) Multiple effect of chromate on the photosynthetic apparatus of *Spirodela polyrhiza* as probed by OJIP chlorophyll a fluorescence measurements. Environ Pollut 115:49–64

Aravind P, Prasad MNV (2004) Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophytes. Plant Sci 166:1321–1327

Armstrong W (1967) The oxidizing activity of roots in waterlogged soils. Physiol Plantarum 20:920–926

Arreghini S, de Cabo L, de Iorio AF, Rendina A, Bonetto C (2001) Effects of Zinc on the growth of bulrush (*Schoenoplectus californicus*) and its distribution between different sediments fractions. Bull Environ Contam Toxicol 67:264–270

Ashraf MA, Maah MJ, Yusoff I (2011) Heavy metals accumulation in plants growing in ex tin mining catchment. Int J Environ Sci Tech 8(2):401–416

Badr NBE, Fawzy M (2008) Bioaccumulation and biosorption of heavy metals and phosphorous by *Potamogeton pectinatus* L. and *Ceratophyllum demersum* L. in two Nile Delta lakes. Fresenius Environ Bull 17:282–292

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements–a review of their distribution, ecology and phytochemistry. Biorecovery 1:811–826

Bargiela MF (2009) Influencia de la relación entre los metales pesados y las sustancias húmicas de ríos de llanura en la movilidad de estos elementos. Dissertation, University of Buenos Aires

Bates S, Tessier A, Campbell P, Buffle J (1982) Zinc adsorption and transport by *Chlamydomonas variabilis* and *Scenedesmus subspicatus* (Chlorophyceae) grown in semicontinuous culture. J Phycol 18:521–529

Bednar AJ, Averett DE, Seiter JM, Lafferty B, Jones WT, Hayes CA, Chappell MA, Clarke JU, Steevens JA (2013) Characterization of metals released from coal fly ash during dredging at the Kingston ash recovery project. Chemosphere 92:1563–1570

Bolan NS, Adriano DC, Naidu R (2003) Role of phosphorus in (Im) mobilization and bioavailability of heavy metals in the soil-plant system. Rev Environ Contam Toxicol 177:1–44

Bonanno G (2013) Comparative performance of trace element bioaccumulation and biomonitoring in the plant species *Typha domingensis*, *Phragmites australis* and *Arundo donax*. Ecotoxicol Environ Saf 97: 124, http://dx.doi.org/10.1016/j.ecoenv.2013.07.017i

Borisova G, Chukina N, Maleva M, Prasad MNV (2014) *Ceratophyllum demersum* and *Potamogeton alpinus* Balb. From Iset' river, rural region, Russia differ in adaptive strategies to heavy metals exposure—a comparative study. Int J Phytorem 16:621

Brueske CC, Barrett GW (1994) Effects of vegetation and hydrologic load on sedimentation patterns in experimental wetland ecosystems. Ecol Eng 3(4):429–447

Bunluesin S, Kruatrachue M, Pokethitiyook P, Lanza GR, Upatham ES, Soonthornsarathool V (2004) Plant screening and comparison of *Ceratophyllum demersum* and *Hydrilla verticillata* for cadmium accumulation. Bull Environ Contam Toxicol 73:591–598

Caffall KH, Mohnen D (2009) The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohyd Res 344: 1879–1900

Calmano W, Hong J, Förstner U (1993) Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. Water Sci Technol 28:223–235

Cardwell AJ, Hawker DW, Greenway M (2002) Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere 48(7):653–663

Carroll MC, Girouard JB, Ulloa JL, Subramaniam JR, Wong PC, Valentine JS, Culotta VC (2004) Mechanisms for activating Cu- and Zn-containing superoxide dismutase in the absence of the CCS Cu chaperone. Proc Natl Acad Sci U S A 101:5964–5969

Casares MV (2013) Biodisponibilidad y toxicidad de metales pesados en aguas naturales con características fisico-quimicas extremas. Bases para su monitoreo y remediación. Acta Toxicol Argentina 21(1):57

Cavedon V (2012) Effects of rigid stems on sediment transport. Dissertation, University of Trento

Cayuela ML, Millner P, Slovin J, Roig A (2007) Duckweed (*Lemna gibba*) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting. Chemosphere 68: 1985–1991

CCME (Canadian Council of Ministers of the Environment) (2002) Canadian sediment quality guidelines for the protection of aquatic life. In: Canadian environmental quality guidelines. http://www.ccme.ca/

Cheng S, Grosse W, Karrenbrock F, Thoennessen M (2002) Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Ecol Eng 18(1):317–325

Chiodi Boudet L, Escalante A, von Haeften G, Moreno V, Gerpe M (2011) Assessment of heavy metal accumulation in two aquatic macrophytes: a field study. J Braz Soc Ecotoxicol 6(1):57–64

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7(7):309–315

Colzi I, Doumett S, Del Bubba M, Fornaini J, Arnetoli M, Gabbrielli R, Gonnelli C (2011) On the role of the cell wall in the phenomenon of copper tolerance in *Silene paradoxa* L. Environ Exp Bot 72(1):77–83

Committee on Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy, National Research Council (1992) Restoration of aquatic ecosystems: science, technology, and public policy. National Academy Press, Washington, DC

Das M, Maiti SK (2008) Metal accumulation in naturally colonizing vegetation in abandoned Cu-tailings ponds at Rakha Mines, East Singhbhum, Jharkhand, India. Land Contam Reclamat 16:135–153

Daskalakis KD, O'Connor TP (1995) Distribution of chemical concentrations in US coastal and estuarine sediment. Mar Environ Res 40(4):381–398

de Deckere E, De Cooman W, Leloup V, Meire P, Schmitt C, von der Ohe PC (2011) Development of sediment quality guidelines for freshwater ecosystems. J Soil Sedim 11:504–517

Deng H, Ye ZH, Wong MH (2004) Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environ Pollut 132:29–40

Denny H, Wilkins D (1987) Zinc tolerance in *Betula* spp. II. Microanalytical studies of zinc uptake into root tissues. New Phytol 106:525–534

Eggleton J, Thomas KV (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ Int 30:973–980

Eid EM, Shaltout KH, El-Sheikh MA, Asaeda T (2012) Seasonal courses of nutrients and heavy metals in water, sediment and above- and below-ground *Typha domingensis* biomass in Lake Burullus (Egypt): perspectives for phytoremediation. Flora 207(11):783–794

Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soils. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68:989–1003

Fonkou T, Agendia P, Kengne I, Akoa A, Derek F, Nya J, Dongmo F (2005) Heavy metal concentrations in some biotic and abiotic components of the Olezoa wetland complex (Yaounde-Cameroon, West Africa). Water Qual Res J Can 40:457–461

Gries C, Kappen L, Lösch R (1990) Mechanism of flood tolerance in reed, *Phragmites australis* (Cav.) Trin. ex Steudel. New Phytol 114:589–593

Guilizzoni P (1991) The role of heavy metals and toxic materials in the physiological ecology of submersed macrophytes. Aquat Bot 41:87–109

Halim M, Conte P, Piccolo A (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere 52(1):265–275

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11

Harmelin-Vivien M, Bodiguel X, Charmasson S, Loizeau V, Mellon-Duval C, Tronczyński J, Cossa D (2012) Differential biomagnification of PCB, PBDE, Hg and Radiocesium in the food web of the European hake from the NW Mediterranean. Mar Pollut Bull 64:974–983

Hosono T, Ch-Ch S, Delinom R, Umezawa Y, Toyota T, Kaneko S, Taniguchi M (2011) Decline in heavy metal contamination in marine sediments in Jakarta Bay, Indonesia due to increasing environmental regulations. Estuar Coast Shelf Sci 92:297–306

Hozhina EI, Khramov AA, Gerasimov PA, Kumarkov AA (2001) Uptake of heavy metals, arsenic and antimony by aquatic plants in the vicinity of ore mining and processing industries. J Geochem Explor 74:153–162

Iorio AF de, Della Vecchia F, Ragay G, Rearte A, Baldassini P (2007) Adsorción simultánea de Cu, Cr, Pb y Zn en sedimentos contaminados del río Matanza-Riachuelo (Argentina). III Reunión Binacional de Ecología "Ecología: la teoría y sus aplicaciones". La Serena, Chile. 30 de septiembre al 4 de octubre.

John R, Ahmad P, Gadgil K, Sharma S (2008) Effect of cadmium and lead on growth, biochemical parameters and uptake in L*emna polyrrhiza* L. Plant Soil Environ 54:262–270

Kabata-Pendias A (2011) Trace elements in soils and plants, 4th edn. CRC Press, Taylor & Francis Group, Boca Raton, FL

Kähkönen MA, Pantsar-Kallio M, Manninen PKG (1997) Analysing heavy metal concentrations in the different parts of *Elodea Canadensis* and surface sediment with PCA in two boreal Lakes in Southern Finland. Chemosphere 35:2645–2656

Kamel AK (2013) Phytoremediation potentiality of aquatic macrophytes in heavy metal contaminated water of El-Temsah Lake, Ismailia, Egypt. Middle East J Sci Res 14(12):1555–1568

Khan AG, Kuek C, Chaudhry TM, Koo CS, Hayes W (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Khotimchenko M, Kovalev V, Khotimchenko Y (2007) Equilibrium studies of sorption of lead(II) ions by different pectin compounds. J Hazard Mater 149:693–699

Kidd PS, Domínguez-Rodríguez MJ, Díez J, Monterroso C (2007) Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere 66:1458–1467

Klink A, Macioł A, Wisłocka M, Krawczyk J (2013) Metal accumulation and distribution in the organs of Typha latifolia L. (cattail) and their potential use in bioindication. Limnologica 43:164–168

Kominkova D, Kuehn KA, Busing N, Steiner D, Gessner MO (2000) Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. Aquat Microb Ecol 22:271–282

Konhauser KO, Mortimer RJG, Morris K, Dunn V (2002) The role of microorganisms during sediment diagenesis: implications for radionuclide mobility. In: Keith-Roach MJ, Livens FR (eds) Interactions of microorganisms with radionuclides. Elsevier, Amsterdam, pp 61–100

Kothyari UC, Hashimoto H, Hayashi K (2009) Effect of tall vegetation on sediment transport by channel flows. J Hydraul Res 47(6): 700–710

Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581:2263–2272

Krzesłowska M (2011) The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol Plant 33:35–51

Kumar JLN, Hiren Soni H, Rita N, Kumar RN, Bhatt I (2008) Macrophytes in phytoremediation of heavy metal contaminated water and sediments in Pariyej Community Reserve, Gujarat, India. Turk J Fish Aquat Sci 8:193–200

Kumar N, Bauddh K, Dwivedi N, Barman SC, Singh DP (2012) Accumulation of metals in selected macrophytes grown in mixture of drain water and tannery effluent and their phytoremediation potential. J Environ Biol 33:923–927

Lahive E, O'Callaghan MJA, Jansen MAK, O'Halloran J (2011) Uptake and partitioning of zinc in Lemnaceae. Ecotoxicology 20:1992–2002

Lambert DH, Baker DE, Cole H Jr (1979) The role of mycorrhizae in the interactions of phosphorus with zinc, cooper and other elements. Soil Sci Soc Am J 43:976–980

Lasat MM, Baker AJM, Kochian LV (1998) Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in Thlaspi caerulescens. Plant Physiol 118:875–883

Lee B-H, Scholz M (2007) What is the role of Phragmites australis in experimental constructed wetland filters treating urban runoff? Ecol Eng 29:87–95

Lei D, Duan C (2008) Restoration potential of pioneer plants growing on lead-zinc mine tailings in Lanping, southwest China. J Environ Sci 20:1202–1209

Lenssen JPM, Menting FBJ, van der Putten WH, Blom CWPM (1999) Effects of sediment type and water level on biomass production of wetland plant species. Aquat Bot 64:151–165

Leonard SS, Harris GK, Shi X (2004) Metal-induced oxidative stress and signal transduction. Free Rad Bio Med 37(12):1921–1942

Levitt J (1980) Responses of plant to environmental stresses. Academic, London

Lottermoser BG, Ashley PM (2011) Trace element uptake by *Eleocharis equisetina* (spike rush) in an abandoned acid mine tailings pond, northeastern Australia: implications for land and water reclamation in tropical regions. Environ Pollut 159:3028–3035

Lu Q (2009) Evaluation of aquatic plants for phytoremediation of eutrophic stormwaters. Dissertation, University of Florida

Maine MA, Duarte MV, Suñe N (2001) Cadmium uptake by floating macrophytes. Water Res 35(11):2629–2634

Maine MA, Suñe NL, Lagger SC (2004) Chromium bioaccumulation: comparison of the capacity of two floating aquatic macrophytes. Water Res 38:1494–1501

Maine MA, Suñe N, Hadad H, Sánchez G, Bonetto C (2006) Nutrient and metal removal in a constructed wetland for wastewater treatment from a metallurgic industry. Ecol Eng 2(6):341–347

Marchand L, Mench M, Jacob DL, Otte ML (2010) Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ Pollut 158:3447–3461

Mazej Z, Germ M (2009) Trace element accumulation and distribution in four aquatic macrophytes. Chemosphere 74:642–647

McGrath SP (1995) Chromium and nickel. In: Alloway BJ (ed) Heavy metals in soils, 2nd edn. Blackie Academic & Professional, Glasgow

McLaren RG, Clucas LM (2001) Fractionation of copper, nickel, and zinc in metal-spiked sewage sludge. J Environ Qual 30:1968–1975

Meharg AA, Cairney JW (2000) Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv Ecol Res 30:69–112

Mishra VK, Tripathi BD (2008) Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour Technol 99:7091–7097

Mishra VK, Upadhyay AR, Pandey SK, Tripathi BD (2008) Concentrations of heavy metals and aquatic macrophytes of Govind Ballabh Pant Sagar an anthropogenic lake affected by coal mining effluent. Environ Monit Assess 141:49–58

Mitsch WJ, Zhang L, Anderson CJ, Altor AE, Hernández ME (2005) Creating riverine wetlands: ecological succession, nutrient retention, and pulsing effects. Ecol Eng 25:510–527

Molisani MM, Rocha R, Machado W, Barreto RC, Lacerda LD (2006) Mercury contents in aquatic macrophytes from two reservoirs in the Paraiba Do Sul: Guandu River system, SE Brazil. Braz J Biol 66:101–107

Mouvet C, Claveri B (1999) Localization of copper accumulated in *Rhynchostegium riparioides* using sequential chemical extraction. Aquat Bot 63:1–10

Olguín EJ, Sánchez-Galván G (2012) Heavy metal removal in phytofiltration and phycoremediation: the need to differentiate between bioadsorption and bioaccumulation. N Biotechnol 30(1):3–8

Olguín EJ, Hernández E, Ramos I (2002) The effect of both different Light conditions and the pH value on the capacity of *Salvinia minima* Baker for removing cadmium, lead and chromium. Acta Biotechnol 22(1–2):121–131

Olsen SR (1991) Micronutriens interactions. In: Mordvedt JJ, Giordano P, Lindsay WL (eds) Micronutrients in agriculture, 2nd edn. Soil Sci Soc America, Madison, WI, Chapter 11

Peruzzi E, Masciandaro G, Macci C, Doni S, Mora Ravelo SG, Peruzzi P, Ceccanti B (2011) Heavy metal fractionation and organic matter stabilization in sewage sludge treatment wetlands. Ecol Eng 37:771–778

Rai PK (2009) Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Crit Rev Env Sci Tec 39:697–753

Rai UN, Tripathi RD, Vajpayee P, Pandey N, Ali MB, Gupta DK (2003) Cadmium accumulation and its phytotoxicity in *Potamogeton pectinatus* L. (Potamogetonaceae). Bull Environ Contam Toxicol 70:566–575

Reddy KR, D'Angelo EM, Harris WG (2000) Biochemistry of wetlands. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton, FL

Reed DT, Tasker IR, Cunnane JC, Vandegrift GF (1992) Environmental restoration and separation science. In: Vandgrift GF, Reed DT, Tasker IR (eds) Environmental remediation removing organic and metal ion pollutants. Am Chem Soc, Washington, DC, pp 1–19

Ren H, Liu H, Qu J, Berg M, Qi W, Xu W (2010) The influence of colloids on the geochemical behavior of metals in polluted water using as an example Yongdingxin River, Tianjin, China. Chemosphere 78:360–367

Rodriguez Salemi V, García AR, Morisio Y, Torres Yamaguchi F, de Iorio AF (2010) Removilización de metales durante la resuspensión de sedimentos anóxicos del Riachuelo: estudio de laboratorio. In: Varni M, Entraigas I, Vives L (eds) Hacia la gestión integral de los recursos hídricos en zonas de llanura. Editorial Martín, Buenos Aires

Sasmaz A, Obek E, Hasar H (2008) The accumulation of heavy metals in *Typha latifolia* L. grown in a stream carrying secondary effluent. Ecol Eng 33:278–284

Sawidis T, Chettri MK, Zachariadis GA, Stratis JA (1995) Heavy metals in aquatic plants and sediments from water systems in Macedonia, Greece. Ecotox Environ Safe 32:73–80

Schaller J, Brackhage C, Mkandawire M, Dudel EG (2011) Metal/metalloid accumulation/remobilization during aquatic litter decomposition in freshwater: a review. Sci Total Environ 409:891–4898

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Sharma SS, Gaur JP (1995) Short communication: potential of Lemna polyrrhiza for removal of heavy metals. Ecol Eng 4:37–43

Srivastava M, Ma LQ, Gonzaga Santos JA (2006) Three new arsenic hyperaccumulating fern. Sci Total Environ 364:24–31

Suteerapataranon S, Bouby M, Geckeis H, Fanghänel T, Grudpan K (2006) Interaction of trace elements in acid mine drainage solution with humic acid. Water Res 40:2044–2054

Tadiso TM, Borgstrøm R, Rosseland BO (2011) Mercury concentrations are low in commercial fish species of Lake Ziway, Ethiopia, but stable isotope data indicated biomagnifications. Ecotox Environ Safe 74:953–959

Taiz L, Zeiger E (2002) Plant physiology, 3rd edn. Sinauer Associates Inc., Sunderland, MA, p 690

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51(7):844–851

Teuchies J, Jacobs S, Oosterlee L, Bervoets L, Meire P (2013) Role of plants in metal cycling in a tidal wetland: implications for phytoremediation. Sci Total Environ 445–446:146–154

Tripathi RD, Chandra P (1991) Chromium uptake by *Spirodela polyrrhiza* L. (Schleiden) in relation to metal chelators and pH. Bull Environ Contam Toxicol 47:764–769

Vajpayee P, Rai UN, Ali MB, Tripathi RD, Yadav V, Sinha S, Singh SN (2001) Chromium-induced physiologic changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. Bull Environ Contam Toxicol 67:246–256

Valea CI (2011) Acumulación de metales pesados en plantas acuáticas nativas: localización intra y extracelular: estrategias de tolerancia. Dissertation, University of Buenos Aires

Vardanyan LG, Ingole BS (2006) Studies on heavy metal accumulation in aquatic macrophytes from Sevan (Armenia) and Carambolim (India) Lake systems. Environ Int 32:208–218

Vazquez MD, López J, Carballeira A (1999) Uptake of heavy metals to the extracellular and intracellular compartments in three species of aquatic bryophyte. Ecotox Environ Safe 44:12–24

Vicari Mellis E, Pessôa da Cruz MC, Casagrande JC (2004) Nickel adsorption by soils in relation to pH, organic matter and iron oxides. Sci Agric (Piracicaba, Braz) 61(2):190–195

Wang W, Gao J, Guo X, Li W, Tian X, Zhang R (2012) Long-term effects and performance of two-stage baffled surface flow constructed wetland treating polluted river. Ecol Eng 49:93–103

Weiss J, Hondzo M, Biesboer D, Semmens M (2006) Laboratory study of heavy metal phytoremediation by three wetland macrophytes. Int J Phytorem 33(3):245–259

Whiting SN, Reeves RD, Richards D, Johnson MS, Cooke JA, Malaisse F, Paton A, Smith JAC, Angle JS, Chaney RL, Ginocchio R, Jaffré T, Johns R, McIntyre T, Purvis OW, Salt DE, Schat H, Zhao FJ, Baker AJM (2004) Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. Restor Ecol 12(1):106–116

Yang J, Ma Z, Ye Z, Guo X, Qiu R (2010) Heavy metal (Pb, Zn) uptake and chemical changes in rhizosphere soils of four wetland plants with different radial oxygen loss. J Environ Sci 22(5):696–702

Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464

Zhang W, Yu L, Hutchinson S, Xu S, Chen Z, Gao X (2001) China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. Geomorphology 41:195–205

# 4 Phytoremediation of Eutrophic Waters

Abid Ali Ansari, Subrata Trivedi, Fareed Ahmad Khan, Sarvajeet Singh Gill, Rubina Perveen, Mudasir Irfan Dar, Zahid Khorshid Abbas, and Hasibur Rehman

## 4.1 Introduction

Water is an indispensable resource and essential life supporting factor. On the hydrological map of the world, eutrophication is one of the great issues causing degradation of freshwater ecosystems. The excessive nutrient enrichment of waters results in change of oligotrophic water bodies into mesotrophic, eutrophic, and finally hypertrophic. The major nutrient sources for enrichment of aquatic ecosystems are sewage, household detergents, and industrial discharges, runoff from agriculture, construction sites, and urban areas. Eutrophication is a threat for water used in fisheries, recreation, industry, and drinking as it causes increased growth of cyanobacteria and aquatic macrophytes resulting in low oxygen, death, and decomposition of aquatic flora and fauna (Ansari and Gill 2014).

The water is an essential life supporting matter in every cell of an organism. It enters into the living organisms via absorption or ingestion. It circulates between biotic and abiotic components of the ecosystem. The misuse and reckless over consumption has resulted into the fast depletion of water resources. The nutrient enrichment of the water bodies caused from the natural and man-made sources is depleting the water resources at a faster pace. The eutrophication is a kind of nutrient enrichment process of any aquatic body which results in an excessive growth of phytoplankton (Ansari and Khan 2014).

The common household detergents are the major anthropogenic source of phosphorus input into the nearby water bodies and sewage treatment plants (Ansari and Khan 2014). Eutrophication refers to natural or artificial addition of nutrients to water bodies and its effects on the aquatic life. When the effects are undesirable, eutrophication may be considered a form of pollution. Based on nutrient status and productivity, an aquatic system can be classified into the following three types: (1) Oligotrophic: water with poor nutrient status and productivity; (2) Mesotrophic: water with moderate nutrient status and productivity; (3) Eutrophic: water with rich nutrient status and high productivity (Naeem et al. 2014).

The European Union (EU) Water Framework Directive (WFD) given directions to prevent deterioration, protect aquatic ecosystems and to promote the sustainable use of water (Andersen et al. 2006). Hypoxia is one of the common effects of eutrophication in aquatic ecosystems and is becoming an increasingly prevalent problem worldwide. The causes of hypoxia are associated with nutrient inputs from both point and non-point sources. Eutrophication may be defined as the sum of the effects of the excessive growth of phytoplankton, benthic algae, and macrophytes leading to imbalanced primary and secondary productivity caused by nutrient enrichment. Most studies about eutrophication primarily focused on dissolved nutrients assimilated rapidly by aquatic plants. The role of complex organic nitrogen leading to the eutrophication of tropical waters has been largely overlooked although nitrogen occurs predominantly as organic nitrogen, due to a higher rate of decomposition of organic matter (Berman and Bronk 2003). The sources of nitrogen in the environment serve to enhance plant growth, and nitrogen acts as a pollutant in water bodies, becoming a serious problem worldwide. The elevated concentrations of ammonium nitrate and other forms of nitrogen enhance the eutrophication of aquatic ecosystems (Glibert et al. 2004; Zhang et al. 2007; Naeem et al. 2014).

A.A. Ansari, Ph.D. (✉) • S. Trivedi, M.Phil., Ph.D. • Z.K. Abbas, Ph.D.
H. Rehman, Ph.D.
Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia
e-mail: aansari@ut.edu.sa; aaansari40@gmail.com

F.A. Khan, M.Phil., Ph.D. • R. Perveen, M.Phil., Ph.D., Student
M.I. Dar, Ph.D., Student
Department of Botany, Environmental Physiology Laboratory, Aligarh Muslim University, Aligarh 202002, UP, India

S.S. Gill
Centre for Biotechnology, Maharshi Dayanand University, Rohtak, Haryana, India

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The domestic waste (rich in phosphate and nitrate) when discharged in water bodies makes them highly productive or "eutrophic." Nutrient enrichment is the starting point of eutrophication in any water body and is followed by uncontrolled growth of primary producers which depletes oxygen owing to decomposition of algal organic matter. Lakes and ponds in many Indian cities are the important sources of freshwater for various purposes and recharge the underground water resources. (Fareed et al. 2014).

Phytotechnologies involving use of plants for pollutant removal gained importance during the last two decades. Over the past many years phytoremediation technology has become an effective method for environmental cleaning due to the plants ability to accumulate the contaminants at the concentration level thousands times higher than background one. Phytoremediation is treatment technology that takes advantage of fact that certain species of plants flourish by accumulating contaminants present in the water. Phytoremediation refers to the natural ability of certain plants called hyper-accumulators or bio-accumulators to degrade or render harmless contaminants in water (Dar and Kumawat 2011). The floating macrophytes are applied most often for treatment of sewage released from different industrial sources and may consider as efficient tool for contaminated waters. The plants are capable to remove many toxic substances from water reservoirs, acquiring the pollutants from waters as food elements, basically through root system using the products of decomposition for their viability (Jamuna and Noorjahan 2009).

Development of aquatic plant systems for nutrient recovery from eutrophic water is essentially required to control eutrophication. The performance of phytoremediation system depends upon many factors such as growth performances of the plants selected for phytoremediation, their nutrient removal potential, efficiency to grow in experimental environment. In order to develop high-efficient nutrients phytoremediation systems aquatic plant species in combinations can be used (Ansari et al. 2014).

Contaminants like heavy metals, nonessential metals, crude oil, inorganic and organic substances and their derivatives could be mitigated in phytoremediation projects running across the globe as it is considered a clean, cost-effective, and environment friendly technology. Aquatic plants propagate rapidly by consuming dissolved nutrients from water and are excellent for harvesting nutrients within a short period of time and for the treatment of waste water by absorbing various nutrients like phosphates, calcium, magnesium, chloride from the waste water (Ansal et al. 2010). By harvesting the plants, nutrients can be permanently removed from the system. During the phytoremediation using aquatic plants an increase in pH value of water occurs usually which supports the growth of aquatic plants interne restoring the aquatic ecosystems (Patel and Kanungo 2010; Kaur et al. 2010). Changes in climate, particularly pH, temperature, and light affect the sustainability of phytoremediation systems (Ansari et al. 2011c, 2014; Feuchtmayr et al. 2009). Aquatic plants are highly capable to remove many organic nutrients which they convert into the substance of the plants as their biomass (El-Kheir et al. 2007).

Nitrogen and Phosphorus are the macronutrients required for the growth and physiological development of plants as they are major components of many metabolic and structural compounds in plant cells. These nutrients play a significant role in the synthesis of chlorophylls, protoplasm, and nucleic acids, and act as the backbone for ATP. Nitrogen deficiency causes decreased cell division and expansion, chlorophyll deficiency, and prolonged dormancy. Nitrogen is an essential plant nutrient for healthy growth and reproduction. An increase in availability of N usually boosts life production, such as increasing the abundance of primary producers in a water body (Camago and Alonso 2006). Aquatic plants can utilize various chemical forms of nitrogen ranging from simple inorganic nitrogen compounds such as ammonium and nitrate to organic nitrogen forms such as polymeric proteins (Paungfoo-Lonhienne et al. 2008). Among all the forms of nitrogen, ammonium and nitrate are the most common ionic (reactive) forms of dissolved inorganic nitrogen in aquatic ecosystems. Nitrogen is present naturally due to atmospheric deposition, surface and groundwater runoff, geological deposits, biological nitrogen fixation, and biodegradation of organic matter (Rabalais 2002).

Various free-floating aquatic macrophytes were studied for their possible use in the removal of different ionic form of nutrients (Gunnarsson and Petersen 2007; Malik 2007) as these free-floating aquatic plants have very high growth rates and rapidly utilize the available nutrients in the water (Malik 2007). The plants having high growth rates provide a good estimation of their nutrient removal capacity form eutrophic waters (Agunbiade et al. 2009). Plants with high bioproductivity are preferred for phytoremediation systems as they can utilize more nutrients to support rapid plant growth (Liao and Chang 2004; Gujarathi et al. 2005; Malik 2007).

Many waters of developed nations have experienced widespread and rapid eutrophication due to the increase in supply of organic matter during the last half of the twentieth century. An aquatic system takes thousands of years to become eutrophic which is a natural process. However, a high rate of nutrients inputs due to anthropogenic activities significantly enhances the condition in a very short period of time (Ansari and Khan 2002, 2006a, b, 2007, 2014). The nutrient input to waters from various sources causes eutrophication and are responsible for degradation of aquatic ecosystems (Ansari and Khan 2009b) and plant biodiversity. The environmental factors viz. nutrients, temperature, pH, dissolved oxygen, carbon dioxide, light within an aquatic ecosystem have a major role in controlling eutrophication in aquatic bodies and limiting the growth and development of aquatic plants (Lau and Lane 2002; Shen 2002; Khan and Ansari 2005; Khan et al. 2014).

In this experiment mono, bi, tri, tetra, and penta-cultures of some free-floating aquatic macrophytes *Eichhornia*, *Lemna*, *Salvinia*, *Spirodela*, and *Wolffia* were applied for the treatment of eutrophic waters. The selected plant species were grown in artificial nutrient media for 21 days to investigate their nutrient removal potential in order to develop sustainable nutrient phytoremediation systems for eutrophic waters. Selecting aquatic free-floating macrophytes would not only result in greater nutrient removal from eutrophic waters but also help to reduce other pollutants and to mitigate the other effects of eutrophication. Hence, in this study some free-floating aquatic macrophytes have been selected to determine their nutrient removal ability under controlled conditions, and to establish the phytoremediation systems for eutrophic waters.

## 4.2 Materials and Methods

Plants selected for this experiment were collected from freshwater bodies, washed thoroughly in lab and cultured for 1 week to acclimatize the experimental conditions. The experiments were conducted in large earthen pots of size 40 × 25 cm (diameter × depth) containing 15 L of freshwater with macronutrients 1 mL/L (Tables 4.1 and 4.2). The experimental pots were placed in a greenhouse in which had average conditions of 30/25 °C day/night temperatures, 65–85 % relative humidity, 12-h photoperiod. Natural irradiance (Photosynthetically Active Radiation, PAR) was provided to the plants and the light levels were maintained at 650 Gmol quanta $m^{-2}$ $s^{-1}$ by supplementing with artificial lighting. Growth medium in all the sets were maintained at pH 7, measured regularly with a pH meter (Elico Limited, Hyderabad) and NaOH or HCl were added to the growth medium to maintain the pH level.

Before inoculation to experimental pots the plants were disinfected by immersing them in NaClO (1 % v/v) and then rinsed with distilled water. The final volume (15 L) of the growing medium in the experimental pots was maintained using distilled water. The initial values of total fresh weight were made uniform and 100 g of each plant species transferred from the maintained stock to each experimental pot. The plants were placed in a nutrient-free solution for 3 days to elicit starvation-induced maximal removal response before transfer. Earthen pots of each treatment were maintained in triplicate.

The experiments were terminated after 21 days. Plants removed from the experimental pots and some part of fresh material was taken for chlorophyll-a estimation following the method of Zhao (2000). And rest of the fresh material was dried at 80 °C in order to obtain dry matter. The nitrogen and phosphorus contents in dry matter of aquatic plants were determined using the method of Lindner (1944) and Fiske and Subba-Row (1925) respectively. The parameters dry matter, nitrogen, phosphorus, and chlorophyll-a contents in aquatic plants used in this experiment was taken separately from each plant and an average was taken according to their combination as mono, di, tri, tetra, and penta-culture species used to develop a phytoremediation system for eutrophic waters. Water samples were collected from a depth of 5–10 cm below the water surface and 25 mL of water sample was collected each time in sterile, screw-capped plastic bottles measuring 50 mL. To minimize turbidity, samples were filtered with a Whatman No. 42 filter paper before storage. After filtration, the samples were refrigerated at 4 °C and analyzed following APHA (2005) within a week of collection. All the data obtained from the research were analyzed statistically for significance following Dospekhov (1984).

**Table 4.1** Composition of stock solution of macronutrients added to freshwater used as growth medium

| Macronutrients | g/L |
|---|---|
| $NH_4H_2PO_4$ | 0.23 |
| $KNO_3$ | 1.02 |
| Ca $(NO_3)$ | 0.492 |
| $MgSO_4 \cdot 7H_2O$ | 0.49 |

**Table 4.2** Physicochemical characteristics of freshwater used as growth medium

| Physicochemical characteristics | mg/L (Except pH and turbidity) |
|---|---|
| pH | 7.1 |
| Turbidity | 12 (NTU) |
| Dissolved oxygen | 7.4 |
| Calcium | 15.7 |
| Magnesium | 21.8 |
| Potassium | 9.3 |
| Chloride | 55.7 |
| Phosphate | 0.07 |
| Nitrate | 0.61 |

*NTU* nephelometric turbidity unit

## 4.3 Results

With the increasing interest related to the use of plants for phytoremediation of Nitrates and Phosphates rich waters, aquatic plants like *Eichhornia*, *Salvinia*, *Spirodela*, *Lemna*, *Wolffia* were selected in this study as they offer great potential for phytoremediation, reproduce vegetatively at a very rapid rate, and have relatively high rate for uptake of nutrients. All the plants showed their high potential to remove nitrate and phosphate from eutrophic waters.

## 4.4 Percent Nutrients (Nitrates and Phosphates) Removal from Eutrophic Waters

In mono-culture (one plant species) phytoremediation system the nutrient removal potential was in order of *Eichhorni a* < *Spirodela* < *Lemna* < *Wolffia* < *Salvinia* where *Eichhornia* remove maximum 63 % nitrates and 55 % phosphates from eutrophic waters. In di-culture (two plant species) phytoremediation systems, nutrient removal potential was in order of *Eichhornia* + *Salvinia* < *Lemna* + *Spirodela* < *Eichhornia* + *Spirodela* < *Eichhornia* + *Lemna* < *Spirodela* + *Salvinia* < *Eichhonia* + *Wolffia* < *Wolffia* + *Salvinia* < *Lemna* + *Salvinia* < *Spirodela* + *Wolffia* < *Lemna* + *Wolffia* but maximum potential was shown by *Eichhornia* + *Salvinia* which can remove up to 75 % nitrates and 62 % phosphates. Highest nutrient removal potential was observed in tri-culture (three plant species) phytoremediation system of *Eichhornia* + *Lemna* + *Spirodela* which removes 92 % nitrates and 78 % phosphates from nutrient media (Figs. 4.1 and 4.2, and Table 4.3).

However, in tri-culture phytoremediation systems nutrient removal potential was in the following order: *Eichhornia* + *Lemna* + *Spirodela* < *Lemna* + *Spirodela* + *Wolffia* < *Salvinia* + *Eichhornia* + *Spirodela* < *Eichhornia* + *Lemna* + *Salvinia* < *Wolffia* + *Salvinia* + *Eichhornia* < *Spirodela* + *Wolffia* + *Eichhornia* < *Eichhornia* + *Lemna* + *Wolffia* < *Wolffia* + *Salvinia* + *Lemna* < *Lemna* + *Spirodela* + *Salvinia* < *Spirodela* + *Wolffia* + *Salvinia*. In tetra-culture (four plant species) phytoremediation systems the nutrient removal potential was in order of *Salvinia* + *Eichhornia* + *Lemna* + *Spirodela* < *Spirodela* + *Wolffia* + *Salvinia* + *Eichhornia* < *Eichhornia* + *Lemna* + *Spirodela* + *Wolffia* < *Lemna* + *Spirodela* + *Wolffia* + *Salvinia* < *Wolffia* + *Salvinia* + *Eichhornia* + *Lemna* and maximum was 88 % for nitrates and 75 % for phosphates of *Salvinia* + *Eichhornia* + *Lemna* + *Spirodela*. The penta-culture (five plant species) (using a combination of *Eichhornia* + *Lemna* + *Spirodela* + *Wolffia* + *Salvinia*) phytoremediation system efficiently removes 85 % nitrate and 81 % of phosphate from the eutrophic water (Figs. 4.1 and 4.2, and Table 4.3).

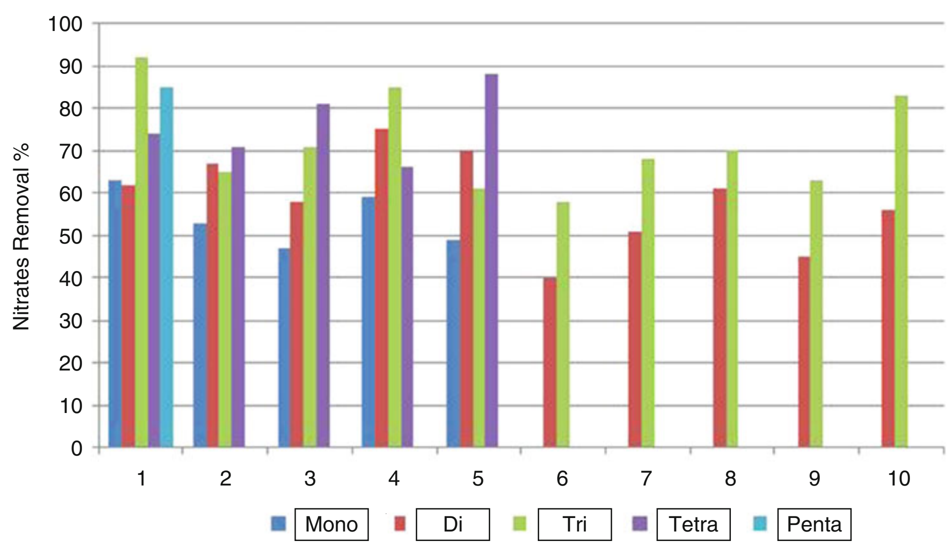


**Fig. 4.1** Nitrates removal potential of free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

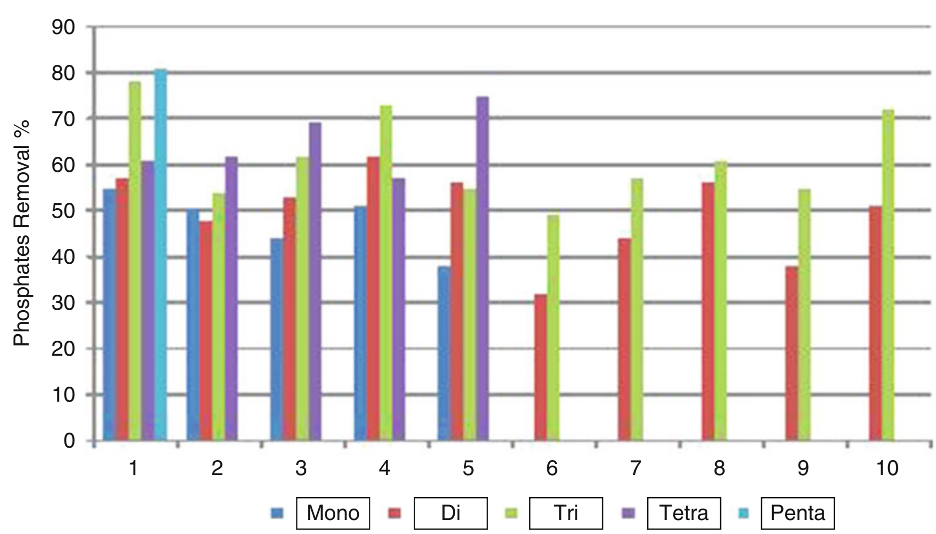


**Fig. 4.2** Phosphates removal potential of free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

**Table 4.3** Percent nutrients (Nitrates and Phosphates) removal from eutrophic waters by mono, di, tri, tetra, and penta-culture species phytoremediation systems

| Mono-culture (single species) | | | Di-culture (two species) | | | Tri-culture (three species) | | | Tetra-culture (four species) | | | Penta-culture (five species) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % Nutrient Removal | | | % Nutrient Removal | | | % Nutrient Removal | | | % Nutrient Removal | | | % Nutrient Removal | |
| | N | P | | N | P | | N | P | | N | P | | N | P |
| E | 63 | 55 | E+L | 62 | 57 | E+L+Sp | 92 | 78 | E+L+Sp+W | 74 | 61 | E+L+Sp+W+Sa | 85 | 81 |
| L | 53 | 50 | E+Sp | 67 | 48 | E+L+W | 65 | 54 | L+Sp+W+Sa | 71 | 62 | | | |
| Sa | 47 | 44 | E+W | 58 | 53 | E+L+Sa | 71 | 62 | Sp+W+Sa+E | 81 | 69 | | | |
| Sp | 59 | 51 | E+Sa | 75 | 62 | L+S+W | 85 | 73 | W+Sa+E+L | 66 | 57 | | | |
| W | 49 | 38 | L+Sp | 70 | 56 | L+Sp+Sa | 61 | 55 | Sa+E+L+Sp | 88 | 75 | | | |
| | | | L+W | 40 | 32 | Sp+W+Sa | 58 | 49 | | | | | | |
| | | | L+Sa | 51 | 44 | Sp+W+E | 68 | 57 | | | | | | |
| | | | Sp+Sa | 61 | 56 | W+Sa+E | 70 | 61 | | | | | | |
| | | | Sp+W | 45 | 38 | W+Sa+L | 63 | 55 | | | | | | |
| | | | W+Sa | 56 | 51 | Sa+E+Sp | 83 | 72 | | | | | | |

E=*Eichhornia*, L=*Lemna*, Sa=*Salvinia*, Sp=*Spirodela*, W=*Wolffia*

## 4.5 Percent Nutrient Uptake by Aquatic Plants from Eutrophic Waters

In mono-culture (one plant species) phytoremediation system the nitrogen and phosphorus uptake was highest (7.2 and 0.85 %) in *Eichhornia*. In di-culture (two plant species) phytoremediation systems, nitrogen and phosphorus uptake was highest (7.6 and 0.95 %) in *Eichhornia*+*Salvinia*. Highest (8.3 and 0.97 %) nitrogen and phosphorus was observed in *Eichhornia*+*Lemna*+*Spirodela* tri-culture (three plant species) phytoremediation system (Figs. 4.3 and 4.4, and Table 4.4).

In tetra-culture (four plant species) phytoremediation systems the nutrient uptake was maximum (7.25 and 0.88 %) in *Salvinia*+*Eichhornia*+*Lemna*+*Spirodela*. The penta-culture (five plant species) phytoremediation system *Eichhornia*+*Lemna*+*Spirodela*+*Wolffia*+*Salvinia* efficiently took up nitrogen and phosphorus (6.9 and 0.79 %); however, other systems were more efficient in taking up the nutrients from eutrophic waters (Figs. 4.3 and 4.4, Table 4.4).

## 4.6 Dry Weight Accumulation and Chlorophyll-a Content in Aquatic Plants Grown in Eutrophic Waters

In mono-culture (one plant species) phytoremediation system the dry matter accumulation and chlorophyll-a content were highest (335 and 1.34 mg/g of fresh matter) in *Eichhornia*. In di-culture (two plant species) phytoremediation systems, dry matter accumulation and chlorophyll-a content were highest (338 and 1.41 mg/g of fresh matter) in *Eichhornia*+*Salvinia*. Highest (342 and 1.40 mg/g of fresh matter) dry matter accumulation and chlorophyll-a content were observed in *Eichhornia*+*Lemna*+*Spirodela* tri-culture (three plant species) phytoremediation system (Figs. 4.5 and 4.6, and Table 4.5).

In tetra-culture (four plant species) phytoremediation systems dry matter accumulation and chlorophyll-a content was maximum (324 and 1.28 mg/g of fresh matter) in *Salvinia*+*Eichhornia*+*Lemna*+*Spirodela*. In penta-culture (five plant species) phytoremediation system (*Eichhornia*+*Lemna*+*Spirodela*+*Wolffia*+*Salvinia*) efficiently accumulate dry matter and chlorophyll-a content (328 and 0.87 mg/g of fresh matter), however, other systems were more efficient in accumulating dry matter and chlorophyll-a content grown in eutrophic waters (Figs. 4.5 and 4.6, Table 4.5).

## 4.7 Discussion

The study indicates that under controlled conditions multi-species phytoremediation systems are more efficient in removing the nutrients from eutrophic waters than the mono-species phytoremediation systems. However, in all types of phytoremediation systems tri-culture phytoremediation system (*Eichhornia*+*Lemna*+*Spirodela*) showed its highest efficiency and may be used for lowering high nutrient levels in eutrophic water. Freshwater aquatic plants are highly capable to remove nitrates and phosphates from waters but the response may be species dependent (Sooknah and Wilkie 2004; Zhang et al. 2009; Konnerup and Brix 2010). Aquatic plants are highly sensitive to pH, temperature and nutrient concentration of the growing media. Nitrate and phosphate removal potential of selected aquatic plants were studied in

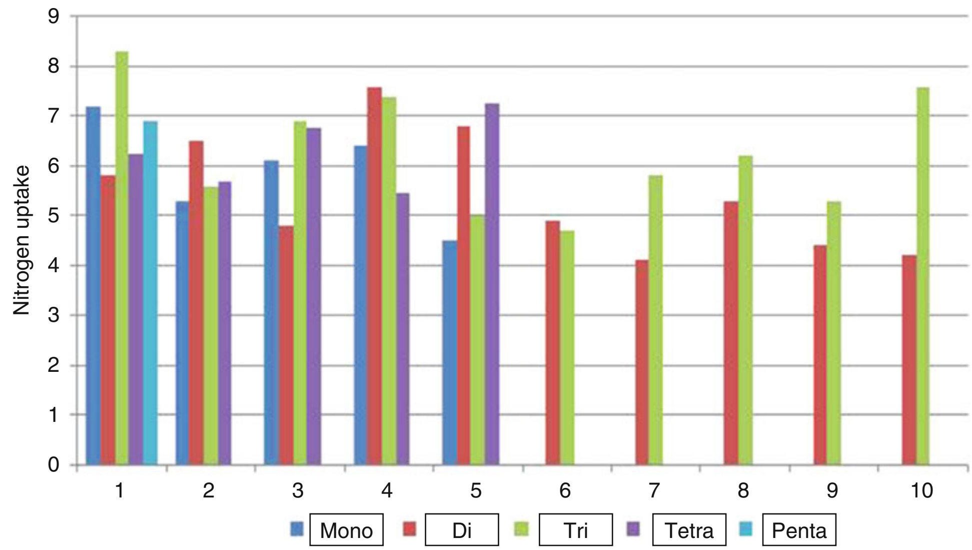


**Fig. 4.3** Nitrogen uptakes (mg/100 mg of dry matter) by free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

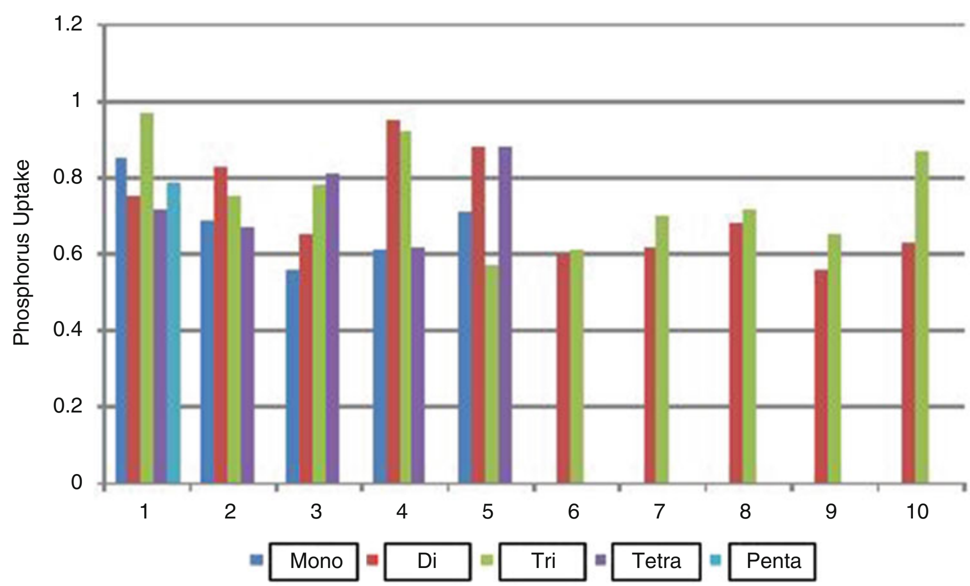


**Fig. 4.4** Phosphorus uptakes (mg/100 mg of dry matter) by free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

**Table 4.4** Nutrient (Nitrogen and Phosphorus) uptake (mg/100 mg of dry matter) by aquatic plants grown in mono, di, tri, tetra, and penta-culture species phytoremediation systems

| Mono-culture (single species) | | | Di-culture (two species) | | | Tri-culture (three species) | | | Tetra-culture (four species) | | | Penta-culture (five species) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % Nutrient uptake | | | % Nutrient uptake | | | % Nutrient uptake | | | % Nutrient uptake | | | % Nutrient uptake | |
| | N | P | | N | P | | N | P | | N | P | | N | P |
| E | 7.2 | 0.85 | E+L | 5.8 | 0.75 | E+L+Sp | 8.3 | 0.97 | E+L+Sp+W | 6.25 | 0.72 | E+L+Sp+W+Sa | 6.9 | 0.79 |
| L | 5.3 | 0.69 | E+Sp | 6.5 | 0.83 | E+L+W | 5.6 | 0.75 | L+Sp+W+Sa | 5.67 | 0.67 | | | |
| Sa | 6.1 | 0.56 | E+W | 4.8 | 0.65 | E+L+Sa | 6.9 | 0.78 | Sp+W+Sa+E | 6.75 | 0.81 | | | |
| Sp | 6.4 | 0.61 | E+Sa | 7.6 | 0.95 | L+S+W | 7.4 | 0.92 | W+Sa+E+L | 5.47 | 0.62 | | | |
| W | 4.5 | 0.71 | L+Sp | 6.8 | 0.88 | L+Sp+Sa | 5.0 | 0.57 | Sa+E+L+Sp | 7.25 | 0.88 | | | |
| | | | L+W | 4.9 | 0.60 | Sp+W+Sa | 4.7 | 0.61 | | | | | | |
| | | | L+Sa | 4.1 | 0.62 | Sp+W+E | 5.8 | 0.70 | | | | | | |
| | | | Sp+Sa | 5.3 | 0.68 | W+Sa+E | 6.2 | 0.72 | | | | | | |
| | | | Sp+W | 4.4 | 0.56 | W+Sa+L | 5.3 | 0.65 | | | | | | |
| | | | W+Sa | 4.2 | 0.63 | Sa+E+Sp | 7.6 | 0.87 | | | | | | |

E = *Eichhornia*, L = *Lemna*, Sa = *Salvinia*, Sp = *Spirodela*, W = *Wolffia*

**Fig. 4.5** Dry matter accumulation (mg/g of fresh matter) of free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

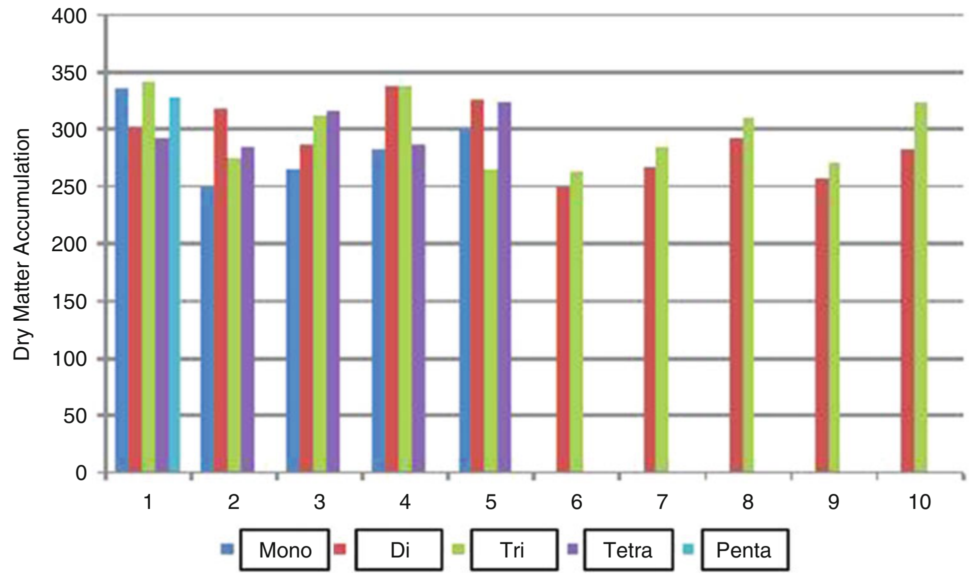


**Fig. 4.6** Chlorophyll-a contents (mg/g of fresh matter) in free-floating aquatic macrophytes grown in mono, di, tri, tetra, and penta-cultures species phytoremediation systems

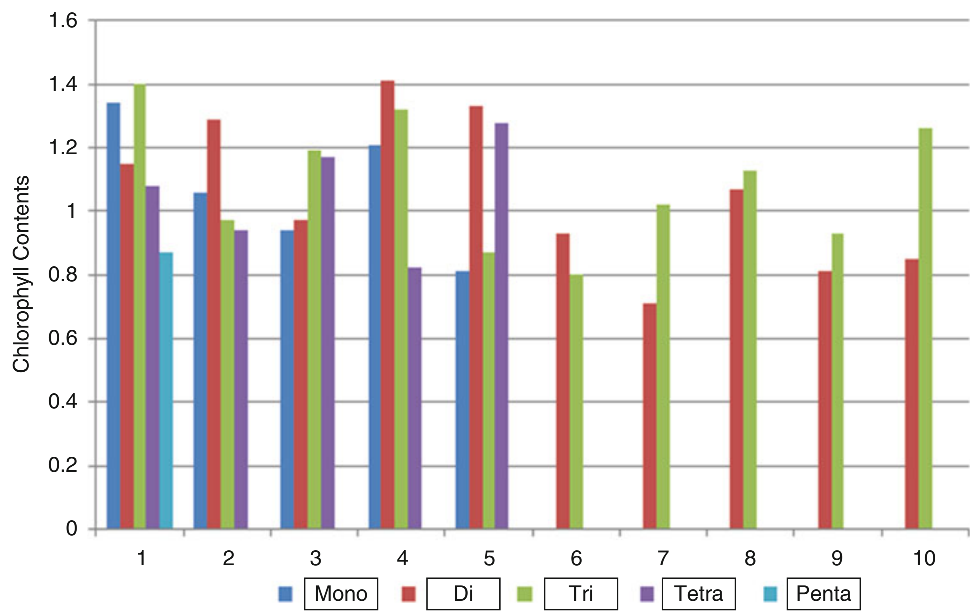


**Table 4.5** Dry matter and chlorophyll-a contents (mg/g of fresh matter) in aquatic plants grown in mono, di, tri, tetra, and penta-culture species phytoremediation systems

| Mono-culture (single species) | | | Di-culture (two species) | | | Tri-culture (three species) | | | Tetra-culture (four species) | | | Penta-culture (five species) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM | Chl-a | | DM | Chl-a | | DM | Chl-a | | DM | Chl-a | | DM | Chl-a |
| E | 335 | 1.34 | E+L | 302 | 1.15 | E+L+Sp | 342 | 1.40 | E+L+Sp+W | 292 | 1.08 | E+L+Sp+W+Sa | 328 | 0.87 |
| L | 250 | 1.06 | E+Sp | 318 | 1.29 | E+L+W | 275 | 0.97 | L+Sp+W+Sa | 284 | 0.94 | | | |
| Sa | 265 | 0.94 | E+W | 287 | 0.97 | E+L+Sa | 313 | 1.19 | Sp+W+Sa+E | 316 | 1.17 | | | |
| Sp | 282 | 1.21 | E+Sa | 338 | 1.41 | L+S+W | 337 | 1.32 | W+Sa+E+L | 287 | 0.82 | | | |
| W | 301 | 0.81 | L+Sp | 326 | 1.33 | L+Sp+Sa | 265 | 0.87 | Sa+E+L+Sp | 324 | 1.28 | | | |
| | | | L+W | 250 | 0.93 | Sp+W+Sa | 264 | 0.80 | | | | | | |
| | | | L+Sa | 267 | 0.71 | Sp+W+E | 285 | 1.02 | | | | | | |
| | | | Sp+Sa | 293 | 1.07 | W+Sa+E | 310 | 1.13 | | | | | | |
| | | | Sp+W | 258 | 0.81 | W+Sa+L | 271 | 0.93 | | | | | | |
| | | | W+Sa | 282 | 0.85 | Sa+E+Sp | 324 | 1.26 | | | | | | |

E = *Eichhornia*, L = *Lemna*, Sa = *Salvinia*, Sp = *Spirodela*, W = *Wolffia*, DM = Dry matter, Chl = Chlorophyll mono, bi, tri, tetra, and penta-culture species phytoremediation systems to investigate the best combination to develop a sustainable phytoremediation system for eutrophic water. Free-floating aquatic macrophytes are highly capable for morphological and physiological adaptations to aquatic environment. They have very high potential to take up and accumulate nutrients through their roots, stems, and leaves and can remove different ionic forms of nutrients especially of nitrogen and phosphorus from aquatic ecosystems (Smith 2007; Ansari and Khan 2006a, 2011, 2013; Ansari and Gill 2014).

Growth responses of aquatic plants reflect the primary productivity which has been considered as a strong indicator of eutrophication (Smith 2007; Ansari and Khan 2006a). The significant enhancement in dry matter, chlorophyll-a, nitrogen, and phosphorus in selected aquatic plants is a direct effect of composition of growth medium (Smith 2007). Waste water containing different forms of nutrients when discharged into the aquatic ecosystems changes the natural quality and quantity of water bring about corresponding changes in natural flora and fauna of the ecosystem (Azzurro et al. 2010).

Contamination of water has become one of the most serious problems of today's civilization. Phytoremediation is cost effective technique that uses plants to remediate contaminants from waste water. According to World Health Organization approximately 1.1 billion people do not have access to safe drinking water and within 15 years, three-fourths of the world's population will face the same problem. Contamination of water by different pollutants alters ecosystem structure and function. As such there has been a great deal of research into finding cost effective methods for the removal of contaminants to improve the quality of water (Abdel-Ghani and EI-Chaghaby 2008; Al-Anber and Matouq 2008). Phytoremediation is a very useful and cost-effective, eco-friendly, and efficient technology in which aquatic plants are used to remediate contaminated water. There are several species of aquatic plants known for their phytoremediation abilities for polluted waters (Riffat et al. 2007; Nouri et al. 2009, 2011). Potential utility for phytoremediation of nutrients by aquatic macrophytes like *Eichhornia crassipes*, *Salvinia natans*, *Spirodela polyrrhiza*, *Lemna minor*, etc. has been tested (Ansari and Khan 2011, 2013; Sooknah and Wilkie 2004; Zimmels et al. 2006; Lu et al. 2010).

Phytoremediation systems using aquatic macrophytes are the major options that have been applied for simultaneously handling of wastewater with the nutrients used for poultry and aquacultural projects (Naphi et al. 2003; Ansal et al. 2010). Aquatic macrophytes may produce many generations of progeny over a very short period of time and multiply their biomass and can remove more than 75 % of total phosphorus and nitrogen in a eutrophied water body (Ansari and Khan 2008, 2009a; Cheng et al. 2002). The use of plants for nutrient uptake is especially valuable because following site remediation, it is possible to identify practical and value-added uses for the plant material (Cheng et al. 2002; Fang et al. 2007; Gulcin et al. 2010).

Phytoremediation systems depend on many factors, including retention time, season, temperature, pH, diversity of species, nutrients loading, hydraulic regimes, plant harvesting, light intensity, etc. (El-Shafai et al. 2007; Ansari and Khan 2009a; Lu et al. 2010). Light reduction in the water column and enhanced organic matter load into the sediments are two main consequences of eutrophication (Olive et al. 2009). Temperature is important environmental factor directly related with the functioning of an aquatic ecosystem (Ansari et al. 2011b). pH controls absorption of nutrients and biochemical reactions taking place in living organisms (Ansari et al. 2011a). The potential of aquatic plants for phytoremediation of various pollutants in water has been determined (Xia and Xiangjuan 2006; Mishra et al. 2007). Aquatic plants are reported for their efficiency to remove about 60–80 % nitrogen (Fox et al. 2008) and about 69 % of potassium from water (Zhou et al. 2007). The pH and temperature significantly control the bio removal of nutrients from waters using aquatic plants (Uysal and Fadime 2009).

A recent meta-analysis examined the effects of nutrients on absolute and relative production of large aquatic ecosystems and found rise in productivity due to increasing eutrophication (Faithfull et al. 2011). A global climate change also enhances freshwater eutrophication (Dokulil and Teubner 2011). Some major problems that humanity is facing in the twenty-first century are related to water quantity and/or water quality issues (UNESCO 2009). Thousands of aquatic ecosystems around the world are suffering due to the excessive inputs of nutrients from human-related uses of the land causing changes in their ecological structure and function (Moss et al. 2011; Esteves 2011).

Many lake managers have adopted the options of increasing macrophytes abundance in order to restore the quality of eutrophic waters (Lau and Lane 2002). The process of eutrophication is directly related with discharge of nutrients in household wastes and sewages, industrial wastes, agricultural and urban runoffs (Ansari and Khan 2006a). A strict control on effluents from the different nutrient sources can mitigate the problem of eutrophication (Stone 2011). The nutrient removal by waste water treatments before release and biological control using free-floating macrophytes may be the cost effective measures to control the eutrophication in aquatic ecosystem.

## 4.8 Conclusions

Multi-species phytoremediation systems are more efficient in removing the nutrients from eutrophic waters than the mono-species phytoremediation systems. However, in all types of phytoremediation systems tri-culture phytoremediation system (*Eichhornia* + *Lemna* + *Spirodela*) were showed highest efficiency in lowering high nutrient levels from eutrophic waters. By removing the rapidly growing free-floating aquatic macrophytes, absorbing high nutrient contents especially nitrates and phosphates from the growing medium, and replacing old with fresh plants at regular intervals, the eutrophic aquatic ecosystem can be restored. This is a preliminary experiment to investigate the nutrients (N and P) removal potential of various free-floating aquatic macrophytes in different combinations. Further we will study the growth of selected aquatic plants (in mono, bi, tri, tetra, and penta-species culture) in response to varying pH, light, temperature, and nutrient concentrations of growing medium. To develop a sustainable nutrient phytoremediation system to improve the quality of eutrophic waters, all the selected plants (in different combinations) will be tested in natural environmental conditions.

## References


Abdel-Ghani NT, EI-Chaghaby GA (2008) The use low cost, environmental friendly materials for the removal of heavy metals from aqueous solutions. Curr World Environ 3:31–38

Agunbiade FO, Olu-Owolabi BI, Adebowale KO (2009) Phytoremediation potential of *Eichhornia crassipes* in metal contaminated coastal water. Bioresour Technol 100:4521–4526

Al-Anber ZA, Matouq MAD (2008) Batch adsorption of cadmium ions from aqueous solution by means of olive cake. J Hazard Mater 151:194–201

Andersen JH, Schluter L, Rtebjerg G (2006) Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. J Plankton Res 28:621–628

Ansal MD, Dhawan A, Kaur VI (2010) Duckweed based bio-remediation of village ponds: an ecologically and economically viable integrated approach for rural development through aquaculture. Livest Res Rural Dev 22:129

Ansari AA, Gill SS (eds) (2014) Eutrophication: causes, consequences and control, vol 2. Springer, The Netherlands, p 262

Ansari AA, Khan FA (2002) Nutritional status and quality of water of a waste water pond in Aligarh showing blooms of *Spirodela polyrrhiza*. J Ecophysiol Occupat Health 2:185–189

Ansari AA, Khan FA (2006a) Studies on the role of selected nutrient sources in the eutrophication of fresh water ecosystem. Nat Env Poll Tech 5:47–52

Ansari AA, Khan FA (2006b) Growth responses of *Spirodela polyrrhiza* to selected detergent at varying temperature and pH conditions. Nat Env Poll Tech 5:399–404

Ansari AA, Khan FA (2007) Eutrophication studies in some freshwater ponds of Aligarh. Ind J Appl Pure Bio 22:21–26

Ansari AA, Khan FA (2008) Remediation of eutrophied water using *Lemna minor* in a controlled environment. Afr J Aquat Sci 33:275–278

Ansari AA, Khan FA (2009a) Remediation of eutrophied water using *Spirodela polyrrhiza* L. Shleid in controlled environment. Pan Am J Aquat Sci 4:52–54

Ansari AA, Khan FA (2009b) Eutrophication studies on Jeffery canal of Aligarh. International Conference on Emerging Technologies in Environmental Science and Engineering. Oct 26–28, Aligarh Muslim University, Aligarh, India, pp. 845–849

Ansari AA, Khan FA (2011) Nutrients phytoremediation of eutrophic waters using *Eichhornia crassipes* in a controlled environment. Int J Environ Sci 2:241–246

Ansari AA, Khan FA (2013) Response of *Eichhornia crassipes* to eutrophic waters receiving nutrients from various sources. Int J Environ Sci 4:39–45

Ansari AA, Khan FA (2014) Household detergents causing eutrophication in freshwater ecosystems. In: Ansari AA, Gill SS (eds) Eutrophication: causes, consequences and control, vol 2. Springer, The Netherlands, pp 139–163

Ansari AA, Gill SS, Khan FA (2011a) Eutrophication: threat to aquatic ecosystems. In: Ansari AA, Gill SS, Lanza GR, Rast W (eds) Eutrophication: causes, consequences and control. Springer, The Netherlands, pp 143–170

Ansari AA, Gill SS, Khan FA, Varshney J (2011b) Aquatic plant diversity in eutrophic ecosystems. In: Ansari AA, Gill SS, Lanza GR, Rast W (eds) Eutrophication: causes, consequences and control. Springer, The Netherlands, pp 247–263

Ansari AA, Gill SS, Lanza GR, Rast W (eds) (2011c) Eutrophication: causes, consequences and control, vol 1. Springer, The Netherlands, pp 143–170

Ansari AA, Gill SS, Khan FA, Naeem M (2014) Phytoremediation systems for the recovery of nutrients from eutrophic waters. In: Ansari AA, Gill SS (eds) Eutrophication: causes, consequences and control, vol 2. Springer, The Netherlands, pp 239–248

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. APHA, AWWA and WEF, New York, NY, 1368 pp

Azzurro E, Matiddi M, Fanelli E, Guidetti P, La Mesa G, Scarpato A, Axiak V (2010) Sewage pollution impact on Mediterranean rocky-reef fish assemblages. Mar Environ Res 69:390–397

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat Microb Ecol 31:279–305

Camago JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831–849

Cheng J, Landesman L, Bergmann BA, Classen JJ, Howard JW, Yamamoto YT (2002) Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. Am Soc Agri Eng 45:1003–1010

Dar SH, Kumawat DM (2011) Sewage treatment potential of water hyacinth (*Eichhornia crassipes*). Res J Environ Sci 5:377–385

Dokulil MT, Teubner K (2011) Eutrophication and climate change: present situation and future scenarios. In: Ansari AA, Singh Gill S, Lanza GR, Rast W (eds) Eutrophication: causes consequences and control. Springer, Dordrecht, pp 1–16

Dospekhov BA (1984) Field experimentation. Mir Publications, Moscow, 351 pp

El-Kheir WA, Ismail G, El-Nour A, Tawfik T, Hammad D (2007) Assessment of the efficiency of duckweed (*Lemna gibba*) in wastewater treatment. Int J Agricul Bio 5:681–689

El-Shafai SA, El-Gohary FA, Nasr FA, Van der Steen NP, Gijzen HJ (2007) Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system. Bioresour Technol 98:798–807

Esteves FA (2011) Fundamentos derd limnologia, 3rd edn. Interciência, Rio de Janeiro, p 790

Faithfull CL, Bergström AK, Vrede T (2011) Effects of nutrient and physical lake characteristics on bacterial and phytoplankton production: a meta-analysis. Limnol Oceanogr 56:1703–1713

Fang YY, Babourina O, Rengel Z, Yang XE, Pu PM (2007) Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. Ann Bot 99:365–370

Feuchtmayr H, Moran R, Hatton K, Connor L, Heyes T, Moss B, Harvey I, Atkinson D (2009) Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow Lake Mesocosms. J Appl Ecol 46: 713–723

Fiske CH, Subba-Row Y (1925) The colorimetric determination of phosphorus. J Bio Chem 66:375–400
Fox LJ, Struik PC, Appletona BL, Rule JH (2008) Nitrogen phytoremediation by water hyacinth (*Eichhornia crassipes* (Mart.) Solms). Wat Air Soil Poll 194:199–207
Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar Ecol-Prog Ser 280:73–83
Gujarathi NP, Haney BJ, Linden JC (2005) Phytoremediation potential of *Myriophyllum aquaticum* and *Pistia stratiotes* to modify antibiotic growth promoters, tetracycline, and oxytetracycline, in aqueous wastewater systems. Int J Phytoremediat 7:99–112
Gulcin I, Kirecci E, Akkemik E, Topal F, Hisar O (2010) Antioxidant, antibacterial and anticandidal activities of an aquatic plant: duckweed (*Lemna minor* L. Lemnaceae). Turk J Biol 34:175–188
Gunnarsson CC, Petersen CM (2007) Water hyacinths as a resource in agriculture and energy production: a literature review. Waste Manag 27:117–119
Jamuna S, Noorjahan CM (2009) Treatment of sewage waste water using water hyacinth *Eichhornia* sp and its reuse for fish culture. Toxicol Int 16:103–106
Kaur L, Gadgil K, Sharma S (2010) Effect of pH and lead concentration on phytoremoval of lead from lead contaminated water by *Lemna minor*. Am Eur J Agric Environ Sci 7:542–550
Khan FA, Ansari AA (2005) Eutrophication: an ecological vision. Bot Rev 71:449–482
Khan FA, Naushin F, Rehman F, Masoodi A, Irfan M, Hashmi F, Ansari AA (2014) Eutrophication: global scenario and local threat to dynamics of aquatic ecosystems. In: Ansasri AA, Gill SS (eds) Eutrophication: causes, consequences and control, vol 2. Springer, The Netherlands, pp 17–28
Konnerup D, Brix H (2010) Nitrogen nutrition of *Canna indica*: effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. Aquat Bot 92:142–148
Lau SSS, Lane SN (2002) Biological and chemical factors influencing shallow lake eutrophication: a long-term study. Sci Tot Env 3:167–181
Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquat Plant Manage 42:60–68
Lindner RC (1944) Rapid analytical methods for inorganic constituents of plant tissues. Plant Physiol 19:76–89
Lu Q, He ZL, Graetz DA, Stoffella PJ, Yang X (2010) Phytoremediation to remove nutrients and improve eutrophic storm waters using water lettuce (Pistia stratiotes L.). Environ Sci Poll Res 17:84–96
Malik A (2007) Environmental challenge - opportunity: the case of water hyacinth. Environ Int 33:122–138
Mishra KK, Rai UN, Prakash O (2007) Bioconcentration and phytotoxicity of Cd in *Eichhornia crassipes*. Env Monitor Assess 130: 237–243
Moss B, Kosten S, Meerhoff M, Battarbee RW, Jeppesen E, Mazzeo N, Havens K, Lacerot G, Liu Z, De Meester L, Paerl H, Scheffer M (2011) Allied attack: climate change and eutrophication. Inl Water 1:101–105
Naeem M, Idrees M, Khan MMA, Moinuddin MA, Ansari AA (2014) Task of mineral nutrients in eutrophication. In: Ansari AA, Gill SS (eds) Eutrophication: causes, consequences and control, vol 2. Springer, The Netherlands, pp 223–237
Naphi I, Dalu J, Ndamba J, Gijzen HJ (2003) An evaluation of duckweed based pond systems an alternative option for decentralized treatment and reuse of wastewater in Zimbabwe. Water Sci Technol 48:115–122
Nouri J, Khorasani N, Lorestani B, Karami M, Hassani AH, Yousefi N (2009) Accumulation of heavy metals in soil and uptake by plant species with Phytoremediation potential. Environ Earth Sci 59:315–323
Nouri J, Lorestani B, Yousefi N, Khorasani N, Hasani AH, Seif S, Cheraghi M (2011) Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead-zinc mine (Hamedan Iran). Environ Earth Sci 62:639–644
Olive I, Garcıa-Sanchez MP, Brun FG, Vergara JJ, Perez-Llorens JL (2009) Interactions of light and organic matter under contrasting resource simulated environments: the importance of clonal traits in the seagrass *Zostera noltii*. Hydrobiologia 629:199–208
Patel DK, Kanungo VK (2010) Phytoremediation potential of Duckweed (*Lemna minor* L: a tiny aquatic plant) in the removal of pollutants from domestic wastewater with special reference to nutrients. Bioscan 5:355–358
Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, Webb RI, Gamage HK, Carroll BJ, Schenk PM, Schmidt S (2008) Plants can use protein as a nitrogen source without assistance from other organisms. Proc Natl Acad Sci U S A 105:4524–4529
Rabalais NN (2002) Nitrogen in aquatic ecosystems. Ambio 31:102–112
Riffat S, Arefin MT, Mahmud R (2007) Phytoremediation of boron contaminated soils by naturally grown weeds. J Soil Nature 1:01–06
Shen DS (2002) Study on limiting factors of water eutrophication of the network of rivers. J Agricul Life Sci 28:94–97
Smith VM (2007) Using primary productivity as an index of coastal eutrophication: the unit of measurement matter. J Plant Res 29:1–6
Sooknah RD, Wilkie AC (2004) Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecol Eng 22:27–42
Stone R (2011) China aims to turn tide against toxic lake pollution. Science 333:210–211
UNESCO (2009) The United Nations world water development report 3: water in a changing world. UNESCO, Par is
Uysal Y, Fadime T (2009) Effect of pH, temperature, and lead concentration on the bioremoval of lead from water using *Lemna minor*. Int J Phytoremediat 11:591–608
Xia H, Xiangjuan M (2006) Phytoremediation of ethion by water hyacinth (*Eichhornia crassipes*) from water. Bioresour Technol 97:1050–1054
Zhang X, Liu P, Yang Y, Chen W (2007) Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. J Environ Sci China 19:902–909
Zhang Z, Rengel Z, Meney K (2009) Kinetics of ammonium, nitrate and phosphorus uptake by *Canna indica* and *Schoenoplectus validus*. Aquat Bot 91:71–74
Zhao SHJ (2000) Detection of chlorophyll pigment. In: Zou Y (ed) Manual of plant physiology experiment. Chinese Agriculture Press, Beijing, pp 72–75
Zhou W, Zhu D, Tan L, Liao S, Hu H, David H (2007) Extraction and retrieval of potassium from water hyacinth (*Eichhornia crassipes*). Bioresour Technol 98:226–231
Zimmels Y, Kirzhner F, Malkovskaja A (2006) Application of *Eichhornia crassipes* and *Pistia stratiotes* for treatment of urban sewage in Israel. J Environ Manage 81:420–428

# 5 Phytoremediation of Water and Wastewater: On-Site and Full-Scale Applications

Gabriel Basílico, Laura de Cabo, and Ana Faggi

## 5.1 Introduction

One of the burning problems of our industrial society is the high consumption of water and the high demand for clean drinking water. Numerous approaches have been taken to reduce water consumption, but in the long run it seems only possible to recycle wastewater into high-quality water. It seems timely to discuss alternative water remediation technologies that are fit for industrial as well as less developed countries to ensure a high quality of drinking water (Schroder et al. 2007). On the other hand, soil and water pollution associated with landfills has reached a very important magnitude.

In the last few decades, pollution associated with both point and non-point sources have been identified as a serious threat to water quality around the world (Wang et al. 2012). Using vegetative buffer strips to reduce the delivery of non-point sources of pollutants from agricultural land to inland water systems has been recognized as a best management practice in the management of agroecosystems (Wang et al. 2012). On the other hand, constructed wetlands are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater (Vymazal 2007). Both systems apply plants capable of reduce nutrients from surface and subsurface water by assimilation, sedimentation, removal and dissipation, and temporary storage.

Municipal wastewater treatment and animal manure produce liquid and solid products. Treated and non-treated effluents are generally discharged into surface water, although increasingly, treated wastewater is used as a source of non-potable water or for indirect drinking water reuse. The effect of wastewater inputs over aquatic systems includes increases in total suspended solids (TSS), biochemical oxygen demand (BOD), and nutrients (Basílico et al. 2013), and in pathogens and metals. Treated sewage sludge can be applied as an organic carbon and nutrient-rich soil amendment or in land-reclamation projects. However, numerous organic contaminants, including pharmaceuticals, detergents, fragrances, antimicrobials, pesticides, and industrial products have been detected in wastewater end products and are known as anthropogenic organic contaminants (AOCs). The potential impacts of the environmental presence of AOCs on humans and wildlife include reproductive impairment, immune deficiencies, and antibiotic resistance among pathogenic bacteria (Kinney et al. 2010).

Depending on the characteristics of the effluents and the system itself, disposal or reuse options of the treated wastewater includes gravity or pressure-dosed irrigation, soil infiltration, greywater reuse, or surface water discharge, among others (Reed et al. 2006).

The aim of this chapter is to discuss several applications of phytoremediation for waste and wastewater treatment through natural systems using macrophytes. Phytoremediation techniques are a low-cost alternative for waste and wastewater treatment and in many cases it could be coupled with nutrient recovery and biomass production (Srivastava et al. 2008).

## 5.2 Phytoremediation Applications

### 5.2.1 Domestic and Municipal Wastewater

Domestic and municipal sewage is a relevant source of liquid wastes and its safe, economical, and effective treatment is one of the most challenging problems faced worldwide (Valipour et al. 2009), in fact billions of people do not get even sanitation services (WHO 2012). Domestic wastewater

G. Basílico (✉) • L. de Cabo, Ph.D. • A. Faggi
Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"–Consejo Nacional de Investigaciones Científicas y Técnicas, Av. Ángel Gallardo 470, CABA, Argentina

Universidad de Flores, Camacuá 245, CABA, Argentina
e-mail: gabrielomarbasilico@hotmail.com

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**Fig. 5.1** La Choza Stream (Argentina) receiving effluents by a modified natural open channel from a WTP located upstream. Site location: –34.66503, –58.982715

is a complex mix of solids and solutes, including wastes from kitchens, bathrooms, laundry, and floor drain (Henry 1999). Beyond the high variability in the composition of these wastewaters, these are characterized by high concentrations of TSS, chemical oxygen demand (COD), BOD, nitrogen, phosphorus, total organic carbon (TOC), presence of human pathogens (Henry 1999), metals (Arroyo et al. 2010), organic contaminants among which highlights emerging contaminants such as pharmaceutical and personal care products (PPCP) (Matamoros and Bayona 2006). Kadlec and Wallace (2009) provide typical values for several water quality variables of municipal wastewater. The effects of the discharge of municipal wastewater treatment plants (WTP) on the receiving aquatic ecosystems depend largely on the magnitude of the discharge and the characteristics of the receiving water body. Unfortunately, situations of streams of low-order receipt WTP effluents with high nutrient levels and high relative discharge values are still common (Fig. 5.1).

#### 5.2.1.1 On-Site Systems

On-site management of domestic wastewater includes several modalities depending on available land or climate limitations, among others. Reed et al. (2006) classify on-site treatment options into four major categories: conventional on-site systems; modified conventional on-site systems; alternative on-site systems; and on-site systems with additional treatment. Phytoremediation techniques would be categorized as alternative systems. A common feature to almost all on-site wastewater treatment is the incorporation of a pretreatment, which in most cases consists of a septic tank. In rural regions, the connection to sewer systems is unfeasible. An approach to wastewater management consists in the use of septic tank followed by a soil infiltration area (O'Luanaigh et al. 2010). This configuration or its variants (e.g., a septic tank followed by a cesspool) can also be found in vast urban and suburban areas of developing countries where sewers do not cover all urban areas.

Before selecting an alternative for wastewater treatment site characteristics, among which highlights permeability, depth, texture, structure, and pore sizes of soil, should be assessed (Reed et al. 2006). Also, terrain slopes, the existence and characteristics of surface water bodies, groundwater levels, vegetation, and landscape of the region should be considered (Reed et al. 2006). The segregation of black and greywaters allows the reuse of greywaters and the adequate treatment of each fraction in relation to its chemical and microbiological characteristics. Several phytoremediation alternatives for on-site treatment of domestic wastewater has been studied and includes the use of evaporation tanks, constructed wetlands (Ye and Li 2009; Paulo et al. 2013) and land application (Tzanakakis et al. 2009) among others.

##### Evapotranspiration Systems

These systems require the use of water-tolerant plant species also capable to evaporate large amounts of water. There are two modalities of evapotranspiration systems: evapotranspiration (ET) and evapotranspiration-absorption (ETA) systems (Reed et al. 2006). Basically ET consists of impermeable recipients containing several layers of different granular substrates such as gravel, coarse and fine sand, and a topsoil layer, where plants grow. In the case of ETA, there is no impermeable barrier (geomembrane, concrete). In both systems configuration promotes not only water loss through transpiration by plants but also by the direct evaporation of water rising by capillary. The area required to build this alternative can be estimated by the following equation (Reed et al. 2006):

$$A = Q / \left(\mathrm{Et} - \mathrm{Pr} + P\right) \tag{5.1}$$

where $A$ is the bed area, $Q$ is the annual flow, Et is the annual potential evapotranspiration, Pr is the annual precipitation rate, and $P$ is the annual percolation. It should be noted that for ET systems, Pr=0. As can be seen, the application of these systems is limited by the amount of available land. Another obvious limitation for the use of these alternatives is that are meaningful only in warm or temperate climates. Paulo et al. (2013) describe a system for treating domestic sewage for nine persons, based on the use of an evaporation tank planted with *Musa cavendishii* (banana tree), *Xanthosoma sagittifolium* ("Taioba"), and *Canna* species. In this case the function of the septic tank is supplied by the same evaporation tank. The authors report good overall system performance during the 400 days of the study and low maintenance requirements (trimming of the plants). The system was efficient in the reduction of COD load; no odors were generated and solids accumulation was not significant.

### Constructed Wetlands (CWs)

Constructed wetlands are commonly used for the treatment of domestic or municipal wastewater as secondary or tertiary treatment (Vymazal 2009; Brix and Arias 2005). Types of CW includes free water surface (FWS), characterized by a water surface exposed to the atmosphere and subsurface flow (SSF), shallow basin and channels filled with porous media (sand and/or gravel). All types of CWs could be used for on-site treatment of domestic or institutional wastewater but in most cases systems consists in horizontal or vertical SSF-CW or hybrid systems with the combination of both. The depth of the porous media is in the range of 0.3–0.9 m in horizontal SSF-CW but often deeper in vertical flow systems. Advantages of SSF over FWS-CW consist in avoid the proliferation of mosquitoes and other insect vectors and eliminates exposure to the wastewater (Reed et al. 2006). Wastewater application varies from continuous flow (most common) to recirculation and batch alternatives.

Species selection for the use in planted systems should be done taking into account native plant species. In the case of FWS-CW several plant species can be used, among which may be mentioned:

Emergent species: *Typha* spp., *Phragmites* spp., *Schoenoplectus* spp., *Carex* spp., *Hydrocotyle* spp., *Scirpus* spp., *Cyperus* spp.;

Submerged species: *Ceratophyllum demersum*, *Elodea* spp., *Potamogeton* spp., *Myriophyllum* spp., *Valisneria americana*;

Floating species: *Lemna* spp., *Spirodela* spp., *Echhornia* spp., *Pistia stratiotes*, *Salvinia* spp., among others.

Design alternatives for SSF-CW includes the use of *Phragmites* or *Scirpus* if N removal is a project requirement or even *Typha* (require higher surface) (Reed et al. 2006).

The use of small-scale constructed wetlands to treat effluent from one or more houses could be a valid alternative that can be applied taking into account weather factors and availability of land. Such systems may be effective for organic matter and nutrients. Gikas and Tsihrintzis (2012) found removal of 96.4 % for BOD, 94.4 % for COD, 90.8 % for total nitrogen (TN), 92.8 % for ammonia, 61.6 % for orthophosphates, and 69.8 % for total phosphorus (TP) in a system designed for two families (eight persons) with three treatment stages: two settling tanks in series, a vertical flow CW (VFCW), and a zeolite tank. The results of the use of constructed wetlands to remove N and P are often variable. Gill et al. (2011) report an N removal of only 29 and 30 % in secondary and tertiary treatment wetlands. In relation to TP, the authors found that removal averaged from 28 to 45 % (higher P removal during summer), with effluent concentrations still found to be high (>5 mg/L on average).

Constructed wetlands are also effective in the removal of metal from domestic wastewater. Yousefi et al. (2013) found metal removal rates of 42 % and 58 % in a constructed wetland treating effluents from a university campus, in relation to hydraulic retention times of 2 and 6 days, respectively, the measured metals were lead and cadmium. In this study, metal removal differences in absence/presence of reeds planted in a granular media were statistically significant (35 % and 65 %, respectively).

### Land Application

The soil is recognized as a biological, physical, and chemical filter (Reed et al. 2006). Land application systems (also land treatment systems, LTS) consist in the discharge of partially treated wastewater into vegetated soils. These slow rate systems combine both treatment and reuse of the effluent and are designed and operated according to the "zero discharge" concept (Tzanakakis et al. 2009).

Assuming that septic tank effluent should be treated, the allowable hydraulic loading rate for LTS is about 10 mm/day and the mass loading rates for BOD, TSS, and TN are 1.5 g/$m^2$/day, 0.8 g/$m^2$/day, and 0.55 g/$m^2$/day, respectively (Reed et al. 2006). To treat greywater septic tank effluent, the allowable hydraulic loading rate is 15 mm/day and the mass loading rates for BOD, TSS, and TN are 1.8 g/$m^2$/day, 0.6 g/$m^2$/day, and 0.22 g/$m^2$/day, respectively (Reed et al. 2006).

In order to reduce environmental risks associated with nutrients releases from land application systems; additional management practices should be adopted. Tzanakakis et al. (2009) recommend the use of plant species with high values of water use efficiency; the adjustment of N application rates to the system capacity for assimilation; interruption of effluent application when plant growth declines or ceases; and the adoption of suitable pre-application treatment schemes (constructed wetlands or retention ponds) aiming to reduce the nutrient concentration in the effluent.

#### 5.2.1.2 Higher Scale Applications

Concepts of phytoremediation could also be implemented in full-scale applications for centralized treatment of municipal wastewater. Brix et al. (2011) describe a full-scale system built in Thailand incorporating a multistage constructed wetland. The system was designed for the treatment of 400 $m^3$/day of wastewater from a business and hotel area. After few years of operation, the system allowed the removal of TSS, BOD, TN, TP, oil and grease, and fecal coliform bacteria from wastewater. However, the authors indicate a high variability of fecal coliform bacteria in influent and effluent, a high concentration of oil and grease in effluent, and a poor TN removal.

Technical and operational problems that can occur in full-scale wastewater treatment systems include clogging of granular media, bypass, lack of ammonia removal, mosquitoes proliferation (only in Free Water Surface Constructed Wetlands), and water quality problems due to metal sulfide precipitation (in SSF-CW) (Reed et al. 2006).

### 5.2.2 Feedlots

The development of modern agriculture with an improved production efficiency and specialization has led to the production of an excess of manure in small areas. A daily manure production (5–6 % of the animal body weight) is equivalent to almost twice the food it eats. Open lot livestock production, in general beef cattle feeding operations or feedlots, constitutes a contributor to surface water impairment due to the accumulation of manure, and transport of contaminants (Rizzo et al. 2012) beyond constitutes a production method questionable from bioethical standpoint.

#### 5.2.2.1 Feedlot Runoff Characteristics

Chemicals of concern in cattle feedlot manure and effluent may include endogenous chemicals such as hormones, non-endogenous natural and synthetic chemicals used to maintain the health and optimum growing conditions for animals. Furthermore, there is potential risk for animals (and hence manure and effluent) to be unintentionally exposed to chemicals in the environment or via contaminated feed products (Khan et al. 2008).

Cattle manure effluents are composed of solids (Koelsch et al. 2006), dissolved organic matter, microorganisms, nutrients, salts, steroidal hormones, antibiotics, ectoparasiticides, mycotoxins, heavy metals, and dioxins (García et al. 2012). Due to high levels of potential pollutants being released toward surface water or groundwater (Garcia et al. 2006; Garcia and de Iorio 2003), the feedlots were specifically defined as "point sources" of water pollution. The main by-products from cattle feedlots are the manure harvested from the surface of the pens and liquid effluent collected during rainfall runoff events. Feedlot runoff quality is weather dependent. Rainfall intensity and duration and, soil retention capacity affect quality and quantity runoff (Koelsch et al. 2006). Depending on variations in management and weather, manure harvesting rates have been reported to vary between 0.41 and 1.05 t dry weight per head per year (Khan et al. 2008). García et al. (2012) found that surface runoff was the dominant process in the feedlot pen soil.

Nutrients represent one of the most ubiquitous components of wastewater from feedlot operation. Then, nutrient management is critical at animal feedlots. Since feedlot pens do not sustain vegetation, vegetative treatment area must be an alternative approach to prevent and control the release of manure—contaminated runoff (Koelsch et al. 2006). Ikenberry and Mankin (2000) defined a VTA (vegetative treatment areas) as a band of planted or indigenous vegetation situated down-slope of cropland or animal production facilities that provides localized erosion protection and contaminant reduction. Planted or indigenous vegetation includes pasture, grassed waterways, cropland, or constructed wetlands that are used to treat runoff through settling, filtration, adsorption, and infiltration. The VTA also allows the recycling of nutrients by plants (Fajardo et al. 2001).

Also, good practice strategies should help reduce nutrient imbalances in feedlots and manure storage areas:

- Alternative feed rations and efficient utilizer of on-farm feeds can offset nutrient inputs as purchased feeds and forages.
- Exporting of manure nutrients to off-farm users can increase managed nutrient outputs.
- Manure treatments allow disposal of manure nutrients in agricultural soils. Some treatment options enhance the value of manure nutrients and complement manure marketing efforts.

The aims of several studies were to evaluate the ability of different plants to remediate a feedlot effluent. Vymazal (2007) compared nutrients removal by constructed wetlands with free-floating plants (FFP), free water surface CWs with emergent plants (FWS) and sub-surface CWs with horizontal (HSSF or HF) and vertical (VSSF or VF) flows. Removal of total nitrogen in studied types of constructed wetlands varied between 40 and 50 % depending on CWs type and in flow loading. Vertical-flow constructed wetlands remove successfully ammonia-N but very limited denitrification takes place in these systems. On the other hand, horizontal-flow constructed wetlands provide good conditions for denitrification but the ability of these systems to nitrify ammonia is very limited. Therefore, various types of constructed wetlands may be combined (hybrid systems) with each other in order to exploit the specific advantages of the individual systems.

The major phosphorus removal processes are sorption, precipitation, plant uptake (with subsequent harvest) and peat/soil accretion. Removal of phosphorus in all types of constructed wetlands is low unless special substrates with high sorption capacity are used. Removal of total phosphorus varied between 40 and 60 % in all types of constructed wetlands with removed load ranging. Removal of both nitrogen and phosphorus via harvesting of aboveground biomass of emergent vegetation is low but it could be substantial for lightly loaded systems (cca 100–200 g N/m$^2$/year and 10–20 g P/m$^2$/year).

### 5.2.2.2 Nutrients Removal

Aquatic systems for wastewater treatment are large basins filled with wastewater undergoing some combination of physical, chemical, and/or biological treatment processes that render the wastewater more acceptable for discharge to the environment. Solids removal via settling basins removed a mean of 64 % of the total solids, 84 % of the TN, 80 % of the total P and 34 % of potassium (K) (Koelsch et al. 2006). Facultative lagoons obtain necessary oxygen for treatment by surface reaeration from the atmosphere, combine sedimentation of particulates with biological degradation, and produce large quantities of algae, which limits the utility of their effluent without further treatment. Aerated lagoons use mechanical equipment to enhance and intensify the biodegradation rate. They do not produce the intense algal load on downstream processes and have smaller areal requirements than facultative systems. Free water surface (FWS) constructed wetlands have also been used, though rarely, for similar reasons (USEPA 2008). Oxic and anoxic decantation ponds are often used to reduce the pollution load of the effluent in intensive farming systems. However, some studies have reported that the levels found in these effluents generate a high impact on surface waters. Rizzo et al. (2012) found that the presence of aquatic plants increases the removal rates of nutrients, organic matter and heavy metals from wastewater in approximately 10–17 days for a feedlot effluent with high organic load.

The main mechanisms of nutrient removal from wastewater in constructed wetlands are microbial processes such as nitrification and denitrification as well as physicochemical processes such as the fixation of phosphate by iron and aluminum in the soil filter. Moreover, plants have a role in nutrient removal. Helophytes are adapted to anoxic rhizosphere conditions and can survive because of their ability to supply their root system with oxygen from the atmosphere. The release of oxygen causes the formation of an oxidative protective film directly on the root surface. This film protects the sensitive root areas from being damaged by toxic components in the anoxic, usually extremely reduced rhizosphere. On the other hand, the macrophytes in constructed wetlands favor aerobic processes such as nitrification near roots. In the zones that are largely free of oxygen, anaerobic processes such as denitrification, sulfate reduction, and/or methanogenesis take place. In subsurface horizontal flow systems the oxidized nitrogen is immediately reduced, preventing the enrichment of nitrite and nitrate (Stottmeister et al. 2003).

Wang et al. (2012) compared the efficiency of trees with pasture buffer strips at reducing nitrate-nitrogen from surface and groundwater flow emanating from the disposal area of Cattle feedlots. Both systems were significantly efficient at reducing nitrate ($NO_3$-N), principally through a significant reduction in soil surface runoff volume (50–57 %) together with a reduction in $NO_3$-N concentration (7–13 %) to give an average reduction in total $NO_3$-N load of 53–61 %.

Lee et al. (2004) studied the subsurface flow constructed wetlands performance at different high loading rates of swine effluent. The wetland vegetated with water hyacinth exhibited higher removal rates than most other wetlands, although it gave low reduction efficiencies. Subsurface flow constructed wetlands removed TP 47–59 %, and TN 10–24 %. Total N organic was mainly removed by physical mechanism. In this wetland, the nitrification was low (1.4 kg/ha/day), due to low oxygen supplied by the plants, although the denitrification could proceed very well, as shown by the very low effluent nitrate N, 1.1–1.7 mg/L. The low growth rate of water hyacinth also made the TN removed by plant uptake very low, only about 0.5 kg/ha/day. The TP removal was also dominated by physical mechanisms. The low growth rate of water hyacinth in this experiment with an extreme high organic load could be toxic to the plants. With respect to relative importance of nutrient uptake by plants, Payer and Weil (1987) state that up to 45 % of phosphorus may be removed in this way, whilst Schwer and Clausen (1989) report approximately 2.5 % of P removal and 15 % of N removal. Equally, the vegetation cover facilitates retention of suspended solids and the nutrients bound to them.

Duckweed (*Lemna punctata*) ponds have been successfully used in the swine waste polishing in small farm of subtemperate climate (southern Brazil). These ponds received the residue after a retention time of 30 days. The removal of total Kjeldahl N and total P were 98 % and 98.8 % respectively. Statistical relationships between the treatment efficiency and the seasons were not found (Mohedano et al. 2012). However, in a humid subtropical with cold winters zone (Shangai, China), another duckweed species (*Spirodela oligorrhiza*) was capable of removing 83.7 % and 89.4 % of TN and TP, respectively, from 6 % swine lagoon water in 8 weeks. In winter, nutrients could still be substantially removed in spite of the limited duckweed growth which was probably attributed to the improved protein accumulation of duckweed plants and the nutrient uptake by the attached biofilm (algae and bacteria) on duckweed and walls of the system (Xu and Shen 2011). However, several plants successfully used in growing seasons almost disappear during the winter. The climate affects plants nutrient removal ability and performance ponds.

#### 5.2.2.3 Solids Removal

Extensive research has been conducted on solids removal by VTA. Total solids are commonly quickly reduced by 70–90 %. The quick reduction can be attributed to a significant reduction in flow velocity due to vegetation retarding the flow and producing soil conditions conducive to infiltration. Variations occur due to site-specific conditions such as vegetation, slope, soil type, size and geometry of filter strip, and influent solids concentration (Koelsch et al. 2006). On the other hand, Rizzo et al. (2012) found more than 70 % of suspended matter removal in both treatments with and without plants for a feedlot effluent. The suspended solids were removed entirely by physical processes, involving sedimentation, filtration, and adsorption in subsurface flow constructed wetland (Lee et al. 2004).

#### 5.2.2.4 COD and BOD Removal

Averaged COD concentration of influent in swine wastewaters was 1,160 mg/L (Lee et al. 2004). The excellent COD removal by a subsurface flow constructed wetland was accomplished by a good cooperation between physical and microbial mechanisms, where the former mechanism made 52–74 % of contribution, and the latter 26–48 % (Lee et al. 2004). The main removal mechanisms were physical separation and microbiological uptake. The organic solids could be settled out and retained in the wetland cell for a longer time, thus allowing an easy biodegradation by attached bacteria in rhizosphere. The treatment of soil infiltration showed reductions of 92 and 93 % in COD and 90 % or more BOD5 after waste effluent had been passed through soil columns (Nuñez Delgado et al. 1995).

The BOD removal percentages were higher than 90 % and COD removal was near to 65 % in treatment with *E. crassipes* and *H. ranunculoides* in the treatment of wastewater from a feedlot effluent (Rizzo et al. 2012). The higher efficiency in the removal of organic load in macrophyte systems could be due to the better conditions of oxygenation provided by macrophytes.

#### 5.2.2.5 Steroidal Hormones Removal

Steroidal hormones potentially present in feedlot manure and effluent include endogenous hormones and some synthetic hormones applied in agriculture. Endogenous hormones are commonly identified in animal excretions including manure and urine (Hoffmann et al. 1997). Both natural and synthetic steroidal hormones are used in many countries as hormonal growth promotants in cattle (Song et al. 2009). They are used to improve feed efficiency, rates of weight gain, and relative proportions of muscle and fat (Lefebvre et al. 2006). Reports of hormonally related abnormalities in a wide range of species have accumulated. Chemical contaminants are believed to be responsible for many of these abnormalities, acting via mechanisms leading to alteration in endocrine function. This phenomenon, known generally as “endocrine disruption,” has been identified by the World Health Organization as an issue of global concern (Khan et al. 2008).

Song et al. (2009) concluded that the performance of wetlands when operating in unsaturated condition was superior to that when operating in water-saturated condition in constructed wetlands. Biodegradation of estrogens is expected to proceed much faster in aerobic horizons. Mass balance calculations indicated that the removal of estrogens in constructed wetlands was very likely attributable largely to biotic processes, a combination of both microbial degradation and plant uptake. Shi et al. (2010) found that the presence of duckweed and algae in wastewater treatment systems accelerates the removal. Estrogens can be quickly sorbed onto algae or duckweed and then the estrogens can be degraded by microorganisms.

#### 5.2.2.6 Antibiotics Removal

Antibiotics are used in veterinary medicine to treat and prevent disease, and for other purposes including growth promotion in food animals (Prescott et al. 2000). Many antibiotic compounds are only partially degraded during metabolism by humans and other animals, and thus are excreted largely unchanged. Accordingly, animal excrements following antibiotic use for treatment or growth promotion are considered to be important sources of these compounds to some affected environments (Khan et al. 2008). Antibiotics in the environment are suspected to induce antibiotic resistance in bacteria, which may cause severe health problems due to an increasing ineffectiveness of antibiotic drugs. An antibiotic-resistant strain of *Clostridium perfringens* was detected in the groundwater below plots of land treated with swine manure. *Myriophyllum aquaticum* (parrot feather) and *Pistia stratiotes* (water lettuce), were used for studying phytoremediation of tetracycline (TC) and oxytetracycline (OTC) from aqueous media. TC and OTC are two of the most commonly used tetracyclines in veterinary medicine. The authors suggest the involvement of root-secreted enzyme(s)/metabolite(s) in degrading/transforming the antibiotics (Gujarathi et al. 2005). When subjected to stress, plants produce reactive oxygen species (ROS) as a part of the defense response. The oxidative response is also used to degrade organic pollutants. Gujarathi et al. (2005) suggests involvement of reactive oxygen species (ROS) in the antibiotic modification process.

#### 5.2.2.7 Pathogen Organisms Removal

Microbiological contamination is a key parameter pertaining to the treatment requirements and safe reuse of effluent. There are few literature data on pathogens in feedlot effluent and most studies have measured only bacterial indicator organisms. These bacterial counts are fairly high. However, a range of pathogens has been measured in manure, soils and water bodies impacted by feedlot runoff. These include *Salmonella* spp., pathogenic *Escherichia coli* H157:O157, *Leptospira* spp., *Campylobacter* spp.; *Cryptosporidium* *parvum*, *Giardia lamblia*, and helminthes worms. Pathogenic contamination of recycled feedlot effluent and the associated risk of disease outbreaks are the most concerning aspects of using recycled water for a cattle drinking supply.

Fajardo et al. (2001) report fecal coliform removal rates between 64 and 87 % when using small-scale simulated runoff events with stockpiled manure. Lim et al. (1997) found that all fecal coliforms were removed in the first 6.1 m of a vegetative treatment area used to treat runoff from a simulated pasture. Average fecal coliform removal in the study reported was 76.6 % (Ikenberry and Mankin 2000).

Kadlec and Wallace (2009) listed the efficiency of the elimination of coliforms and streptococci in various systems of constructed wetlands. More than 90 % of the coliforms and more than 80 % of the fecal streptococci were eliminated. The findings of Thurston et al. (1996) regarding the comparison of a pond system with a subsurface flow planted soil filter. The planted soil filter is more efficient at eliminating bacteria than the *Lemna* pond.

#### 5.2.2.8 Metal Removal

Some metals in livestock excreta may be derived from the animal diet, either intentionally or as a result of contamination. However, metals are more likely to be derived from the ingestion of contaminated soil by the animal. Practices such as using composted municipal waste as feedlot bedding also have the potential to contribute to the presence of heavy metals in contaminated manure (Khan et al. 2008). Rizzo et al. (2012) found that the presence of *E. crassipes* and *H. ranunculoides* increases the removal rates of Cu, Zn, and Cr from wastewater in approximately 10–17 days for a feedlot effluent with high organic load. Due to the fact that suspended matter could be associated with metals, their removal from the water column could mitigate the action of suspended matter as a vector of contaminants.

### 5.2.3 Landfills

During the past decade much effort has been made to test a diverse range of techniques to remediate gas emissions, soil and groundwater contamination in landfills, and metals by industrial waste or illegal dumping. In such cases phytoremediation using a vegetation cover has been frequently used to mitigate the effects of leachates (Nagendran et al. 2006).

The composition of leachate is site specific and can vary significantly due to the different waste sources and stages of waste decomposition (Christensen et al. 2001). Landfill's leachate is normally a potentially highly polluting liquid because it contains high concentrations of dissolved and suspended organic matter, inorganic chemicals, and metals (Licht and Isebrands 2005). In addition, leachate has a high COD and BOD (Licht and Isebrands 2005; Andreottola and Cannas 1992).

Phytoremediation techniques by using vegetative caps are useful when the completed landfill closes, especially if species with high leaf area index and transpiration activity are selected (Borjesson 1999). Vegetative caps, are used as evapotranspiration landfill covers and are designed to store water until it is either transpired through vegetation or evaporated from the soil surface (Blight and Fourie 2005; Licht et al. 2001; Nixon et al. 2001; Albright et al. 2004; Preston and McBride 2004). Therefore, as the vegetative layer interrupts the flow patterns minimizing percolation, groundwater contamination can be reduced (Hupe et al. 2001). In addition, vegetative caps are useful against erosion, prevent runoff and dust blow, sequester $CO_2$, meliorate climate and, as an extended greenery, add aesthetic value to the place. It is a win-win technology, being at the same time cost effective and environmental friendly (Nagendran et al. 2006).

When trees are employed, poplar (*Populus* spp.) and willow species (*Salix* spp.) are normally used because they have an optimal evapotranspiration performance. Poulsen and Møldrup (2005) showed that such plantations can increase the evapotranspiration and thereby reduce percolation rates by up to 47 % in comparison with a grass cover.

In metal contaminated soils, higher plants used as cover, can be useful for metals uptake into their biomass. If necessary, plants can subsequently be harvested and removed from the site (Pulford and Watson 2003). Moreover, plants can stabilize inorganic contaminants in the rhizosphere, preventing contaminants to pollute the groundwater (Alvarenga et al. 2008).

There are good examples around the world of cities that have recovered landfills as compensatory mitigation actions. Germany, Hong Kong, Korea, and the USA, among others, have successfully converted landfills to parks featuring recreational, educational, and conservation activities. Nature reserves, sports fields, golf courses, ski slopes, sculpture gardens, etc. have been created with the successful installation of green covers as initial stages in habitat restoration or creation programs (Zedler and Callaway 1999). Some others famous landfill-to-park site projects are underway in NYC, USA like Staten Island's Freshkills and Jamaica Bay. Over 400 acres of land on the eastern coast of Brooklyn are under a long-term restoration process to turn the toxic Jamaica Bay landfills into a family friendly park. The sites operated as working landfills in the 1950s and 1960s, though oil contaminated with PCBs and metals leached into Jamaica Bay and the landfills were closed in 1980. Now, a prairie-like landscape composed of native grasses, flowers, and saplings is the result of a restoration effort since 2004.

In closed landfills the principal restoration targets are to produce a dense, self sustaining vegetation cover, returning a damaged ecosystem to a more natural condition helping to reestablish native populations, communities, and ecosystem processes (Byers et al. 2006).

The most appropriate restoration technique for ecological diversity is considered to be a combination of interventions

**Fig. 5.2** Spontaneous plants growing on an unclosed landfill in Buenos Aires province temporarily covered with a plastic liner

followed by natural successional stages (Simmons 1999). Sites restored with a mixture of herbs, grasses, shrubs, and trees show a more attractive appearance than only grass species and thereby contribute to biodiversity and to the visual amenity of the area (Simmons 1990).

A mixture of native plants are usually preferred, because native vegetation is more adapted than alien plants to regional conditions, and more resilient to disturbances such as extreme weather, species competition, and disease. In temperate climates the combination of warm- and cool-season species in the selected plant mixture guarantees water uptake throughout the entire growing season, which enhances transpiration.

Seed mix of native warm and cool season grasses generate a diverse grassland community which provides habitat for several species of grassland birds with declining populations. Seed mix examples from commercial vendors are available (e.g., *Andropogon gerardii*, *Schizachyrium scoparium*, *Panicum virgatum*, *Sorghastrum nutans*, *Elymus canadensis*, and *Chamaecrista fasciculata*).

Because seed mix should be selected based on their tolerance of the chemical contamination in the soils adjusted to site specific and seasonal conditions, it is necessary to know which plants to use at a local level. In countries where this information is not available, plants that grow spontaneously on dump sites are potential candidates to be proposed (Fig. 5.2).

**Table 5.1** Spontaneous plants growing on an unclosed landfill in Buenos Aires metropolitan area

| Native species | Exotic |
|---|---|
| Forbs: *Solidago chilensis*, *Verbena intermedia*, *Aster squamatus*, *Physalis viscosa*, *Baccharis pingrae*, *B. salicifolia*, *Pluchea sagittalis*, *Salpichroa origanifolia*, *Oxypetalum solanoides* | Forbs: *Chenopodium ambrossioides*, *Brassica campestris*, *Chenopodium album*, *Carthamus lanatus*, *Carduus acanthoides*, *Centaurium pulchellum*, *Lotus glaber* |
| Grasses: *Paspalum dilatatum*, *Jarava plumosa*, *Echinochloa polystachya*, *Cortaderia selloana*, *Stipa neesiana*, *Typha* sp. | Grasses: *Cynodon dactylon*, *Sorghum halepense*, *Lolium multiflorum*, *Setaria geniculata*, *Festuca arundinacea*, *Dactylis glomerata* |
| Shrub: *Solanum glaucophyllum* | |
| Tree: *Salix humboldtiana* | |

Figure 5.2 shows spontaneous and alien plants growing on an unclosed landfill in Buenos Aires province (Argentina). Most of them are hydrophilic pioneers having the potential to cover disturbed sites quickly (Fig. 5.2, Table 5.1), such an information can be useful when designing the final vegetative cap.

## 5.3 Harvested Biomass Use

Globally, both food shortages as water pollution problems treatable through phytoremediation occur simultaneously in the same location. Therefore, the use of aquatic plants as a food source for cattle is a promising alternative. The plant biomass in wetlands can subsequently be put to viable economic use, for example as a source of energy or as raw material for the paper industry. These are issues that will naturally vary from one country to the next depending on the various socioeconomic and climatic conditions that apply (Stottmeister et al. 2003). Among aquatic plants, the floating ones are easier to manage and harvest. Aquatic plants present a high growth rate. For example, the total biomass duckweed harvested was 5.30 times that of the starting amount after 8 weeks (Xu and Shen 2011). The duckweed ponds constructed for swine waste polishing produced over 68 t/ha/year of dry biomass, with 35 % of crude protein content, which represents a productivity of 24 t CP ha/year. Due to the high rate of nutrient removal, and also the high protein biomass production, duckweed ponds reveal a great potential for the polishing and valorization of swine waste (Mohedano et al. 2012).

Composting and compaction has been proposed as a post-harvest biomass treatment by some authors (Kadlec and Wallace 2009) especially for biomass harvested after treatments of wastewater rich in nutrients.

Combustion is a crude method of burning the biomass, but it should be under controlled conditions, whereby volume is reduced to 2–5 % and the ash can be disposed of properly. Combustion is an important sub routes for organized generation of electrical and thermal energy. Recovery of this energy from biomass by burning could help make phytoextraction more cost-effective.

## 5.4 Concluding Remarks

Waste and wastewater produce an enormous amount of organic matter relatively easy to remedy. Also, the effluents represent a source of emerging contaminants and pathogens more difficult for treatment. Application of plants in remediation of soils and water constitutes a valuable tool. However, the treatment selection depends upon climate, hydrology, type of soil, type of effluent, geomorphological features, inundation level, and applied plants. Native plants are more resilient to disturbances, ensure a more diverse community, and are visually more enjoyable. However, one of the major complications of phytoremediation of substrates having excess organic matter and nutrients is the overgrowth of vegetation. Then, the programmed harvest should be taken into account and the fate of harvested material should be considered in management plan. Obtaining energy, composting, use as livestock feed and fertilizer are some of the possible destinations. However, the presence of toxics and pathogens must be analyzed prior to the use of harvested material.

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## References

Albright WH, Benson CH, Gee GW, Roesler AC, Abichou T, Apiwantragoon P, Lyles BF, Rock SA (2004) Field water balance of landfill final covers. J Environ Qual 33:2317–2332

Alvarenga P, Palma P, Goncalves AP, Baiao N, Fernandes RM, De Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2008) Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72:1774–1781

Andreottola G, Cannas P (1992) Chemical and biological characteristics of landfill leachate. In: Christensen TH, Cossu R, Stegmann R (eds) Landfilling of waste: leachate. Elsevier Science, London

Arroyo P, Ansola G, de Luis E (2010) Effectiveness of a full-scale constructed wetland for the removal of metals from domestic wastewater. Water Air Soil Pollut 210(1–4):473–481

Basílico G, de Cabo L, Faggi A (2013) Impacts of composite wastewater on a Pampean stream (Argentina) and phytoremediation alternative with *Spirodela intermedia* Koch (Lemnaceae) growing in batch reactors. J Environ Manag 115:53–59

Blight GE, Fourie AB (2005) Experimental landfill caps for semi-arid and arid climates. Waste Manag Res 23:113–125

Borjesson P (1999) Environmental effects of energy crop cultivation in Sweden: identification and quantification. Biomass Bioenergy 16:137–154

Brix H, Arias CA (2005) The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: new Danish guidelines. Ecol Eng 25:491–500

Brix H, Koottatep T, Fryd O, Laugesen CH (2011) The flower and the butterfly constructed wetland system at Koh Phi Phi-System design and lessons learned during implementation and operation. Ecol Eng 37:729–735

Byers JE, Cuddington K, Jones CG, Talley TS, Hastings A, Lambrinos Crooks JA, Wilson WG (2006) Using ecosystem engineers to restore ecological systems. Trends Ecol Evol 21(9):493–500

Christensen TH, Kjeldsen P, Bjerg DJ, Christensen JB, Baun A, Albrechsten HJ, Heron G (2001) Review: Biogeochemistry of landfill leachate plumes. Appl Geochem 16:659–718

Fajardo JJ, Bauder JW, Cash SD (2001) Managing nitrate and bacteria in runoff from livestock confinement areas with vegetative filter strips. J Soil Water Conserv 56(3):185–191

Garcia AR, de Iorio AF (2003) Phosphorus distribution in sediments of Morales Stream tributary of the Matanza-Riachuelo River, Argentina. The influence of organic point source contamination. Hydrobiologia 492:129–138

Garcia AR, Massobrio MJ, de Iorio AF (2006) Comportamiento de nutrientes derivados de actividades de engorde a corral (feedlot) en Hapludoles de la provincia de Bs. As.-Argentina. Agrochimica 50(3–4):148–157

García AR, Maisonnave R, Massobrio MJ, Fabrizio de Iorio AR (2012) Field-scale evaluation of water fluxes and manure solution leaching in feedlot pen soils. J Environ Qual 41:1–9

Gikas GD, Tsihrintzis VA (2012) A small-size vertical flow constructed wetland for on-site treatment of household wastewater. Ecol Eng 44:337–343

Gill LW, O'Luanaigh N, Johnston PM (2011) On-site wastewater treatment using subsurface flow constructed wetlands in Ireland. J Environ Sci Health A Tox Hazard Subst Environ Eng 46(7):723–728

Gujarathi N, Haney BJ, Park HJ, Wickramasinghe SR, Linden J (2005) Hairy roots of *Helianthus annuus*: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Prog 21:775–780

Henry JG (1999) Contaminación del agua. In: Henry JG, Heinke GW (eds) Ingeniería ambiental. Prentice Hall, México

Hoffmann B, dePinho TG, Schuler G (1997) Determination of free and conjugated oestrogens in peripheral blood plasma, feces and urine of cattle throughout pregnancy. Exp Clin Endocrinol Diabetes 105:296–303

Hupe K, Unger A, Heyer KU, Stegmann R (2001) Alternative Oberflächen-abdichtungssysteme für *in situ* stabilisierte Deponiekörper—Versuchsfelder auf der Altdeponie Kuhstedt. In: Belüftung von Altdeponien zur in situ Stabilisierung. Tagung am 31.05.2001 in Kuhstedt, Landkreis Rotenburg (Wümme), Band 3 der Schriftenreihe Abfall aktuell, Hrsg.: R. Stegmann, Verlag Abfall aktuell, Stuttgart

Ikenberry CD, Mankin KR (2000) Review of vegetative filter strip performance for animal waste treatment. ASAE Paper No. MC00128. ASAE, St. Joseph

Kadlec RH, Wallace S (2009) Treatment wetlands, 2nd edn. CRC Press Taylor & Francis Group, Boca Raton, FL

Khan DJ, Roser DJ, Davies CM, Peters GM, Stuetz RM, Tucker R, Ashbolt NJ (2008) Review article: chemical contaminants in feedlot wastes: concentrations, effects and attenuation. Environ Int 34:839–859

Kinney CA, Furlong ET, Kolpin DW, Zaugg SD, Burkhardt MR, Bossio JP, Werner SL (2010) Earthworms: diagnostic indicators of wastewater derived anthropogenic organic contaminants in terrestrial environments. In: Halden R (ed) Contaminants of emerging concern in the environment: ecological and human health considerations, ACS Symposium Series. American Chemical Society, Washington, DC

Koelsch RK, Lorimer J, Mankin K (2006) Vegetative treatment systems for open lot runoff: review of literature. Conference presentations and white papers: Biological systems engineering. Paper 5. http://digitalcommons.unl.edu/biosysengpres/5. Accessed 24 May 2013

Lee CY, Lee CC, Lee FY, Tseng SK, Liao CJ (2004) Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. Bioresour Technol 92:173–179

Lefebvre B, Malouin F, Roy G, Giguere K, Diarra MS (2006) Growth performance and shedding of some pathogenic bacteria in feedlot cattle treated with different growth-promoting agents. J Food Prot 69:1256–1264

Licht LA, Isebrands JG (2005) Linking phytoremediated pollutant removal to biomass economic opportunities. Biomass Bioenergy 28:203–218

Licht L, Aitchison PE, Schnabel W, English M, Kaempf M (2001) Landfill capping with woodland ecosystems. Pract Period Hazard, Toxic, Radioact Waste Manag 5:175–184

Lim TT, Edwards DR, Workman SR, Larson BT, Dunn L (1997) Vegetated filter strip removal of cattle manure constituents in runoff. Trans ASAE 41(5):1375–1381

Matamoros V, Bayona J (2006) Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ Sci Technol 40:5811–5816

Mohedano R, Rejane HR, Costa FA, Tavares PBF (2012) High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. Bioresour Technol 112:98–104

Nagendran R, Selvam A, Joseph K, Chiemchaisri C (2006) Phytoremediation and rehabilitation of municipal solid waste landfills and dumpsites: a brief review. Waste Manag 26(12):1357–1369

Nixon DJ, Stephens W, Tyrrel SF, Brierley EDR (2001) The potential for short rotation energy forestry on restored landfill caps. Bioresour Technol 77:237–245

Nuñez Delgado A, López Periago E, Diaz-Fierros Viqueira F (1995) Vegetated filter strips for wastewater purification: a review. Bioresour Technol 51:13–22

O'Luanaigh ND, Goodhue R, Gill LW (2010) Nutrient removal from on-site domestic wastewater in horizontal subsurface flow reed beds in Ireland. Ecol Eng 36:1266–1276

Paulo PL, Azevedo C, Begosso L, Galbiati AF, Boncz MA (2013) Natural systems treating greywater and blackwater on-site: integrating treatment, reuse and landscaping. Ecol Eng 50:95–100

Payer FS, Weil RR (1987) Phosphorus renovation of wastewater by overland flow land application. J Environ Qual 16:391–397

Poulsen TG, Møldrup P (2005) Factors affecting water balance and percolate production for a landfill in operation. Waste Manag Res 23:72–78

Prescott JF, Baggot JD, Walker RD (2000) Antimicrobial therapy in veterinary medicine. Iowa State University Press, Ames

Preston GM, McBride RA (2004) Assessing the use of poplar tree systems as a landfill evapotranspiration barrier with the SHAW model. Waste Manag Res 22:291–305

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540

Reed SC, Crites RW, Middlebrooks EJ (2006) Natural wastewater treatment systems. CRC Press Taylor & Francis Group, Boca Raton, FL

Rizzo PF, Arreghini S, Serafini RJM, Bres PA, Crespo DE, Fabrizio de Iorio AR (2012) Remediation of feedlot effluents using aquatic plants. Rev Fac Cien Agric 44(2):47–64

Schroder P, Navarro-Aviñó J, Azaizeh H, Goldhirsh AV, DiGregorio S, Komives T, Langergraber G, Lenz A, Maestri E, Memon AR, Ranalli A, Sebastiani L, Smrcek S, Vanek T, Vuilleumier S, Wissing F (2007) Using phytoremediation technologies to upgrade waste water treatment in Europe. Environ Sci Pollut Res 14(7):490–497

Schwer CB, Clausen JC (1989) Vegetative filter treatment of dairy milkhouse wastewater. J Environ Qual 18(4):446–451

Shi W, Wang L, Rousseau D, Lens PNL (2010) Removal of estrone, 17α-ethinylestradiol, and 17β-estradiol in algae and duckweed-based wastewater treatment systems. Environ Sci Pollut Res 17(4):824–833

Simmons E (1990) From rubbish tip to wildlife reserve. Waste Manag 80(3):220–221

Simmons E (1999) Restoration of landfill sites for ecological diversity. Waste Manag Res 17:511–519

Song HL, Nakano K, Taniguchi T, Nomura M, Nishimura O (2009) Estrogen removal from treated municipal effluent in small-scale constructed wetland with different depth. Bioresour Technol 100:2945–2951

Srivastava J, Gupta A, Chandra H (2008) Managing water quality with aquatic macrophytes. Rev Environ Sci Biotechnol 7(3):255–266

Stottmeister U, Wießner A, Kuschk P, Kappelmeyer U, Kästner M, Bederski O, Müller RA, Moormann H (2003) Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol Adv 22:93–117

Thurston JA, Falabi JA, Gerba CP, Foster KE, Karpiscak MM (1996) Fate of indicator microorganisms, *Giardia*, and *Cryptosporidium* in two constructed wetlands. 5th International conference on wetland systems for water pollution control, Vienna, Poster 30-1

Tzanakakis VA, Paranychianakis NV, Angelakis AN (2009) Nutrient removal and biomass production in land treatment systems receiving domestic effluent. Ecol Eng 35(10):1485–1492

USEPA (2008) Onsite wastewater treatment systems technology fact sheet 7: stabilization ponds, FWS constructed wetlands, and other aquatic systems. US Environmental Protection Agency. http://www.epa.gov/nrmrl/pubs/625r00008/html/html/tfs7.htm. Accessed 24 July 2013

Valipour A, Kalyan Raman V, Ghole VS (2009) A new approach in wetland systems for domestic wastewater treatment using *Phragmites* sp. Ecol Eng 35(12):1797–1803

Vymazal J (2007) Removal of nutrients in various types of constructed wetlands. Sci Total Environ 380:48–65

Vymazal J (2009) The use constructed wetlands with horizontal subsurface flow for various types of wastewater. Ecol Eng 35:1–17

Wang L, Duggin JA, Nie D (2012) Nitrate-nitrogen reduction by established tree and pasture buffer strips associated with a cattle feedlot effluent disposal area near Armidale, NSW Australia. J Environ Manag 99:1–9

WHO (2012) Informe 2012 acerca de los progresos sobre el agua potable y saneamiento. World Health Organization. http://www.who.int/water_sanitation_health/monitoring/jmp2012/es/. Accessed 15 Apr 2013

Xu J, Shen G (2011) Growing duckweed in swine wastewater for nutrient recovery and biomass production. Bioresour Technol 102:848–853

Ye F, Li Y (2009) Enhancement of nitrogen removal in towery hybrid constructed wetland to treat domestic wastewater for small rural communities. Ecol Eng 35(7):1043–1050

Yousefi Z, Mashayekh Salehi A, Mohamadpur Tahamtan RA (2013) Efficacy of hybrid constructed wetland in removing plumb and cadmium from wastewater in the campus of Mazandaran University of medical sciences. J Mazandaran Univ Med Sci 23(97):258–269

Zedler JB, Callaway JC (1999) Tracking wetland restoration: do mitigation sites follow desired trajectories? Restor Ecol 7:69–73

# Part II

# Phytoremediation Using Plants, Microbial Assemblages, and Novel Applications in Soil and Water

# 6 Interaction Algae–Bacteria Consortia: A New Application of Heavy Metals Bioremediation

Ana Lucia Rengifo-Gallego
and Enrique Javier Peña Salamanca

## 6.1 Introduction

Environmental pollution has different origins; industrial activities, metal complexes and effluents, chemical discharges, and sediments are all results of human activities and discharges to rivers, sea, land, soil, air, and in several instances, contact with animals, plants, and humans (Karigar et al. 2011). In the search for biological agents and processes for bioremediation, metabolism pathways of bacteria are highly involved and are the keystone in microbial–algae interactions. Mechanisms as active transport efflux pumps, biotransformation, biomineralization, and intra- and extracellular sequestration, and the production of enzymes and membrane characteristics are parts in this complex web of interactions. Bioremediation and phytoremediation now appear as appealing technologies inasmuch as they are based on the use of living organisms, microorganisms, plants, and their enzymatic set (Karigar et al. 2011). In this chapter, we enlist different examples and recent research advances about algae–bacteria consortia. Additionally, we documented previous experiences in our laboratory working with algae–bacteria consortia for Chromium removal isolated from natural algal populations along the Pacific coast of Colombia (Peña-Salamanca et al. 2011).

A.L. Rengifo-Gallego, B.Sc., Biological. Sciences.
Ph.D. Student. (✉)
Departament of Biology, Biology of Plants and Microorganism Research Group, Universidad del Valle, Cali, Valle del Cauca, Colombia

Biology Department, Biology of Plants and Microorganism Research Group, Universidad del Valle, Bogota, Cundinamarca, Colombia
e-mail: anarengifog@gmail.com

E.J.P. Salamanca, Ph.D.
Department of Biology, Universidad del Valle, Calle 13 N° 100-00, Cali, Valle, Colombia

## 6.2 Algae–Bacteria Interactions

Algae–bacteria interactions, wherein phytoplankton may represent a microhabitat for aquatic bacteria, were first studied by Cole (Cole 1982). In a schematic form, the interactions occurring in the phycosphere are principally bacterial metabolism products and chelators with algae cells, and allelopathic substances from algae to bacteria. Algae can present exosymbionts and endosymbionts; planktonic bacteria attach and develop chemotaxis with them. Natural processes such as *lysis*, excretion, and mechanical damage provide nutrients (and substances) to the growth media that contribute in the same measure (In uptake form) to Planktonic bacteria. However, additional processes including enzymatic attack and dissolution are also found in this web of interactions. At present, environmental bioremediation has been used to explore the bacterial consortia role in many different ways. For example, in contaminants such as BTEX (Littlejohns and Daugulis 2008), Crude oil (Tang et al. 2010; Rahman et al. 2002), hydrocarbonates (Diesel) (Richard and Vogel 1999), PAHs (Vázquez et al. 2013), Phenol (Ambujom 2001), Nitrates (Rajakumar et al. 2008), organophosphate pesticides (Yañez-Ocampo et al. 2009), TNT (trinitroaromatic compounds; (Robertson and Jjemba 2005), and Toxic and heavy metals (Singh et al. 2012; Johnson et al. 2007), many bioremediation processes are in evaluation.

Several genera such as *Achromobacter* (*A. anthropic* Richard and Vogel 1999), *Acinetobacter* sp. (*A. baumannii* Kim et al. 2009, *A. johnsonii* Robertson and Jjemba 2005), *Alcaligenes* sp. (Rajakumar et al. 2008), *Arthrobacter* sp. (Iwahashi et al. 2003), *Bacillus* (sp. YW4, *B. subtilis*, *B. cereus* Rajakumar et al. 2008), *Brevundimonas* (Vázquez et al. 2013), *Burkholderia* sp., *Buttiauxella* (*B. Izardii* strain MHF ENV 19), *Clostridium* sp. *Klebsiella* (*K. pneumonidae*, *K. oxytoca* Kim et al. 2009), *Enterobacter* (*E. agglomerans*), *Escherichia* (*E. coli*), *Hydrogenophaga* sp., *Pseudomonas* (sp. KW1, *P. mendocina*, *P. aeruginosa*, *P. putida*, *P. denitrificans*, *P. fluorescens III*, *P. stutzeri*, *P. gessardii*, *P. migulae T*, *P. lini*

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T, *P. frederiksbergensis*, *P. fragi T*, *P. paucimobilis*, *P. vesicularis*, *P. capacia* Ambujom 2001), *Rhodococcus* (Ambujom 2001), *Pedobacter* (*P. piscium*), *Sphingomonas* (*S. paucimobilis* Wang et al. 2004), *Stenotrophomonas* (*S. rhizophila*, *S. maltophilia* Kim et al. 2009), *Streptomyces* (Ambujom 2001), and *Xanthomonas* (*X. maltophilia* Wang et al. 2004), are involved with a high level of efficiency.

In the same way, bacterial–microalgal and bacterial–fungal consortiums (e.g., *Ceriporiopsis subvermispora* and bacterium *Cellulomonas* sp., *Azospirillum brasilense*; including artificial consortiums—*Scenedesmus obliquus* GH2 and Sphingomonas GY2B and *Burkholderia cepacia* GS3C, Pseudomonas GP3A and *Pandoraea pnomenusa* GP3B (Tang et al. 2010)), have been treated with different environmental contaminants and have contributed valuable information regarding the development of systems from matrices recovery (land and water).

Ahemad (2012) describes seven mechanisms of microbial cell and heavy metal interaction: Biotransformation, Bioaccumulation, Biomineralization, Biosorption, Bioleaching, Biodegradation of chelating agents, and microbially enhanced chemisorption of metals. According to Cole (1982) indirect interactions with the host bacteria occur in the phycosphere as endosymbiosis processes.

### 6.2.1 Algae–Bacteria Consortiums: Taxa, Characteristics, and Biochemistry

The algae–bacteria consortia are being studied recently with details in selected groups of bacteria (Taxa). Goecke et al. (2013), using phylogenetic studies, determined at species level what taxa are involved in important interactions between algae and bacteria. They defined algae as an important environment for bacteria, and distributed them among six bacterial phyla: Bacteriodetes (42 spp.), Proteobacteria (36 spp.), and Firmicutes, Actinobacteria, Verrumicrobia, and Planctomycetes (23 spp.). They concluded that the species and strains carry out similar metabolic functions and then colonize similar algae taxa and groups. Additionally, Alphaproteobacteria in the Roseobacter clade could to be the most adapted because it is the most widely reported and described around microalgae in relation to phytoplankton.

In a study case, Beceiro-González et al. (2000) showed the interaction between metallic species and biological substrates; they started with *Chlorella vulgaris* and arsenic(III) and determined that the algae has the capacity for selective separation of As(III). phosphate and nitrates showed chemical similarity to arsenate and arsenate. Compounds play an important role in the interaction mechanisms. The authors demonstrated that *C. vulgaris* and As(III) have two interactions in the bigger process: As(III) retention process and As(III) transformation process. the first one presents two compounds: Absorption (transport across the membrane) and Adsorption (Binding to the cell-wall surface). The second one, Absorption (Oxidation into the cell and subsequent expulsion, only when the cell is alive) and Adsorption (oxidation in the cell–wall surface in cells with and without). 50 % of As remains in *C. vulgaris;* 25 % exists as retained by the algae. The total As(III) separation by *C. vulgaris* turns out to be higher than that proposed, and would be approximately 75 %. This kind of work helps us to understand the importance of bacteria contributions to the algae process, as Cole showed in his review; different strategies between allelophatic, metabolic secondaries and chemotactic substances are involved in the retro alimentation metabolism in algae. Bacteria can assimilate to different ions and contaminants, and change the structure to convert them in other substances. Iqbal et al. (1993) worked with a unicellular red algae *Porphyridium cruentum* and the interactions with strains of bacteria living in the capsule: this study showed the importance of the presence of bacteria in the production of polysaccharide (inhibitory effects depending from strain). *P. cruentum* confirmed that the most important factor for bacterial growth within the algal cultures was their extracellular polysaccharide and that there was no competition between algae and bacteria for any other nutrient present in the medium; coexisting bacteria did not affect growth and starch production of the algal member but did increase biomass accumulation. In conclusion, they proved that algal productivity (growth and polysaccharide production) is independent of bacteria. In contrast, bacteria are dependent for growth on *P. cruentum.*

In 2009, Nakajima et al. studied an ecological mechanism that evolved in an endosymbiotic association, and conducted a long-term microcosm culture with the stages of the ecosystem development using *Chorella vulgaris*, *Eschericha coli,* and *Tetrahymena thermophila* for a period of three years. In their study, they showed experimental evidence that an auto-/heterotrophic endosymbiosis evolves in a mature stage of ecosystem development through the advantage of efficient material/energy transfers among participating organisms and proximity.

The autonomous dynamics of ecosystem development favored the association, not the experimental operation. Hollants et al. (2013) pointed out a host specificity and coevolution of Flavobacteriaceae endosymbionts within *Bryopsis*; they examined 128 green algal samples of Flavobacteriaceae endosymbionts, including 146 *Bryopsis* samples covering 23 different species, and 92 additional samples of Bryopsidales (genera *Avrainvillea*, *Boodleopsis*, *Caulerpa*, *Chorodesmis*, *Codium*, *Derbesia*, *Halimeda*, *Rhipilia*, *Tydemania,* and *Udotea*), Dasycladales (*Acetabularia*, *Bornetella,* and *Neomeris*), Cladophorales (*Aegagropila*, *Anadyomene*, *Apjohnia*, *Boergesenia*, *Boodlea*, *Chaetomorpha*, *Cladophora*, *Cladophoropsis*, *Dictyosphaeria*, *Ernodesmis*, *Microdyction*, *Rhizoclonium*, *Siphonocladus,* and *Valonia*), and Ulvales (*Ulva*).

They provide strong evidence for a non random association between *Bryopsis* and its Flavobacteriaceae endosymbionts, whereby more closely related host species predominantly harbor genetically similar endosymbiosis, suggestive of coevolution; they recognized that this physiological ground remains unknown and proposed the possibility that Flavobacteriaceae endosymbionts offer the algal host an adaptive advantage. Other important endosymbiosis was investigated recently; Liang et al. (2013) studied a new algae–bacteria interaction, a combined system of *Chlorella vulgaris* and *Bacillus licheniformis*. They demonstrated that the system achieved better removal efficiencies of $NH_4^+$ and TP than a single algae or bacteria system did. *B. licheniformis* could promote the growth of *C. vulgaris* in the combined system. They examined pH and Chl *a*; when pH was reduced to acid from neutral, the removal efficiency of both substances was higher (together) with the same behavior in Chl *a*. In conclusion, all this indicates that an algae–bacteria combined system has potential in nutrient removal from wastewater and pH control is crucial in the process. In wastewater artificial treatment, Karya et al. (2013) proposed a Photo-oxygenation to support nitrification in an algal–bacterial consortium in a mixed culture of *Scenedesmus* and nitrifiers; without aeration an open photo bioreactor can be maintained, for the microorganism culture achieved 100 % nitrification, algae produce oxygen at a rate of 0.46 kg/m$^3$/day (which is higher than in highest rate in algae or stabilization ponds), the maximum ammonium conversion rate was 7.7 mg/L h, the influent alkalinity was 400 mg $CaCO_3$/L, and on either side of the reactor a light intensity of ±60 μmol/m$^2$s, a mass balance showed that ammonium removal was mainly by nitrification (81–85 %) rather than by uptake for algae growth.

Tang et al. (2010) showed the construction of an artificial microalgal–bacterial consortium that efficiently degrades crude oil, using *Scenedesmus obliquus* GH2, *Sphingomonas* GY2B, *Burkholderia cepacia* GS3C, and a mixed culture GP3 (*Pseudomonas* GP3 and *Pandoraea pnomenusa* GP3B). For the consortium construction, bacteria combined with a unialgal culture. The algae showed different effects on oil degradation, signaling a possible reason for these advantages. Degradation properties of mixed cultures versus pure cultures may produce some extracellular matter that can inhibit the activity of the inoculated microbes. In the same way, another possibility is that the intermediate products produced by the different degradation mechanism for the same carbon source by different strains could inhibit the degradation ability of other strains. They demonstrated that the combination of four oil component-degrading bacteria combined with axenic *S. obliquus* GH2 produced a highly efficient, broadly hydrocarbon-utilizing artificial microalgal–bacterial consortium. In 1999, Safonova et al., using a collection of algal strains, tested the ability of growth in a mineral media (1 % of black oil): alcanotrophic bacteria were stimulated to grow, and in consequence bacteria stimulated the algae growth. *Phormidium* sp. ES-19 stimulates cell growth in *Stichococcus minor* ES-19. This growth stimulation was observed in 17 strains and was found not only for the *Chlorella* sp. ES-3m-2 strain. The Natural association of algae (AS-45 and AS-47) with the addition of *Rhodococcus* sp. 7HX and artificial association of *Kirchneriella obese* ES-60 with alcanotrophic bacteria D1-7 destroyed the black oil more efficiently in comparison with *Rhodococcus* sp. 7HX; a total of 15 algae genera were tested and in the presence of bacteria, the number of algal strains with a high resistance level increased, and was found to restore the growth of sensitivity to black oil algae strains and to stimulate growth in tolerant strains. the representatives of Chlorophyceae and Cyanophyceae were most tolerant to black oil, whereas the majority of Xantophyceae were sensitive.

Tujula et al. (2010) examined the variability and abundance of the epiphytic bacterial community associated with *Ulvacean* alga; quantification of the *Alphaproteobacteria*, *Gammaproteobacteria,* and *Bacteroidetes* showed that the first one comprised on average 70 % of the cells in the microbial community. four distinct morphotypes were noted; the average proportion of the third-mentioned cells was 13 % and very few of the second-mentioned cells (<1 %) were detected in this study. The marine–alphaproteobacterial lineage, which includes *Roseobacter* (12 %), was selected as it was represented by many of the DGGE (denaturing gradient gel electrophoresis) band sequences determined; the consistent detection of these sequences indicates that this sub population may have an important role in the function of the bacterial community on *U. australis* (marine bacteria sessile host interactions). The key members of the epiphytic community (from DGGE analysis) were affiliated with *Alphaproteobacteria* and the *Bacteriodetes* and were related to the importance of bacteria for the normal morphological growth of *Ulva*. Many bacteria were observed in microcolonies in association with the algal intracellular cell-wall junctions (data not shown) and were related to the accumulation of nutrients in the depression between cells on the algal surface. In Addition, *Alphaproteobacteria* was the most abundant group on the surface of *U. australis* but it is difficult to relate that dominance with functional phenotypes, because they are morphologically and metabolically extremely diverse. Admiraal et al. (1999) worked with microbenthic algae and bacteria in a metal–polluted stream, examining short-term toxicity of Zinc; colonized glass-discs and samples of natural assemblages on coarse sand were used to test zinc tolerance. This parameter was characterized by measuring inhibition of $^{14}$C-incorporation in microalgae and inhibition of $^{3}$H-thymidine incorporation in bacteria. Algae from the strongly polluted site were only slightly affected by the highest test concentrations of zinc, in contrast to other algal communities. The high resistance shown by the community in extreme pollution levels, suggests a strong degree of resilience, part of that in micro- and macrobenthic communities to metal stress. It could be due to inorganic waste, providing material for the sorption and chelation of metal ions. Alternatively, the input of this organic waste may also stimulate heterotrophs to secrete protective mucus or detoxify metals to such a degree that benthic organisms could survive in this habitat. Table 6.1 summarizes the algal–bacterial species and Interaction levels.

#### 6.2.1.1 Bacterial Taxa Characteristics

Table 6.2 summarizes the most important characteristics of bacteria taxa implicated in interaction-type consortiums with algae species. The majority belong to marine bacteria, and most of them are part- or entero-bacteria groups, and physically are gram-positive and gram-negative with a short advantage from this last group. The major descriptions about this taxa explain the extreme media, habitat or niche, where these bacteria can live. In a recent investigation, most bacteria found in associations of this type belong to *Alphaproteobacteria* and Roseobacter clade, *Proteobacteria,* and *Firmicutes*.

#### 6.2.1.2 Types of Methodologies to Taxa Identification and Algae–Bacteria Interaction

Table 6.3 summarizes the most common methodologies used to understand algae–bacteria interactions. It is common to choose strains directly from labs and banks of strains; in those cases the experimentation is designed with artificial experiments and long time periods, but when the type and taxon of bacteria are unknown, it uses 16S rRNA sequences and DGGE and then makes comparisons in GeneBank, generally.

### 6.2.2 Interaction Between *Bostrychia calliptera* and Bacterial Consortia

#### 6.2.2.1 Experimental Description and Important Results

In order to determine the effect of algae–bacteria association in the chromium bioaccumulative process in Buenaventura Bay, bacterial populations associated with *Bostrychia calliptera* from the mouth of the Rio Dagua, Colombia (3° 51′39.3″ N; 77° 03′ 56.7″ W and 3° 51′50″; N 77° 04′07.9″) were monitored in vitro. Specimens of algae were obtained from material adhering to the pneumatophores of *Avicennia germinans* (Verbenaceae) and *Rizophora mangle* (Rhizophoraceae). Tests were conducted in synthetic seawater with two levels of chromium, 5 and 10 ppm, using bioreactors (125 mL Erlenmeyer flasks) with artificial seawater, in four treatments including unprocessed plant material (Algae–Bacteria), antibiotic plant material (algae), sediment and/or suspended material from the algae surface (Bacteria) and a control without the presence of *B. calliptera* or bacteria (White). The experimental design was a two-factor factorial model with repeated measures on one factor. We monitored the behavior of microbial populations and the rate of decrease in the concentration of chromium in ppm, using plaque counts and atomic absorption spectroscopy (AAS), respectively (Rengifo-Gallego et al. 2012).

Significant differences were obtained for the bacteria population to the total concentration of chromium in the systems Algae–bacteria and bacteria, with the Algae–bacteria being the most efficient system in the degradation of the chromium concentration to 5 ppm and more effective in the treatment bacteria at 10 ppm. From the results, it was concluded that there is a possible positive interaction between the bacteria associated with the surface of the red alga *B. calliptera* in the process of accumulation of chromium in environmental levels and greater efficiency of metal degradation in the bacteria system at higher levels in ex situ conditions (Rengifo-Gallego et al. 2012).

#### 6.2.2.2 Discussion

In evaluating the chromium percentage removal in the algae–bacteria association with two chromium concentrations, bacterial growth appeared to be different in both metal concentrations examined; the microbial population was higher in the second concentration (10 ppm) and in the rate of chromium removal. this was approximated at both concentrations (62.85 % and 68.55 % at 5 to 10 ppm, respectively) at the same value. This would mean, for the first case, that under natural conditions, the selection pressure on the bacterial consortium rests on the same concentration of the contaminant and while controlling—in in vitro conditions—the concentration of bacterial cells per milliliter and the second, any similarity in the percentage of removal is supported by the results found by Acosta et al. (2005). They indicate that in the case of the *Cryptococcus neoformans* and *Helminthosporium* sp. Fungus, the metal removal rate in the hexavalent state is not dependent on the total concentration in the medium but on the innate ability of the species; in this case both the alga *B. calliptera* as the bacterial consortium have limited absorption capacity of up to 4.5 ppm in liquid medium for 192 h (Rengifo-Gallego et al. 2012).

The results allow us to observe that an Alga-Bacterium system has higher system efficiency regarding an Alga–Antibiotic system, indicating that the bacteria associated with *B. calliptera* possess participation in the bioaccumulative process of the algae, given that the percentage reduction of chromium is higher in Algae systems than in bacteria (87 % at 10 ppm) in bacteria-free systems, such as seaweed antibiotic (65.3 %, 10 ppm). Therefore, the findings suggest the existence of a possible positive interaction between the

**Table 6.1** Comparative scheme, algal–bacterial species and interaction levels

| Alga sp. | Bacteria sp. | Interaction typology | Interaction level | Reference |
|---|---|---|---|---|
| Microalgae<br>Phytoplankton<br>Macroalgae | Bacteriodetes (42 spp.), Proteobacteria (36 spp.) and firmicutes, Actinobacteria, Verrucomicrobia, and Planctomycetes (23 spp.)<br>Roseobacter clade (Alphaproteobacteria)<br>32 % of Roseobacter clade spp. | Similar metabolic functions were carried out and found to colonize similar algal taxa or algal groups.<br>Adapts for life in close association with phytoplankton.<br>Able to decompose macroalgal polysaccharides. | Endosymbiosis/ectosymbiosis. | Goecke et al. (2013) |
| *Chlorella vulgaris* | Algae–bacteria | Accumulation of arsenic species: metallic speciation or metallic. | Ectosymbiosis | Beceiro-González et al. (2000) |
| *Porhyridium cruentum* | Associated bacteria<br>Achromobacter, Arthrobacter, Microbacterium, or Cellulomonas/Oerskovia | Some specificity for their sugar requirements, constituents of extracellular polysaccharide.<br>Theoretically, bacteria in certain combinations have some effect on growth and polysaccharide production; they are unable to change the rheological property of the polysaccharide. | Bacteria living in the capsule of the algae (examined in batch culture)–Symbiosis | Iqbal et al. (1993) |
| *Chlorella vulgaris* | *Escherichia coli*, and *Tetrahymena thermophilia* (ciliated protozoan) | Direct/indirect interactions among ecosystem components led to a reduction in dissolved $O_2$ and food (*E. coli*) available to the *T. thermophile*, which gave a selective advantage to the organism in the endosymbiotic association. | Endosymbiosis. | Nakajima et al. (2009) |
| Ulvacean<br>*Ulva australis* | Alphaproteobacteria (70%)–*Roseobacter clade*<br>Bacteroidetes (13 %) | Microbial community of seaweed surface; members of both taxa are part of this stable sub-population, and are likely to have an important role in function of this. | Symbiosis–Ecological relations, enter microbial community, subgroups. | Tujula et al. (2010) |
| *Chlorella vulgaris* | *Bacillus licheniformis* | 78 % of $NH_4^+$ could be removed in the combined system, 29 % in a single algae system, and only 1 % in a single bacteria system. Approximately 92 % of TP was removed in the combined system, compared with 55 % and 78 % in the single algae and bacteria system, respectively.<br>*B. licheniformis* was proven to be a growth-promoting bacterium for *C. vulgaris* by comparing Chl *a* concentration in the single and combined systems. | Endosymbiosis. | Liang et al. (2013) |
| Microbenthic algae: *Synedra acus* Kützing *Gongrosira* sp., especially *Stigeoclonium* cf *tenue* Kützing, while other algae were rare<br>Green algae: *Stigeochonium tenue*<br>*Chironomus riparius* | Microbenthic bacteria | Assembles of microalgae and bacteria colonizing glass discs suspended in river water and natural river sands were both suitable for testing the short-term toxicity of metals. The response to zinc was measured effectively using $^{3}$H-thymidine incorporation by bacteria, but $^{14}$C-$HCO_3$ incorporation by microalgae was inhibited only at high test concentrations.<br>Assemblages of in situ bacteria growing under high zinc and cadmium concentrations proved to be more resistant to zinc in short-term exposures indicating selection for tolerant life forms.<br>Toxic effects of zinc (and cadmium) are prevented by metal binding in the biofilm, stimulated by the simultaneous pollution with organic waste water and precipitation of iron in dense layers of cells, mucus, and detritus. | Ectosymbiosis (no specified)–Biofilm | Admiraal et al. (1999) |

(continued)

**Table 6.1** (continued)

| Alga sp. | Bacteria sp. | Interaction typology | Interaction level | Reference |
|---|---|---|---|---|
| *Stichococcus minor* ES-19<br>*Chorella* sp. ES-3, ES-27, ES-30<br>*Scenedesmus quadricauda* ES-59, ES-79, ES-80<br>*Nostoc* sp. ES-79-2, U-15/3<br>*Phormidium* sp. ES-90, 0-b-1 | Alcanotrophic bacteria<br>*Rhodococcus* sp. 7HX<br>*Kirchneriella obessa* ES-60 + alcanotriphic bacteria DI-7<br>*Scenedesmus,* abundant in associations AS-45, AS-47 (*Phormidium*) | The presence of alcanotrophic bacteria was found to restore reproduction in algae sensitive to black oil, and stimulate cell growth in tolerant algal strains.<br>Combinations of alcanotrophic bacteria with certain algal strains that tolerate elevated amounts of black oil demonstrated maximum efficiency of oil destruction. | Algae–alcanotrophic bacteria interaction. | Safonova et al. (1999) |
| *Scenedesmus obliquus* GH2 | *Sphingomonas* GY2B<br>*Burkholderia cepacia* GS3C<br>*Pandoraea pnomenusa* GP3B | For construction or the consortium, bacteria combined with unialgal or axenic algae showed different effects on oil degradation. Unialgal GH2 was not cooperative with GS3C and GP3. However, the bacterial strain co cultured with axenic GH2 enhanced the degradation of crude oil.<br>The combination of four oil component-degrading bacteria combined with axenic *S. obliquus* GH2 produced a highly efficient, broadly hydrocarbon-utilizing, artificial microalgal–bacterial consortium that is capable of crude oil degradation. | Artificial microalgal–bacterial consortium. Extracellular interaction | Tang et al. (2010) |
| *Scenedesmus quadricauda* | Nitrifying bacteria | Oxygenation by photosynthesis in a mixed culture of nitrifiers was investigated for its potential to support nitrification.<br>A mixed culture of algae and nitrifying micro organisms can be maintained in a photo bioreactor fed with artificial wastewater (50 mg/L $NH_4^+$-N) and achieve 100 % nitrification, without aeration. | Artificial algal–bacteria consortium treating artificial wastewater | Karya et al. (2013) |
| *Bryopsis* | *Flavobacteriaceae* | Flavobacteriaceae endosymbiosis, restricted to *Bryopsis*, is a complex host–symbiont evolutionary association, whereby the closely related host predominantly harbors genetically similar endosymbiosis.<br>Bacterial genotypes are rarely confined to a single *Bryopsis* species and most *Bryopsis* species harbored several Flavobacteriacea, obscuring a clear pattern of coevolution. | Evolutionary endosymbiosis | Hollants et al. (2013) |

**Table 6.2** Bacterial taxa descriptions

| Taxon | Type of bacteria | | | |
|---|---|---|---|---|
| | Entero bacteria | Marine bacteria | Gram-positive | Gram-negative |
| Bacteriodetes | X | X | | X |
| Proteobacteria | X | X | | X |
| Firmicutes | X | e.g., *Lactobacillus* sp., *Clostridium* sp. | Mayor | Without cell wall |
| Actinobacteria | | | X | |
| Verrucomicrobia | X | X | | X |
| Planctomycetes | | X | | No peptidoglycan |
| Alphaproteobacteria | | X | | Major |
| Roseobacter clade (Alphaproteobacteria) | | X | | X |
| *Achromobacter* sp. | X | X | | X |
| *Arthrobacter* sp. | | | X | |
| *Microbacterium* or Cellulomonas/ Oerskovia | | | X | |
| *Escherichia coli* | X | X | | X |
| *Bacillus licheniformis* | | | X | |
| *Rhodococcus* sp. | | X | X | |
| *Sphingomonas* | | X | | X |
| *Burkholderia cepacia* | X | | | X |
| *Pandoraea pnomenusa* | | | | X |
| *Flavobacteriaceae* | | X | | X |

bacterial consortium found in the *B. calliptera* surface and the chromium reduction process, as demonstrated in the work of Arias and Tebo (2003), Cervantes (1991), Kwak et al. (2003), and Paul (2004).

At a higher than normal concentration (*normal for natural bacteria habitat* 10 ppm) the Algae–bacteria system is more efficient than the CBN system, allowing us to identify the importance of bacterial presence on the surface of algae, compared to the levels of degradation presented in an isolated algae and bacterial consortium. Bacterial concentration was higher in algae-free media (CBN combination) on the algae surface, likewise the number of morphotypes observed, thus allowing the identification of the importance of these microorganisms in terms of the algae life cycle, as was observed by Tujula et al. (2010) in *Ulva*.

Bacteria with the ability to reduce chromium (VI) to Cr(III) (Cervantes 1991; Arias and Tebo 2003; Kwak et al. 2003) and bio adsorb (Cabrera et al. 2007; Rabbania et al. 2005), we could consider that in the Algae–bacteria system, bacteria can act as a mediator in the reduction process, changing the oxidation state of the Cr (VI) to Cr (III), facilitating seaweed biosorption (Acosta et al. 2005); it was also determined that the Bacteria system was able to bio adsorb chromium, confirming the observations of Lee et al. (2006) and a reduction in the capacity of the metal is suspected, inasmuch as authors including Zhu et al. (2008) report the reduction of chromium by various bacterial groups

Fude et al. (1994) reported the formation of an associated amorphous precipitate surface bacteria, that was identified as related metal precipitates to the reduction process; this feature was observed as gray clusters for efficiency testing and would be related to extracellular reduction of Chromium. According to Shen and Wang (1993), Cr (III) formed by *Escherichia coli* ATCC 33456 from the reduction of Cr (VI) probably adheres to the surface of cells, indicating that previous studies showed that when the Cr (VI) is reduced to Cr (III), Cr (III) cannot be removed intracellularly when the cell membrane remains intact. Aaseth et al. (1982), allowed us to reinforce the deduction that the chromium reduction in this case could be presented extracellularly.

#### 6.2.2.3 Conclusions

There is a positive interaction between the red alga *Bostrychia calliptera* and bacterial consortium probably associated with its surface as a biofilm, with the capacity for Bio-adsorption/reduction of Cr (VI) within a period of 7 days (89.91 %) in vitro.

The chromium removal efficiency was 87 % for Algae-bacteria (AB) to 10 ppm, 68.55 % for natural bacterial consortium (CBN) to 10 ppm, 65.3 % for Alga–antibiotic 10 ppm, and 62.85 for CBN to 5 ppm in 7 days, demonstrating a higher removal efficiency of Algae–bacteria consortia. In contrast we found a removal efficiency of 89.91 % (in 12 days) for a selected bacterial consortium to 7 ppm.

These results suggest further studies to evaluate the effectiveness of this consortium for heavy-metal removal processes. The study also confirms the role of this consortium in the process of reducing Chromium for biotechnological applications.

**Table 6.3** Common methodologies used to understand algal–bacteria interactions

| Algal sp. | Bacteria sp. | Methodology<br>Identification taxa | Interaction study | Reference |
|---|---|---|---|---|
| Microalgae<br>Phytoplankton<br>Macroalgae | Bacteriodetes (42 spp.), Proteobacteria (36 spp.) and firmicutes, Actinobacteria, Verrucomicrobia, and Planctomycetes (23 spp.)<br>Roseobacter clade (Alphaproteobacteria) 32 % of Roseobacter clade spp. | 16S rRNA sequences—GeneBank. | None. | Goecke et al. (2013) |
| *Chlorella vulgaris* | Algae–bacteria | None. | HPLC-HG-AAS. | Beceiro-Gonzáles et al. (2000) |
| *Porhyridium cruentum* | Associated bacteria Achromobacter, Arthrobacter, Microbacterium, or Cellulomonas/ Oerskovia | Culture Collection for Algae and Protozoa (CCAP), Cambridge, England. | Turbidity measurements, Viscosity (Cannon-Fenske viscometer), Antibiotics (ampicillin, erythromycin, cloxacillin, and methicillin), Photobioreactors.<br>HPLC.<br>TEM (Transmission electron microscopy). | Iqbal et al. (1993) |
| *Chlorella vulgaris* | *Escherichia coli* and *Tetrahymena thermophilia* (ciliated protozoan) | None. | Mixed cultures, microcosm—microscope observations, DO. | Nakajima et al. (2009) |
| Ulvacean. *Ulva australis* | Alphaproteobacteria (70 %)-Roseobacter clade<br>Bacteroidetes (13 %) | 16S rRNA—FastDNA spin kit for soil (QBiogene, Montreal, QC, Canada).<br>PCR and DGGE (Denaturing gradient gel electrophoresis).<br>CARD-FISH (catalyzed reporter deposition fluorescence in situ hybridization). | Seasonal sampling and comparison of microbial communities (Summer, Autumn, Winter and Spring); Comparison card-fish bacterial groups with total number of SYBR Green II-stained cells of surface of *Ulva australis*. | Tujula et al. (2010) |
| *Chlorella vulgaris* | *Bacillus licheniformis* | None. *C. vulgaris* (FACHB-6) was obtained from freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. *B. licheniformis* was obtained from Hunan Institute of Microbiology. | Algae–bacteria combined system in a 6-days experiment. Increase/decrease of chlorophyll *a* concentration, in the single and combined systems. pH control (to neutral, natural tendency to acid). Assay of $NH_4^+$, TP removal ability. | Liang et al. (2013) |

| | | | | |
|---|---|---|---|---|
| Microbenthic algae:<br>*Synedra acus* Kützing<br>*Gongrosira* sp., especially<br>*Stigeoclonium* cf *tenue* Kützing, whereas other algae were rare<br>Green algae:<br>*Stigeochonium tenue*<br>*Chironomus riparius* | Microbenthic bacteria: No taxa id. | None. Quadruple samples of epipsammon and epilithon were examined in a 1-mL counting chamber under an epifluorescence microscope to count the numbers of microalgal cells. Cell counts per microscope field were expressed per mL sand or $cm^{-2}$ glass surface.<br>Microbenthic activity: Radionuclides were measured in a TRI-CARB 1600 TR liquid scintillation (Packard, US) analyzer as counts per minute. | Colonized glass-disc and samples of natural assemblages on coarse sand were used to test zinc tolerance. Tolerance was characterized by measuring inhibition of $^{14}C$-incorporation in microalgae and inhibition of $^{3}H$-thymidine incorporation in bacteria.<br>*Experimental Scheme*<br>1. Testing the activity of communities on sand glass exposed to (1) light or dark, (2) addition of 1 mg $L^{-1}$ pf $FeCl_2$ solution 2 h before the activity measurement (to examine the potential role of iron bacteria), and (3) site water or aged upstream water.<br>2. Testing the activity of communities exposed to a geometric concentration series of zinc (3.2–1,000 μM) in aged upstream water. Communities were pre-exposed for 2 h to zinc before the activities were measured. | Admiraal et al. (1999) |
| *Stichococcus minor* ES-19<br>*Chorella* sp. ES-3, ES-27, ES-30<br>*Scenedesmus quadricauda* ES-59, ES-79, ES-80<br>*Nostoc* sp. ES-79-2, U-15/3<br>*Phormidium* sp. ES-90, 0-b-1 | Alcanotrophic bacteria<br>*Rhodococcus* sp. 7HX<br>*Kirchneriella obessa* ES-60 + alcanotriphic bacteria DI-7<br>*Scenedesmus,* abundant in associations AS-45, AS-47 (*Phormidium*) | Collection of algal strains tested for the ability to grow in a mineral medium containing 1 % black oil. | Pure and mixed culture, with standard and alcanotrophic bacterial strains. | Safonova et al. (1999) |
| *Scenedesmus obliquus* GH2 | *Sphingomonas* GY2B<br>*Burkholderia cepacia* GS3C<br>*Pandoraea pnomenusa* GP3B | None. *Scenedesmus obliquus* GH2 was isolated for surface water of Huangpu Port (Guangzhou, China) and deposited in the China Center from Type Culture Collection (CCTCC No. M 209253).<br>From their lab were obtained *Sphingomonas* GY2B (CCTCC No. 206019), *Pseudomonas* GP3 and *Pandoraea pnomenusa* GP3B (here referred to as GP3) (CCTCC No. M 207166 and M 207167), *Bulkholderia cepacia* GS3C (CCTCC No. 207169). | Biodegradation potential was studied in microalgal and bacterial culture (single and mixed). An analysis of oil degradation was carried out by classifying the major oil components into groups based on related structures. | Tang et al. (2010) |

(continued)

**Table 6.3** (continued)

| Algal sp. | Bacteria sp. | Methodology: Identification taxa | Methodology: Interaction study | Reference |
|---|---|---|---|---|
| *Scenedesmus quadricauda* | Nitrifying bacteria | None. *Scenedesmus quadricauda* was obtained from the laboratory of UNESCO-IHE, Delft (The Netherlands). An enriched culture of nitrifying bacteria was obtained from activated sludge of the Harnascholder Wastewater Treatment Plan (Delft, The Netherlands). | Using Photo Bioreactors. Chlorophyll a determined to Dutch standard method NEN 6472 and 65220, Nitrite ($NO^{-}_{2}$-N), total suspended solids (TSS), and volatile suspended solids (VSS) were analyzed according to standard methods for the examination of water and wastewater. Nitrate ($NO^{-}_{3}$-N) analyzed on a Dionex ICS-100, Total nitrogen (TN) was determined according to NEN 6472. | Karya et al. (2013) |
| Flavobacteriaceae | *Bryopsis* | 16s rRNA, optimized PCR protocol to directly and specifically amplify Flavobacteriaceae. They specifically analyzed the PCR protocol and exclusively amplify Flavobacteriaceae endophytic sequences in non-surface sterilized, natural *Bryopsis* samples. | 238 green algal samples were screened for presence of Flavobacteriacea endosymbionts, including 146 *Bryopsis* samples covering different species, and 92 additional samples of Bryopsidales and Ulvales. | Hollants et al. (2013) |

## References


Aaseth J, Alexander J, Norseth T (1982) Uptake of 5Cr-chromate by human erythrocytes—a role of glutathione. Acta Pharmacol Toxicol 50:310–315

Acosta M, Moctezuma-Zárate G, Gutiérrez C, Rodríguez X (2005) Bioadsorción de Cromo (VI) en Solución Acuosa por la Biomasa Celular de Cryptococcus neoformans y Helminthosporium Sp. Inf Tecnol 16(1):11–15

Admiraal W, Blanck H, Buckert-de Jong M, Guasch H, Ivorra N, Lehmann V, Nystrom BAH, Paulsson M, Sabater S (1999) Short-term toxicity of Zinc to microbenthic algae and bacteria in a metal polluted stream. Water Resour 33(9):1989–1996

Ahemad M (2012) Implications of bacterial resistance against heavy metals in bioremediation: a review. IIOAB J 3(3):39–46

Ambujom S (2001) Studies on composition and stability of a large membered bacterial consortium degrading phenol. Microbiol Res 156:293–301

Arias Y, Tebo B (2003) Cr(VI) reduction by sulfidogenic and nonsulfidogenic microbial consortia. Appl Environ Microbiol 69(3):1847–1853

Beceiro-Gonzáles E, Taboada-de la Calzada A, Alonso-Rodríguez E, López-Mahía P, Muniategui-Lorenzo S, Prada-Rodríguez D (2000) Interaction between metallic species and biological substrates: approximation to possible interaction mechanism between the alga Chlorella vulgaris and arsenic(II). Trends Anal Chem 19(8):475–480

Cabrera G, Viera M, Gómez J, Cantero D, Donati E (2007) Bacterial removal of chromium (VI) and (III) in a continuous system. Biodegradation 18:505–513

Cervantes C (1991) Bacterial interactions with chromate. Antonie Van Leeuwenhoek 59:229–233

Cole J (1982) Interactions between bacteria and algae in aquatic ecosystems. Annu Rev Ecol Syst 13:291–314

Fude L, Harris B, Urrutia M, Beveridge T (1994) Reduction of Cr (VI) by a consortium of sulfate-reducing bacteria (SRB III). Appl Environ Microbiol 60(5):1525–1531

Goecke F, Thiel V, Wiese J, Labes A, Imhoff JF (2013) Algae as an important environment for bacteria—phylogenetic relationships among new bacterial species isolated from algae. Phycologia 52:14–24

Hollants J, Leliaert F, Verbruggen H, De Clerck O, Willems A (2013) Host specificity and coevolution of Flavobacteriaceae endosymbionts within the siphonous green seaweed Bryopsis. Molecular Phylogenetics and Evolution 67:608–614

Iqbal M, Grey D, Sepan-Sarkissian G, Fowler MW (1993) Interactions between the cellular red algae Porphyridium cruentum and associated bacteria. Eur J Phycol 28(1):63–68

Iwahashi M, Katsuragi T, Tani Y, Tsutsumi K, Kakiuchi K (2003) Mechanism for degradation of poly(sodium acrylate) by bacterial consortium No. L7-98. J Biosci Bioeng 95(5):483–487

Johnson KJ, Szymanowski JES, Borrok D, Huynh TQ, Fein JB (2007) Proton and metal adsorption onto bacterial consortia: stability constants for metal–bacterial surface complexes. Chem Geol 239:13–26

Karya NGAI, Van der Steen NP, Lens PNL (2013) Photo-oxygenation to support nitrification in an algal-bacterial consortium treating artificial wastewater. Bioresour Technol 134:244–250

Karigar C, Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Research. p 11

Kim YM, Ahn CK, Woo SH, Jung GY, Park JM (2009) Synergic degradation of phenanthrene by consortia of newly isolated bacterial strains. J Biotechnol 144:293–298

Kwak Y, Lee D, Kim H (2003) Vibrio harveyi nitroreductase is also a chromate reductase. Appl Environ Microbiol 69(8):4390–4395

Lee S, Lee J, Lee J, Chon H (2006) Effects of indigenous bacteria on Cr(VI) reduction in Cr-contaminated sediment with industrial wastes. J Geochem Explor 88:41–44

Liang Z, Liu Y, Ge F, Xu Y, Tao N, Peng F, Wong M (2013) Efficiency assessment and pH effect in removing nitrogen and phosphorus by algae-bacteria combined system of Chlorella vulgaris and Bacillus licheniformis. Chemosphere 92(10):1383–1389

Littlejohns JV, Daugulis AJ (2008) Kinetics and interactions of BTEX compounds during degradation by a bacterial consortium. Process Biochem 43(2008):1068–1076

Nakajima T, Akiko S, Matsuoka H (2009) Auto-/heterotrophic endosymbiosis evolves in a mature stage of ecosystem development in a microcosm composed of an alga, a bacterium and a ciliate. BioSystems 96:127–135

Paul A (2004) Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiol Res 159:347–354

Peña-Salamanca EJ, Rengifo-Gallego AL, Benitez-Campo N (2011) Detoxification mechanisms of heavy metals by algal-bacteria consortia (chapter 28). Se-Kwon Kim (ed). Handbook of marine macroalgae. Biotechnology and applied phycology. Wiley, New York, NY, pp 592. ISBN 978-0-470-97918

Rabbania M, Ghafouriana H, Sadeghib S, Nazerib Y (2005) Biosorption of chromium (III) by new bacterial strain (NRC-BT-2). Int Congr Ser 1276:268–269

Rahman KSM, Thahira-Rahman J, Lakshmanaperumalsamy P, Banat IM (2002) Towards efficient crude oil degradation by a mixed bacterial consortium. Bioresour Technol 85:257–261

Rajakumar S, Ayyasamy PM, Shanthi K, Thavamani P, Velmurugan P, Song YC, Lakshmanaperumalsamy P (2008) Nitrate removal efficiency of bacterial consortium (*Pseudomonas* sp. KW1 and *Bacillus* sp. YW4) in synthetic nitrate-rich water. J Hazard Mater 157:553–563

Rengifo-Gallego AL, Peña-Salamanca EJ, Benitez-Campo N (2012) Efecto de la asociación alga-bacteria *Bostrychia calliptera* (Rhodomelaceae) en el porcentaje de remoción de cromo en laboratorio. Int J Trop Biol 60(3):1055–1064

Richard JY, Vogel TM (1999) Characterization of a soil bacterial consortium capable of degrading diesel fuel. Int Biodeterior Biodegradation 44:93–100

Robertson BK, Jjemba PK (2005) Enhanced bioavailability of sorbed 2,4,6-trinitrotoluene (TNT) by a bacterial consortium. Chemosphere 58:263–270

Safonova ETh, Dmitrieva IA, Kvitko KV (1999) The interaction of algae with alcanotrophic bacteria in black oil decomposition. Resources, Conservation and Recycling 27:193–201

Shen H, Wang Y (1993) Characterization of enzymatic reduction of hexavalent chromium by Escherichia coli ATCC 33456. Appl Environ Microbiol 59(11):3771–3777

Singh PK, Singh AL, Kumar A, Singh MP (2012) Mixed bacterial consortium as an emerging tool to remove hazardous trace metals from coal. Fuel 102:227–230

Tang X, He LY, Tao XQ, Dang Z, Guo CL, Lu GN, Yi XY (2010) Construction of an artificial microalgal-bacterial consortium that efficiently degrades crude oil. J Hazard Mater 181:1158–1162

Tujula N, Crocetti G, Burke C, Thomas T, Holsntrom C, Kjelleberg S (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. ISME J 4:301–311

Vázquez S, Nogales B, Ruberto L, Mestre C, Christie-Oleza J, Ferrero J, Bosch R, Mac Cormack WP (2013) Characterization of bacterial consortia from diesel-contaminated Antarctic soils: towards the design of tailored formulas for bioaugmentation. Int Biodeterior Biodegradation 77:22–30

Wang Y, Fan Y, Gu JD (2004) Dimethyl phthalate ester degradation by two planktonic and immobilized bacterial consortia. Int Biodeterior Biodegradation 53:93–101

Yañez-Ocampo G, Sanchez-Salinas E, Jimenez-Tobon GA, Penninckx M, Ortiz-Hernandez ML (2009) Removal of two organophosphate pesticides by a bacterial consortium immobilized in alginate or tezontle. J Hazard Mater 168:1554–1561

Zhu W, Chai L, Yang Z, Ma Z, Chai L (2008) Reduction of high concentrations of chromate by Leucobacter sp. CRB1 isolated from Changsha, China. World J Microbiol Biotechnol 24:991–996

# 7 Heavy Metals Phytoremediation from Urban Waste Leachate by the Common Reed (*Phragmites australis*)

Amin Mojiri, Hamidi Abdul Aziz, Ramlah Bt Mohd Tajuddin, Shahin Gavanji, and Ali Gholami

## 7.1 Introduction

The use of plants for remediation of soils and waters contaminated with heavy metals, has gained acceptance in the past two decades as a cost-effective and noninvasive technique (Mojiri 2012). This approach is emerging as an innovative tool with great potential that is most useful when contaminants are within the root zone of the plants (top 3–6 ft). Furthermore, phytoremediation is energy efficient, cost-effective, aesthetically pleasing technique of remediation sites with low to moderate levels of pollution. The technique of phytoremediation exploits the use of either naturally occurring metal hyperaccumulator plants or genetically engineered plants (Setia et al. 2008). A variety of contaminated waters can be phytoremediated, counting sewage and municipal wastewater, agricultural runoff/drainage water, industrial wastewater, coal pile runoff, landfill leachate, mine drainage, and groundwater plumes (Olguin and Galvan 2010).

A. Mojiri, Ph.D. (✉)
School of Civil Engineering, Universiti Sains Malaysia, Nibong Tebal 14300, Penang, Malaysia
e-mail: amin.mojiri@gmail.com

H.A. Aziz, Ph.D.
School of Civil Engineering, Universiti Sains Malaysia, Nibong Tebal 14300, Penang, Malaysia

Solid Waste Management Cluster, Engineering Campus, Universiti Sains Malaysia, 14300, Penang, Malaysia

R.B.M. Tajuddin, Ph.D.
Faculty of Civil Engineering, Universiti Technology Mara (UiTM), Shah Alam 40450, Selangor Darul Ehsan, Malaysia

S. Gavanji, M.Sc.
Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran

A. Gholami, Ph.D.
Department of Soil Science, Khuzestan Science and Research Branch, Islamic Azad University, Khouzestan, Iran

A rising method for polluted area remediation is phytoextraction (Ok and Kim 2007). Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants, and it has been recognized as an appropriated approach to remove pollutants from soil, sediment, and sludge (Singh et al. 2011). Plants may play a vital role in metal removal through absorption, cation exchange, filtration, and chemical changes through the root. There is evidence that wetland plants such as *Typha latifolia*, *Cyperus malaccensis*, and *Phragmites australis* can accumulate heavy metals in their tissues (Mojiri et al. 2013a; Yadav and Chandra 2011).

Introduced Phragmites is a vigorous plant that, once established, rapidly takes over, creating dense patches that consume available growing space and push out other plants, including the native subspecies. It also alters wetland hydrology, increases the potential for fire, and may reduce and degrade wetland wildlife habitat due, in part, to its dense growth habit (Swearingen and Saltonstall 2010).

Phragmites australis (Fig. 7.1), or Common Reed, is a large perennial rhizomatous grass that grows 5–20 ft (1.5–3 m) tall. Its leaves are broad and sheath like, 0.4–1.6 in. (1–4 cm) wide at their base. Phragmites has gray-green foliage during the growing season. New stems grow in the spring, and its rhizomes spread horizontally during the growing season. It flowers in late June, with bushy panicles and seeds forming by August to early fall. During this time, energy stores are translocated from the leaves and stems to the rhizomes of the plant. *Phragmites australis* is a strong colonizer, producing an abundance of wind-dispersed seeds, though its seed viability is typically low and it exhibits an interannual variation in fecundity (URI CELS Outreach Center 2012).

Burkea et al. (2000) studied release of metals by the leaves of the Salt marsh grasses *Spartina alterniflora* and *Phragmites australis*.

The aims of the study were to investigate the heavy metals removal from urban waste leachate by Common Reed and optimization of process parameters using the response surface methodology (RSM).

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**Fig. 7.1** Common reed in a wetland

## 7.2 Materials and Methods

### 7.2.1 Sample Preparation

The plants were transplanted into pots containing 10 L of mixed urban waste leachate and water (mixed 80 percentages of waste leachate with 20 % of water; V:V), and aeration was done in 2011. Central composite design and response surface methodology were used in order to clarify the nature of the response surface in the experimental design and explain the optimal conditions of the independent variables. Different number of *Phragmites australis* transplanting in each pot (2–4) and different lengths of time for taking samples (24–72 h) were used.

### 7.2.2 Laboratory Analysis

The plant tissues were prepared for laboratory analysis by Wet Digestion method (Campbell and Plank 1998). Iron (Fe), manganese (Mn), and cadmium (Cu), and nickel (Ni) in waste leachate and plant tissues were carried out using a flame atomic absorption spectrometer (Varian Spectra 20 Plus, Mulgrave, Australia) in accordance to the Standard Methods (APHA 2005). Waste leachate and water properties are shown in Table 7.1.

### 7.2.3 Statistical Analysis

Central composite design (CCD) and Response surface methodology (RSM) were employed in order to clarify the nature of the response surface in the experimental design and elucidate the optimal conditions of the independent variables. CCD was established through Design Expert Software (6.0.7). The behavior of the system is described through equation 1 an empirical second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_i^2 + \sum_{i=1}\sum_{i\neq j=1}\beta_{ij} x_i x_{ij} + \varepsilon. \qquad (7.1)$$

where $Y$ is the response; $X_i$ and $X_j$ are the variables; $\beta_0$ is a constant coefficient; $\beta_j$, $\beta_{jj}$, and $\beta_{ij}$ are the interaction coefficients of linear, quadratic, and second-order terms, respectively; $k$ is the number of study factors; and e is the error (Mojiri et al. 2013b).

The results were completely analyzed by analysis of variance (ANOVA) in the Design Expert Software. Number of *Phragmites australis* transplanting (2, 3, and 4) and times for taking samples (24, 48, and 72 h) were used. To carry out an adequate analysis, three dependent parameters (reducing Fe, Mn, Cu, and Ni concentration in leachate) were measured as responses (Table 7.2).

Descriptive statistical analysis including mean comparison of Fe, Mn, Cu, and Ni accumulation in the roots and shoots of the plants using Duncan's Multiple Range Test (DMRT) was conducted using the SPSS software.

## 7.3 Results and Discussions

Waste leachate properties before the experiment, the results of the experiments, ANOVA results for response parameter, and comparing the means of Fe, Mn, Cu, and Ni accumulation in *Phragmites australis* roots and shoots are shown in Tables 7.2.

In this work, the RSM was used for analyzing the correlation between the variables (number of *Phragmites australis* transplanting and the lengths of time for taking samples) and the important process response (the amount of removed Fe, Mn, Cu, and Ni). Predicted vs. actual values plot for metal removals are shown in Figs. 7.2 and 7.3. Considerable model terms were preferred to achieve the best fit in a particular model. CCD permitted the development of mathematical equations where predicted results ($Y$) were evaluated as a function of the number of *Phragmites australis* transplanting ($A$) and the lengths of time for taking samples ($B$). The results were computed as the sum of a constant, two first order effects (terms in $A$ and $B$), one interaction effect ($AB$), and two second-order effects ($A^2$ and $B^2$), as shown in the equation (Table 7.3). The results were analyzed by ANOVA to determine the accuracy of fit.

The model was significant at the 5 % confidence level because probability values were less than 0.05. The lack of fit (LOF) F-test explains variation of the data around the modified model. LOF would be significant, if the model did not fit the data well. Generally, large probability values for LOF (>0.05) explained that the F-statistic was insignificant, implying a significant model relationship between variables and process responses.

**Table 7.1** Waste leachate and water properties

| pH | EC (dS $m^{-1}$) | N (mg/L) | $BOD_5$ (mg/L) | Fe (mg/L) | Mn (mg/L) | Cu (mg/L) | Ni (mg/L) |
|---|---|---|---|---|---|---|---|
| Water | | | | | | | |
| 7.04 | 0.20 | ND | – | ND | ND | ND | ND |
| Urban waste leachate | | | | | | | |
| 6.12 | 26.32 | 0.64 | 28.18 | 57.03 | 14.31 | 7.9 | 1.29 |

ND: not detected, MDL: 10 μg/L

**Table 7.2** Experimental variables and results for the removal metals

| | Variables | | Response | | | |
|---|---|---|---|---|---|---|
| Run | A: number of plants transplanting | B: Time for taking samples (h) | Amount of Fe removed. (mg/kg) | Amount of Mn removed. (mg/kg) | Amount of Cu removed.(mg/kg) | Amount of Ni Removed (mg/kg) |
| 1 | 2.0 | 48.0 | 15.809 | 4.946 | 3.605 | 0.426 |
| 2 | 3.0 | 48.0 | 18.601 | 6.026 | 4.941 | 0.598 |
| 3 | 3.0 | 48.0 | 18.742 | 5.941 | 5.009 | 0.584 |
| 4 | 3.0 | 48.0 | 18.697 | 6.123 | 4.932 | 0.590 |
| 5 | 3.0 | 72.0 | 22.072 | 8.063 | 4.982 | 0.764 |
| 6 | 2.0 | 24.0 | 11.670 | 3.081 | 2.241 | 0.311 |
| 7 | 3.0 | 48.0 | 18.691 | 6.001 | 3.872 | 0.598 |
| 8 | 4.0 | 72.0 | 25.013 | 9.810 | 6.318 | 0.914 |
| 9 | 2.0 | 72.0 | 19.898 | 6.525 | 4.004 | 0.689 |
| 10 | 4.0 | 24.0 | 16.761 | 5.292 | 4.023 | 0.537 |
| 11 | 4.0 | 48.0 | 20.786 | 6.984 | 4.218 | 0.701 |
| 12 | 3.0 | 48.0 | 18.532 | 6.014 | 4.928 | 0.601 |
| 13 | 3.0 | 24.0 | 13.761 | 3.633 | 2.919 | 0.398 |

### 7.3.1 Iron (Fe) Removed

Iron is a natural constituent of the Earth's crust and is present in varying concentrations in all ecosystems. They are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Human activity has drastically changed the biogeochemical cycles and balance of some metals (Anusha 2011). Iron (II) ions have a high solubility in the aquatic environment and can be absorbed by plants and living organisms (Bulai and Cioanca 2011).

The amount of removed Fe ranged from 11.67 mg/kg (two plants transplanting, and 24 h of time for taking samples) to 25.01 mg/kg (four plants transplanting, and 72 h of time for taking samples). The phytoremediation of Fe increased when the number of plants transplanting and time for taking samples were increased.

### 7.3.2 Manganese (Mn) Removed

Manganese ions exist in wastewaters from numerous industries, chiefly pyrolusite ($MnO_2$) treatment, ink and dyes, glass and ceramics, paint and varnish, steel alloy dry cell batteries, firework and match, and in metal galvanization plant waste matters (Taffarel and Rubio 2009).

The amount of removed Mn ranged from 3.08 mg/kg (two plants transplanting, and 24 h of time for taking samples) to 9.81 mg/kg (four plants transplanting, and 72 h of time for taking samples). The phytoremediation of Mn increased when the number of plants transplanting and time for taking samples were increased.

### 7.3.3 Copper (Cu) Removed

Copper can be found in many wastewater sources including printed circuit board manufacturing, electronics plating, painting manufacturing, and printing operations. This compound can be removed from wastewater by some methods (Yahyaa and Rosebi 2010).

The amount of removed Cu ranged from 2.24 mg/kg (two plants transplanting, and 24 h of time for taking samples) to 6.31 mg/kg (four plants transplanting and 72 h of time for taking samples). The phytoremediation of Cu increased when the number of plants transplanting and time for taking samples were increased.

### 7.3.4 Nickel (Ni) Removed

In the environment, Ni is found primarily combined with oxygen (oxides) or sulfur (sulfides) (Ministry of the Environment 2001). Elevated levels of Ni ($Ni^{++}$) can pose a major threat to both human health and the environment (Hussain et al. 2010).

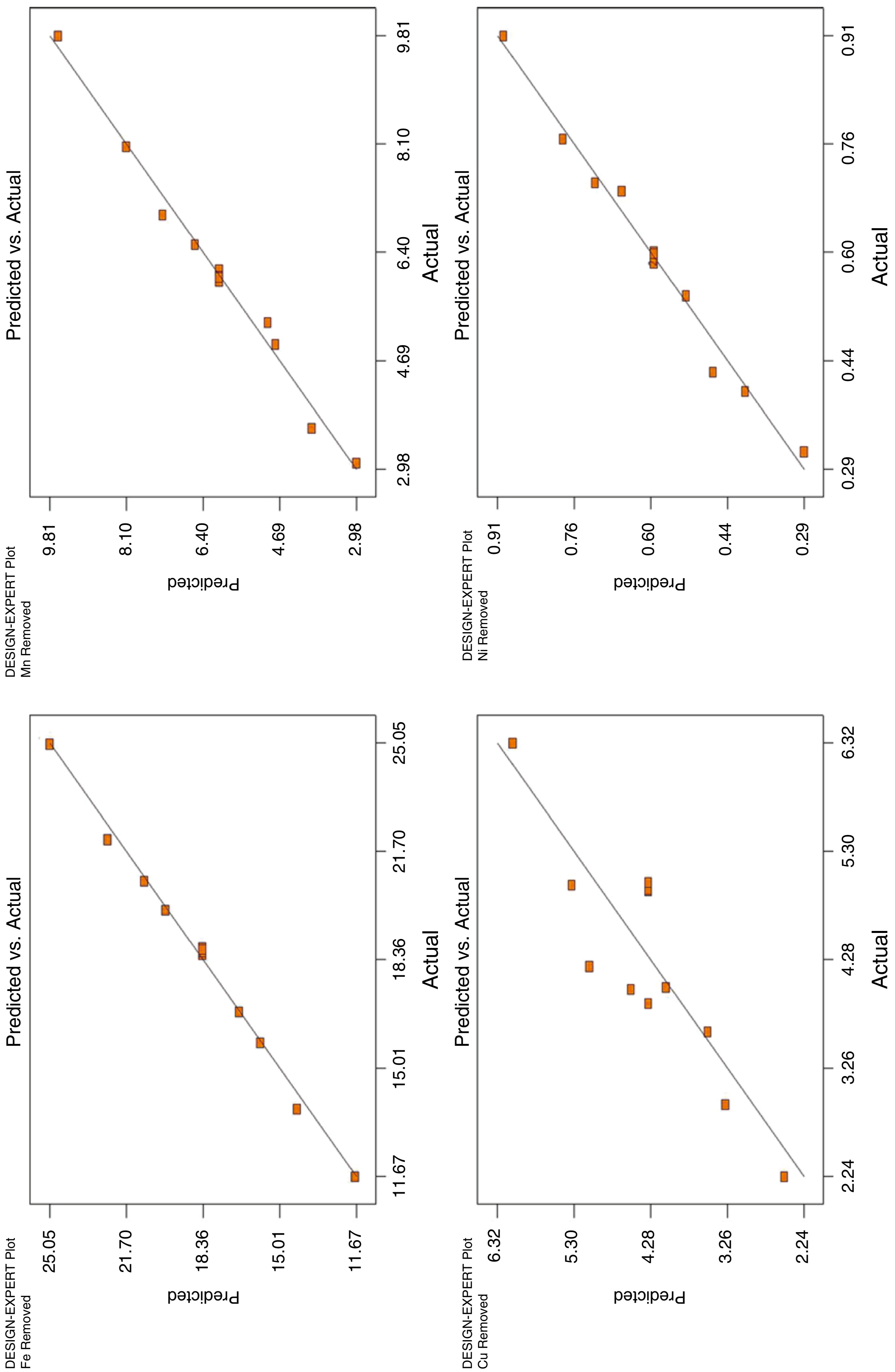


**Fig. 7.2** The design expert statistical plots—predicted versus actual plot: (**a**) Fe, (**b**) Mn, (**c**) Cu, (**d**) Ni

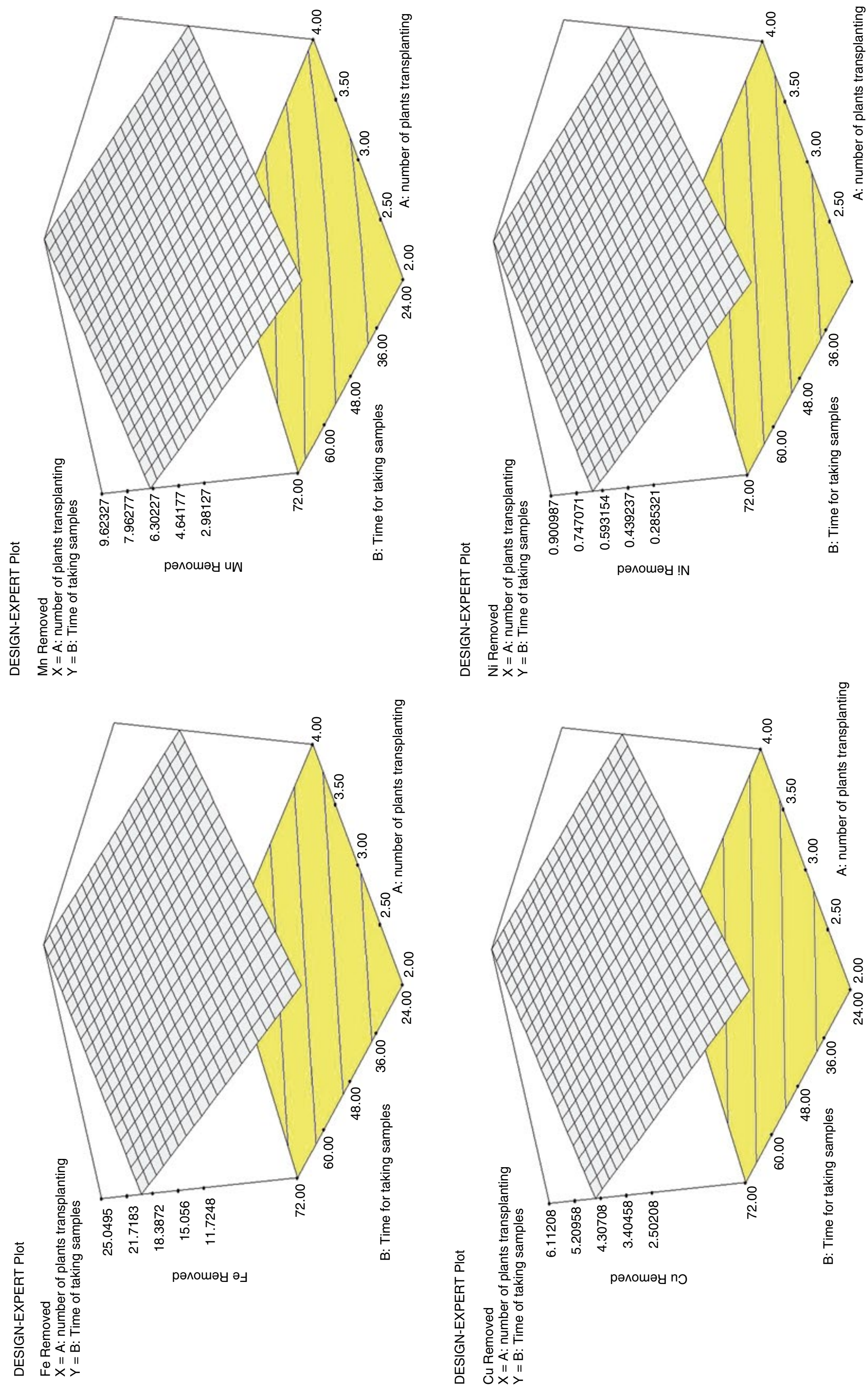


**Fig. 7.3** The 3D surface plots of heavy metal removal: **(a)** Fe, **(b)** Mn, **(c)** Cu, **(d)** Ni

**Table 7.3** ANOVA results for response parameter

| Response | Final equation in terms of actual factors | Prob. | $R^2$ | Adj.$R^2$ | SD | CV | PRESS | Prob.LOF |
|---|---|---|---|---|---|---|---|---|
| Fe removal | $1.444+2.406A+0.231B+0.018A^2-0.0006B^2+0.0002AB$ | 0.0001 | 0.9968 | 0.9946 | 0.25 | 1.38 | 2.93 | 0.0075 |
| Mn removal | $0.901-0.060A+0.050B+0.129A^2+0.00002B^2+0.011AB$ | 0.0001 | 0.9910 | 0.9830 | 0.23 | 3.73 | 3.12 | 0.0044 |
| Cu removal | $-3.161+2.511A+0.074B-0.332A^2-0.0005B^2+0.005AB$ | 0.0146 | 0.8220 | 0.6962 | 0.58 | 13.44 | 13.26 | 0.2568 |
| Ni Removal | $-0.089+0.124A+0.004B-0.0005A^2+0.00002B^2-0.00001AB$ | 0.0001 | 0.9876 | 0.9787 | 0.02 | 3.90 | 0.02 | 0.0051 |

*Prob* probability of error, $R^2$ coefficient of determination, *Ad.* $R^2$ adjusted $R^2$, *Adec*. P. adequate precision, *SD* Standard deviation, *CV* Coefficient of variance, *PRESS* predicted residual error sum of square, *Prob. LOF* probability of lack of fit
Where *A* is number of *Phragmites australis* transplanting, and *B* is time for taking samples

**Table 7.4** Comparison the heavy metals TF in *Phragmites australis* after 24, 48, and 72 h

| Metals (mg/L) | Time (h) | Plants | | TF | Time (h) | Plants | | TF | Time (h) | Plants | | TF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | Root | Shoot | – | 48 | Root | Shoot | – | 72 | Root | Shoot | – |
| Fe | | 2.401a+ | 1.132e | 0.47 | | 3.981a | 4.676e | 1.17 | | 6.103a | 7.989e | 1.30 |
| Mn | | 1.102b | 0.502f | 0.45 | | 2.109b | 1.891f | 0.89 | | 4.711b | 5.295f | 1.12 |
| Cu | | 0.966c | 0.334g | 0.34 | | 1.893c | 1.324g | 0.69 | | 4.091c | 4.505g | 1.10 |
| Ni | | 0.101d | 0.039h | 0.38 | | 0.313d | 0.132h | 0.42 | | 0.602d | 0.611h | 1.01 |

+Numbers followed by same letters in each column are not significantly ($P<0.05$) different according to the DMR test

The amount of removed Ni ranged from 0.31 mg/kg (two plants transplanting, and 24 h of time for taking samples) to 0.91 mg/kg (four plants transplanting and 72 h of time for taking samples). The phytoremediation of Ni increased when the number of plants transplanting and time for taking samples were increased.

### 7.3.5 Uptake of Heavy Metals by Common Reed

Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1,000 times more than those taken up by non-accumulator (excluder) plants. The uptake performance by plant can be greatly improved (Tangahu et al. 2011).

The concentrations of Fe (ppm) in the roots of *Phragmites australis* were 2.40, 3.98, and 6.10, and in the shoots of *Phragmites australis* were 1.13, 4.67, and 7.98, after 24, 48, and 72 h, respectively.

The concentrations of Mn (ppm) in the roots of *Phragmites australis* were 1.10, 1.10, and 4.71, and in the shoots of *Phragmites australis* were 0.50, 1.89, and 5.29, after 24, 48, and 72 h, respectively.

The concentrations of Cu (ppm) in the roots of *Phragmites australis* were 0.96, 1.89, and 4.09 and in shoots of *Phragmites australis* were 0.33, 1.32, and 4.50, after 24, 48, and 72 h, respectively.

The concentrations of Ni (ppm) in the roots of *Phragmites australis* were 0.10, 0.31, and 0.60, and in the shoots of *Phragmites australis* were 0.03, 0.13, and 0.61, after 24, 48, and 72 h, respectively.

### 7.3.6 Translocation Factor (TF)

The efficiency of phytoremediation can be quantified by calculating translocation factor. The TF expresses the capacity of a plant to store the MTE in its upper part. This is defined as the ratio of metal concentration in the upper part to that in the roots (Chakroun et al. 2010). The translocation factor indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots. It is calculated as follows (Padmavathiamma and Li 2007).

$$\text{Translocation Factor}\left(\text{TF}\right)=\frac{C_{\text{Shoot}}}{C_{\text{Root}}} \tag{7.2}$$

where $C_{\text{shoot}}$ is the concentration of the metal in plant shoots and $C_{\text{root}}$ is the concentration of the metal in plant roots.

Based on Table 7.4, translocation factors (TF) were more than 1 in treatment number 3, and in treatment number 2 just for Fe. A translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part (Jamil et al. 2009). According to Yoon et al. (2006), only plant species with TF greater than 1 have the potential to be used for phytoextraction.

## 7.4 Conclusions

Phytoremediation of heavy metals from urban waste leachate by *Phragmites australis* was studied. CCD and RSM were used in the design of experiments, statistical analysis and optimization of the parameters. The factors were number of *Phragmites australis* transplanting (2, 3, and 4) and time for taking samples (24, 48, and 72 h); while the responses were removals of Fe, Mn, Cu, and Ni. The findings clarified that the *Phragmites australis* is an effective accumulator plant for phytoremediation of Fe, Mn, Cu, and Ni. Statistical analysis via Design Expert Software (6.0.7) showed that the optimum conditions for the number of *Phragmites australis* is transplanting and the time for taking samples were 4.00 and 72.00 h, respectively. For the optimized factors, the amount of removed pollutants Fe, Mn, Cu, and Ni (ppm) were 25.04, 9.62, 6.11, and 0.90 mg/kg, respectively.

## References


Anusha G (2011) Removal of iron from wastewater using bael fruit shell as adsorbent. 2011 2nd international conference on environmental science and technology IPCBEE, vol.6© IACSIT Press, Singapore

APHA (2005) Standard methods for examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Bulai P, Cioanca ER (2011) Iron removal from wastewater using chelating resin purolite S930. TEHNOMUS—new technologies and products in machine manufacturing technologies

Burkea DJ, Weisa JS, Weisb P (2000) Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. Estuar Coast Shelf Sci 51(2):153–159

Chakroun HK, Souissi F, Bouchardon JL, Souissi R, Moutte J, Faure O, Remon E, Abdeljaoused S (2010) Transfer and accumulation of lead, zinc, cadmium, and copper in plants growing in abandoned mining-district area. Afr J Environ Sci Technol 4(10):651–659

Campbell CR, Plank CO (1998) Preparation of plant tissue for laboratory analysis. In: Kalra YP (ed) Handbook of reference method for plant analysis. CRC, Boca Raton, FL, pp 37–49

Hussain ST, Mahmood T, Malik SA (2010) Phytoremediation technologies for Ni++ by water hyacinth. Afr J Biotechnol 9(50):8648–8660

Jamil S, Abhilash PC, Singh N, Sharma PN (2009) *Jatropha curcas*: a potential crop for phytoremediation of coal fly ash. J Hazard Mater 172:269–275

Ministry of the Environment (2001) Nickel in the environment. Ontario, fact sheet, March 2001

Mojiri A (2012) Phytoremediation of heavy metals from municipal wastewater by *Typha domingensis*. Afr J Microbiol Res 6(3):643–647

Mojiri A, Aziz HA, Aziz SQ, Selamat MRB, Gholami A, Aboutorab M (2013a) Phytoremediation of soil contaminated with nickel by *Lepidium sativum*; optimization by response surface methodology. Global NEST Journal 15(1):69–75

Mojiri A, Aziz HA, Zahed MA, Aziz SQ, Selamat MRB (2013b) Phytoremediation of heavy metals from urban waste leachate by southern cattail. Int J Sci Res Environ Sci 1(4):63–70

Ok YS, Kim JG (2007) Enhancement of cadmium phytoextraction from contaminated soils with *Artemisia princeps* var. orientalis. J Appl Sci 7(2):263–268

Olguin EJ, Galvan GS (2010) Aquatic phytoremediation: novel insights in tropical and subtropical regions. Pure Appl Chem 82(1):27–38

Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut 184:105–126

Setia RC, Kuar N, Setia N, Nayyar H (2008) Heavy metal toxicity in plants and phytoremediation. Crop improvement: strategies and applications. I.K. International Publishing House Pvt. Ltd., New Delhi, pp 206–218

Singh D, Gupta R, Tiwari A (2011) Phytoremediation of lead from wastewater using aquatic plants. Int J Biomed Res 7:411–421

Swearingen J, Saltonstall K (2010) Phragmites field guide: distinguishing native and exotic forms of common reed (*Phragmites australis*) in the United States. Plant conservation alliance: weeds gone wild. http://www.nps.gov/plants/alien/pubs/index.htm

Taffarel SR, Rubio J (2009) On the removal of $Mn^{2+}$ ions by adsorption onto natural and activated Chilean zeolites. Miner Eng 22:336–343

Tangahu BV, Abdullah SRS, Basir H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. Article ID 939161, 31p

URI CELS Outreach Center (2012) Common reed (Phragmites australis) control fact sheet. Accessed [http://www.uri.edu/cels/ceoc/documents/commonReed.pdf]

Yadav S, Chandra R (2011) Heavy metals accumulation and ecophysiological effect on *Typha angustifolia* L. and *Cyperus esculentus* L. growing in distillery and tannery effluent polluted natural wetland site, Unnao, India. Environ Earth Sci 62:1235–1243

Yahyaa N, Rosebi AF (2010) Copper removal from hazardous waste landfill leachate using peat as an adsorbent. Health Environ J 1(2):51–53

Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464

# 8 Cyanobacteria as Potential Options for Wastewater Treatment

Anjuli Sood, Nirmal Renuka, Radha Prasanna, and Amrik Singh Ahluwalia

## 8.1 Introduction

Environmental issues such as increase in population, industrialisation, excessive generation of wastewater are posing a major threat to the sustainability of developing countries. Discharge of pollutants/polluted water without appropriate treatment into freshwater resources has led to the degradation of pristine water bodies. This unregulated release of agricultural, sewage and industrial wastewater magnifies the problem of water pollution, besides making the water unfit for drinking, irrigation and aquatic life (Egun 2010). Since in developing countries, like India, a large proportion of rural population is dependent on freshwater bodies for drinking and their day to day activities, this assumes significance even in the health sector.

Continuous increase in the demand for water due to increasing population, industrialisation and agricultural use coupled with a high degree of variability in the availability of water in developing countries is reducing per capita accessibility of water. According to the reports from India, 26.5 billion litres of untreated wastewater is discharged into water bodies every day, with a huge gap between available treatment capacity and sewage generated; only 30 % of total sewage generated is being treated in urban areas, leaving aside the rural scenario (CPCB 2009). Apart from insufficient treatment capacity, this untreated wastewater is usually rich in nutrients especially nitrogen (N) and phosphorus (P) in the form of nitrate, nitrite, ammonia/ammonium and phosphorus, which leads to the eutrophication of water bodies (Yang et al. 2008).

In general, the treatment of wastewater involves three stages: primary treatment that mainly involves the settling and the removal of solids, secondary treatment for the removal of organic content and tertiary treatment to reduce the levels of phosphorus and nitrogen. The technologies for wastewater treatment, such as the activated sludge process with N and P removal, are too costly to provide a satisfactory solution for the growing sewage water problems in developing countries (Wei et al. 2008). Therefore, there is an urgent need to develop eco-friendly and economic technologies for wastewater treatment, which would require simple infrastructure, lesser inputs and with potential acceptance at commercial level. The methods employed for wastewater treatment comprise physical, chemical and biological, which are put to use depending upon the extent and type of pollution. In general, both physical and chemical methods are costly. Another disadvantage of chemical methods is the increase in the overall load of dissolved constituents. In this respect, the use of biological methods alone or in combination for wastewater treatment is a better option.

Phytoremediation is the use of plants or microorganisms (microalgae/cyanobacteria/bacteria/fungi) for the removal of contaminants (nutrients, organic compounds, heavy metals) from the wastewaters (Rawat et al. 2011; Sood et al. 2012; Renuka et al. 2013a). It is a promising technology and may offer an inexpensive alternative to conventional forms of tertiary wastewater treatments. The use of microorganisms for wastewater treatment has an edge over the macrophytes, which suffers from the serious drawback of disposal of huge generated biomass. Therefore, the use of photosynthetic microorganisms/microalgae is promising as they play an important role in the self purification of natural waters. The microalgae use solar energy to supply oxygen required for aerobic degradation and recycle the nutrients responsible for eutrophication into potentially valuable biomass. Among microalgae, cyanobacteria (blue green algae), can be better

A. Sood, Ph.D. (✉) • R. Prasanna, Ph.D.
Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: anjulisood@gmail.com

N. Renuka, M.Phil., Ph.D. • A.S. Ahluwalia, Ph.D.
Department of Botany, Panjab University, Chandigarh 160014, India

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candidates for wastewater treatment, because of their wide distribution and ability to sustain in adverse environmental conditions. Their unique characteristics further prove their potentialities;

(a) They have simple growth requirements, therefore, N and P rich wastewaters can be used as cultivation media for their biomass production.
(b) These are photoautotrophic organisms, have ability to fix atmospheric $CO_2$, therefore help in the mitigation of greenhouse gases.
(c) Due to their oxygenic photoautrophic nature, they supply oxygen to photosynthetic bacteria and heterotrophs which degrade other organic compounds during secondary treatment. Therefore, they not only help in treating the wastewater in totality but also support the growth of other micro/macro communities residing in the same habitat.
(d) They serve dual roles of bioremediation of wastewater and generating biomass for various applications viz. feed, pharmaceutical, nutraceutical, industries biofuel and biofertilizers (Rawat et al. 2011).

The use of cyanobacteria in wastewater treatment is an eco-friendly process with no secondary pollution as long as the biomass produced is reused, and it allows efficient nutrient recycling (Munoz and Guieysse 2008). This solar driven technology is also cost effective as compared to other physical and chemical remediation methods (Han et al. 2007). The high requirement of N and P for the growth of cyanobacteria further strengthens their ability to use the nutrient rich wastewater as a medium for multiplication of these organisms. At the same time, assimilated nitrogen and phosphorus can be recycled into algal biomass as biofertilizer (Pittman et al. 2011), after retrieval of heavy metals and treated water can be discharged into water body or can be utilised in hydroelectric water plants.

The use of cyanobacteria for wastewater treatment is not new to the scientific community; this idea was proposed by Caldwell (1946). Oswald reported their role in wastewater treatment in 1955. In the last decade, researchers have been engaged in screening the cyanobacterial diversity from various wastewaters, with an aim to develop eco-friendly wastewater treatment technologies (Magana-Arachchi et al. 2011; Tiwari and Chauhan 2006).

## 8.2 Cyanobacterial Diversity in Aquatic Bodies

Cyanobacteria constitute a broad group of prokaryotic photosynthetic organisms with some of them possessing the unique ability of nitrogen fixation. They are ubiquitous and can be found in almost every type of habitat (aquatic and terrestrial; freshwater, marine, damp soil, bare soil and desert). They occur as plankton and sometimes in the form of phototropic biofilms. They are able to survive in extreme environmental conditions because of their unusual characteristics such as

(a) Presence of outer mucilaginous sheath outside the cell wall helps them to survive in harsh conditions.
(b) Nitrogen fixing ability helps them to survive in low N conditions.
(c) Production of allelochemicals that helps to compete with other organisms in vicinity.

The huge diversity of cyanobacteria in various ecological habitats has been explored by scientists worldwide (Pandey and Pandey 2002; Mukherjee et al. 2010; Dadheech et al. 2013). Many of the studies available reveal the dominance of cyanobacteria in different freshwater and contaminated habitats and/or reservoirs.

### 8.2.1 World Scenario

Table 8.1 summarises the reports on cyanobacterial diversity in various aquatic bodies of different countries. Lopez-Archilla et al. (2004) studied the phytoplankton diversity of a hypereutrophic shallow lake, Santa Olalla (southwestern Spain) and reported the presence of several species of green algae, diatoms, euglenoids and cyanobacteria. Among Cyanophyta, Nostocales and Chroococcales dominated the environment. Dadheech et al. (2013) studied the cyanobacterial diversity of Lake Bogoria, Kenya employing microscopic, sequencing and metagenomic studies. They revealed that most of the phylogenetic lineages of cyanobacteria occurred exclusively in the Bogoria hot springs suggesting a high degree of endemism. The prevalent phylotypes were mainly members of the Oscillatoriales (*Leptolyngbya*, *Spirulina*, *Oscillatoria*-like and *Planktothricoides*) and Chroococcales (Dadheech et al. 2013). Different reports on eutrophic lakes of China (Taihu, Chaohu and Ulungur Lakes) revealed the invariable predominance of *Microcystis* sp. (Ye et al. 2009; Shi et al. 2010; Lin et al. 2011). Magana-Arachchi et al. (2011) studied the cyanobacterial diversity of Gregory Lake, Nuwara Eliya, Sri Lanka which is eutrophied due to agricultural and industrial activities. They reported the occurrence of species of *Synechococcus*, *Microcystis*, *Calothrix*, *Leptolyngbya*, *Limnothrix*. Miller and McMahon (2011) investigated the genetic diversity of cyanobacteria of four eutrophic lakes and reported the presence of species of *Microcystis*, *Aphanizomenon*, *Chroococcus*, *Anabaena* and *Cylindrospermopsis* (Table 8.1). Taton et al. (2003) studied the cyanobacterial diversity of Fryxell, Antarctica, employing 16s RNA. Different cyanobacterial species were: *Nostoc* sp., *Hydrocoryne* cf. *spongiosa*, *Nodularia* cf. *harveyana*, *Leptolyngbya* spp., *Phormidium* cf. *autumnale*. Analyses of cyanobacterial diversity of Pamvotis, Greece using PCR amplification of internal transcribed spacers (ITS) region

**Table 8.1** Cyanobacterial diversity of some aquatic bodies of the world

| Location | Type of contamination | Commonly occurring cyanobacterial genera | References |
|---|---|---|---|
| Shallow lake, Santa Olalla, Southwestern Spain | NA | *Anabaena* spp., *Anabaenopsis* spp., *Aphanocapsa delicatissima*, *Aphanothece clathrata*, *Chroococcus disperses*, *Leptolyngbya* sp., *Limnothrix amphigranulata*, *Merismopedia tenuissima*, *Microcystis aeruginosa*, *Oscillatoria* sp., *Pseudanabaena limnetica*, *Raphidiopsis mediterranea* | Lopez-Archilla et al. (2004) |
| Lake Bogoria, Kenya | NA | *Leptolyngbya*, *Spirulina*, *Oscillatoria*-like, *Planktothricoides*, *Synechocystis*, *Arthrospira and Anabaenopsis* | Dadheech et al. (2013) |
| Lake Taihu and Lake Chaohu, China | NA | *Microcystis* | Shi et al. (2010) |
| Lake Ulungur, Xinjiang, China | NA | *Microcystis* spp. | Lin et al. (2011) |
| Lake Taihu, China | Agricultural intensification and industrial pollution | *Microcystis* sp. | Ye et al. (2009) |
| Lake Gregory, Nuwara Eliya, Sri Lanka | Agricultural and industrial activities | *Synechococcus* sp., *Microcystis aeruginosa*, *Calothrix* sp., *Leptolyngbya* sp., *Limnothrix* sp. | Magana-Arachchi et al. (2011) |
| Mendota, Kegoonsa, Wingra and Monona Lakes | Agricultural and urban run off | *Microcystis*, *Aphanizomenon*, *Chroococcus*, *Anabaena and Cylindrospermopsis* | Miller and McMahon (2011) |
| Lake Fryxell, McMurdo Dry Valleys, Antarctica | NA | *Nostoc* sp., *Hydrocoryne* cf. *spongiosa*, *Nodularia* cf. *harveyana*, *Leptolyngbya* spp., *Phormidium* cf. *autumnale* | Taton et al. (2003) |
| Lake Pamvotis (suburban Mediterranean Lake), Greece | NA | *Microcystis* sp., *Anabaena* sp./*Aphanizomenon* sp. | Vareli et al. (2009) |
| Lake Loosdrecht, the Netherlands | NA | *Aphanizomenon*, *Planktothrix*, *Microcystis*, *Synechococcus*, *Prochlorothrix hollandica*,"*Oscillatoria limnetica* like", *Limnothrix*/*Pseudanabaena* group | Zwart et al. (2005) |
| Western basin of Lake Erie | NA | *Microcystis and Planktothrix* | Rinta-Kanto and Wilhelm (2006) |
| Lake Mendota, Wisconsin, USA | Agricultural and urban run off | *Aphanizomenon* and *Microcystis* | Beversdorf et al. (2013) |

*NA* not available

revealed the presence of species of *Microcystis* sp., *Anabaena* sp./*Aphanizomenon* sp. The study of cyanobacterial diversity of Loosdrecht Lake, Netherlands using analysis of small subunit rRNA gene sequences revealed a diverse consortium of cyanobacteria belonging to *Aphanizomenon*, *Planktothrix*, *Microcystis*, *Synechococcus* (Zwart et al. 2005). A eutrophic lake (due to agricultural and urban run-off) Mendota, Wisconsin, USA harboured *Aphanizomenon* and *Microcystis* as commonly occurring cyanobacterial species which showed alternate dominance (Beversdorf et al. 2013).

### 8.2.2 Indian Scenario

The reports on cyanobacterial diversity in different aquatic bodies (lakes) of India are summarised in Table 8.2 and Fig. 8.1. The studies on cyanobacterial diversity of freshwater lakes and water bodies of Himalayan region revealed the presence of species belonging to Chroococcaceae, Oscillatoriaceae, Nostocaceae and Rivulariaceae (Sidhu and Ahluwalia 2011). *Oscillatoria sancta*, *Lyngbya major* were the dominant forms followed by *Chroococcus minutes*, *Aphanothece castagnei*, *Microcystis aeruginosa* among non-heterocystous and *Nostoc punctiforme*, *Calothrix braunii* and *Anabaena iyengarii* among the heterocystous forms. Seasonal variation in phytoplanktonic diversity in Kitham Lake, Agra, Uttar Pradesh was studied by Tiwari and Chauhan (2006), which mainly receives organic pollutants. The lake harbours microalgae belonging to different divisions and among cyanobacteria genus *Oscillatoria* was represented by maximum species. Pichhola Lake, Udaipur, Rajasthan (India), receives sewage and industrial effluent and harbours different types of microalgae including cyanobacteria. Species of *Microcystis* sp. and *Coccochlaris* sp. were found to be the dominant ones (Sharma et al. 2011). In another study on Baghdara and Swaroop Sagar Lakes (Udaipur, Rajasthan), Pandey and Pandey (2002) observed difference in the cyanobacterial diversity in both the lakes was due to the difference in nature of contaminated sources. They found that high species diversity in Lake Baghdara was due to the input of nutrients from natural resources, whereas lower species diversity in Swaroop Sagar Lake was due to eutrophication caused due to as a result of mixing of sewage. *Microcystis aeruginosa*, *Phormidium* sp. and *Anabaena flos-aquae* were the dominant taxa of the Swaroop Sagar Lake (Pandey and Pandey 2002).

Akoijam and Singh (2011) reported that Lohtak Lake in Manipur is a reservoir for various kinds of organisms

**Table 8.2** Cyanobacterial diversity in different aquatic bodies (lakes) of India

| Location | Type of contamination | Commonly occuring cyanobacteria | References |
|---|---|---|---|
| Lakes and freshwater bodies of lower western Himachal | Freshwater lakes and water bodies | *Oscillatoria sancta*, *Lyngbya Chroococcus minutus*, *Aphanothece castagnei*, *Microcystis aeruginosa Nostoc punctiforme*, *Calothrix braunii* and *Anabaena iyengarii* | Sidhu and Ahluwalia (2011) |
| Kitham Lake, Agra | Water reservoir with organic pollution | *Oscillatoria* spp. | Tiwari and Chauhan (2006) |
| Lake Pichhola, Udaipur, Rajasthan | Artificial freshwater lake sewage systems, overgrowth of hyacinths, industrial waste pollution, deforestation and heavy lakeshore development | *Microsystis* sp. and *Coccochloris* sp. | Sharma et al. (2011) |
| Swaroop Sagar Lake Udaipur, India | Sewage inputs | *Microcystis aeruginosa*, *Phormidium* sp. *and Anabaena flos-aquae* | Pandey and Pandey (2002) |
| Loktak Lake, Manipur | Loktak Lake is the largest freshwater wetland in the North-Eastern region of India | *Anabaena*, *Nostoc*, *Calothrix*, *Cylindrospermum* and *Mastigocladus* | Akoijam and Singh (2011) |
| Eutrophic lake of Ranchi | influx of sewage water | *Microcystis*, *Gloeocapsa*, *Spirulina inflata* | Mukherjee et al. (2010) |
| Wetlands of Sambalpur District, Odisha | Wetland contaminated with industrial untreated wastewater | *Anabaena variabilis*, *Calothrix* sp., *Calothrix elenkini*, *Chroococcus minimus*, *Dermocarpa* sp., *Gloeothece* sp., *Gloeotrichia* sp., *Microcystis* sp., *Synechocystis crassa*, *Westiellopsis prolifica* | Mishra and Das (2011) |
| Urban Lakes of Solapur City, Maharashtra, India | Sewage water | *Oscillatoria raoi*, *O. amoena*, *O. amphibia*, *O. salina*, *O. limosa*, *O. tenuis*, *Lyngbya commune*, *L. corticola*, *L. major*, *Gloeocapsa decorticans*, *G. samoensis*, *Nostoc linckia*, *Phormidium fragile*, *Microcystis incerta*, *Oocystis gigas*, *Scytonema* sp., *Spirulina* sp. | Jagtap et al. (2012) |
| Anbazari and Phutala Lake, Nagpur, India | Anthropogenic impacts such as fishing, boating, swimming, immersion of idols, flowers, garlands, etc. | *Anabaena*, *Oscillatoria*, *Lyngbya*, *Phormidium* and *Microcystis* | Maske et al. (2010) |
| Padmatheertha Pond and Vellayani Lake, Thiruvananthapuram city, India | NA | *Microcystis* sp. | Aneesh and Manilal (2013) |

*NA* not available

including cyanobacteria. Species of *Anabaena*, *Nostoc*, *Calothrix*, *Cylindrospermum* and *Mastigocladius* were commonly occurring either as planktonic, benthic, epilithic or epiphytic forms. Phytoplankton diversity of polluted eutrophic lake in Ranchi revealed *Microcystis*, *Gloeocapsa*, *Spirulina inflata* as dominant forms due to continuous influx of sewage water (Mukherjee et al. 2010). Heavy metal contaminated sites and wetland of Sambalpur, Odisha (Odissa) is also inhabited by cyanobacterial strains (Mishra and Das 2011). The family Chroococcaceae was represented by nine genera, followed by Nostocaceae, Rivulariaceae, Oscillatoriaceae, Scytonemataceae and Stigonemataceae. The most abundant cyanobacterial species were *Anabaena variabilis*, *Calothrix* sp., *C. elenkini*, *Chroococcus minimus*, *Dermocarpa* sp., *Gloeothece* sp., *Gloeotrichia* sp., *Microcystis* sp., *Synechocystis crassa*, *Westiellopsis prolifica* (Mishra and Das 2011).

Jagtap et al. (2012) studied the microalgal diversity of Urban Lakes of Solapur City, Maharashtra, India. They observed the species of *Oscillatoria raoi*, *O. amoena*, *O. amphibia*, *O. salina*, *O. limosa*, *O. tenuis*, *Lyngbya commune*, *L. corticola*, *L. major*, *Gloeocapsa decorticans*, *G. samoensis*, *Nostoc linckia*, *Phormidium fragile*, *Microcystis incerta*, *Oocystis gigas*, *Scytonema* sp., *Spirulina* sp. *Oscillatoria* was represented by maximum number of species. In the lakes at Nagpur, Maharashtra, India, *Anabaena*, *Oscillatoria*, *Lyngbya*, *Phormidium* and *Microcystis* were reported as dominant forms (Maske et al. 2010). At Vellayani Lake, Thiruvananthapuram, Kerala, *Microcystis* and *Scenedesmus* were observed to be the most dominating genera throughout the year (Aneesh and Manilal 2013).

Tables 8.1 and 8.2, *Microcystis*, *Planktothrix*, *Oscillatoria*, *Phormidium*, *Nostoc*, *Anabaena*, *Gloeocapsa* and *Chroococcus* were among the most commonly reported cyanobacteria in the water bodies (lakes) of world and India receiving contaminants from different sources. The available reports reflected that *Microcystis* was invariably present in all eutrophic lakes.

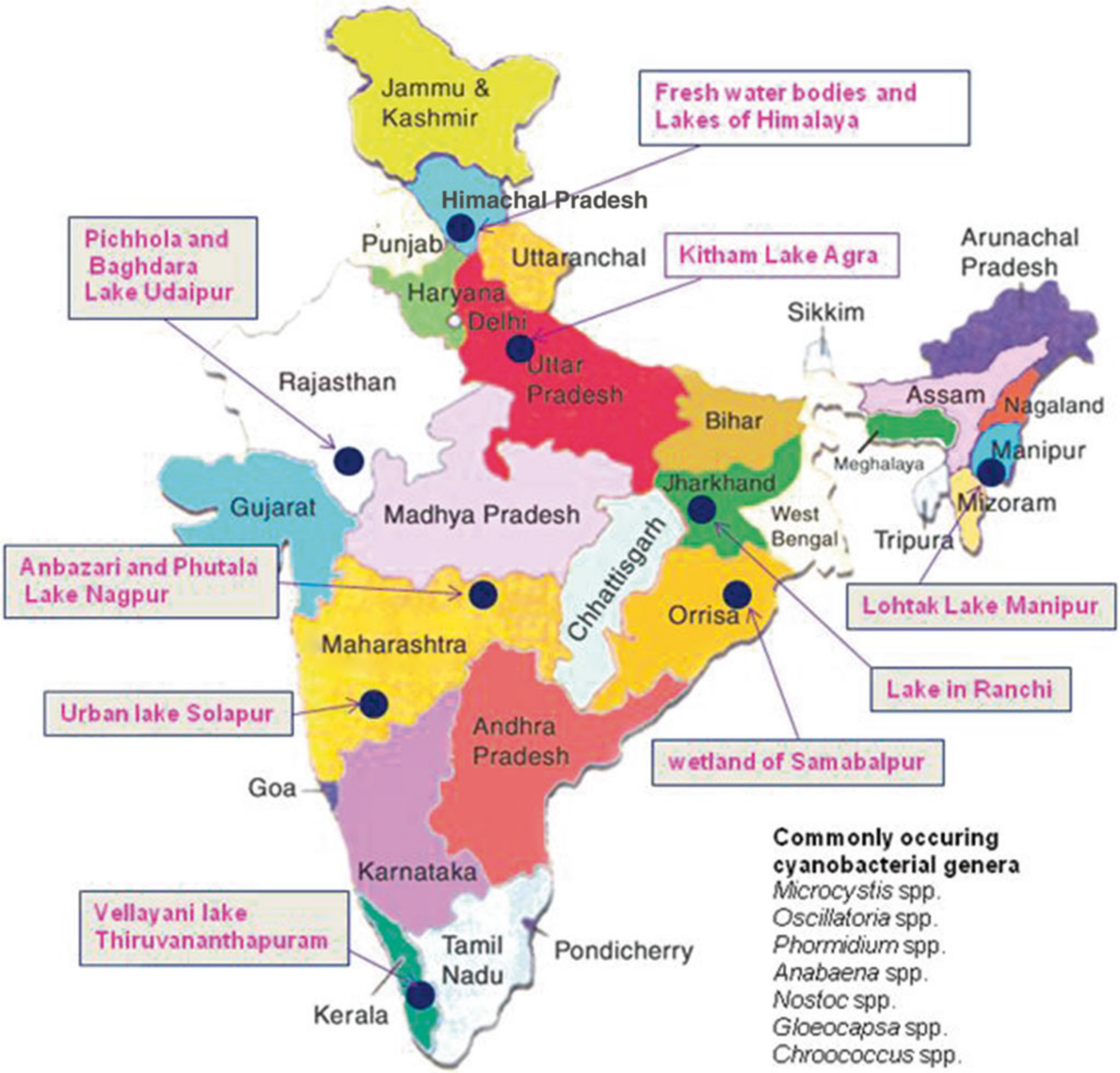


**Fig. 8.1** Site map of different lakes of India and commonly occuring cyanobacteria

### 8.2.3 Characteristics Features Involved in the Dominance of Cyanobacteria in Contaminated Habitats (Fig. 8.2)

Commonly occurring cyanobacteria include:

(a) *Microcystis* spp.
  - Presence of mucilage outside cell wall.
  - Release of allelochemical microcystin.

(b) *Oscillatoria* spp.
  - Presence of mucilaginous layer outer to the cell wall.
  - Release of allelochemicals—anatoxin and fatty acids.

(c) *Phormidium* spp.
  - Release of allelochemicals.

(d) *Nostoc* spp. and *Planktothrix* spp.
  - Presence of mucilaginous sheath.
  - Release of allelochemicals.

(e) *Anabaena* spp.
  - Presence of mucilaginous sheath outside cell wall.
  - Release of allelochemicals viz. extracellular peptides, anatoxin, hydroxamate chelators, microcystin.

(f) *Gloeocapsa* spp. and *Chroococcus* spp.
  - Presence of mucilaginous sheath.

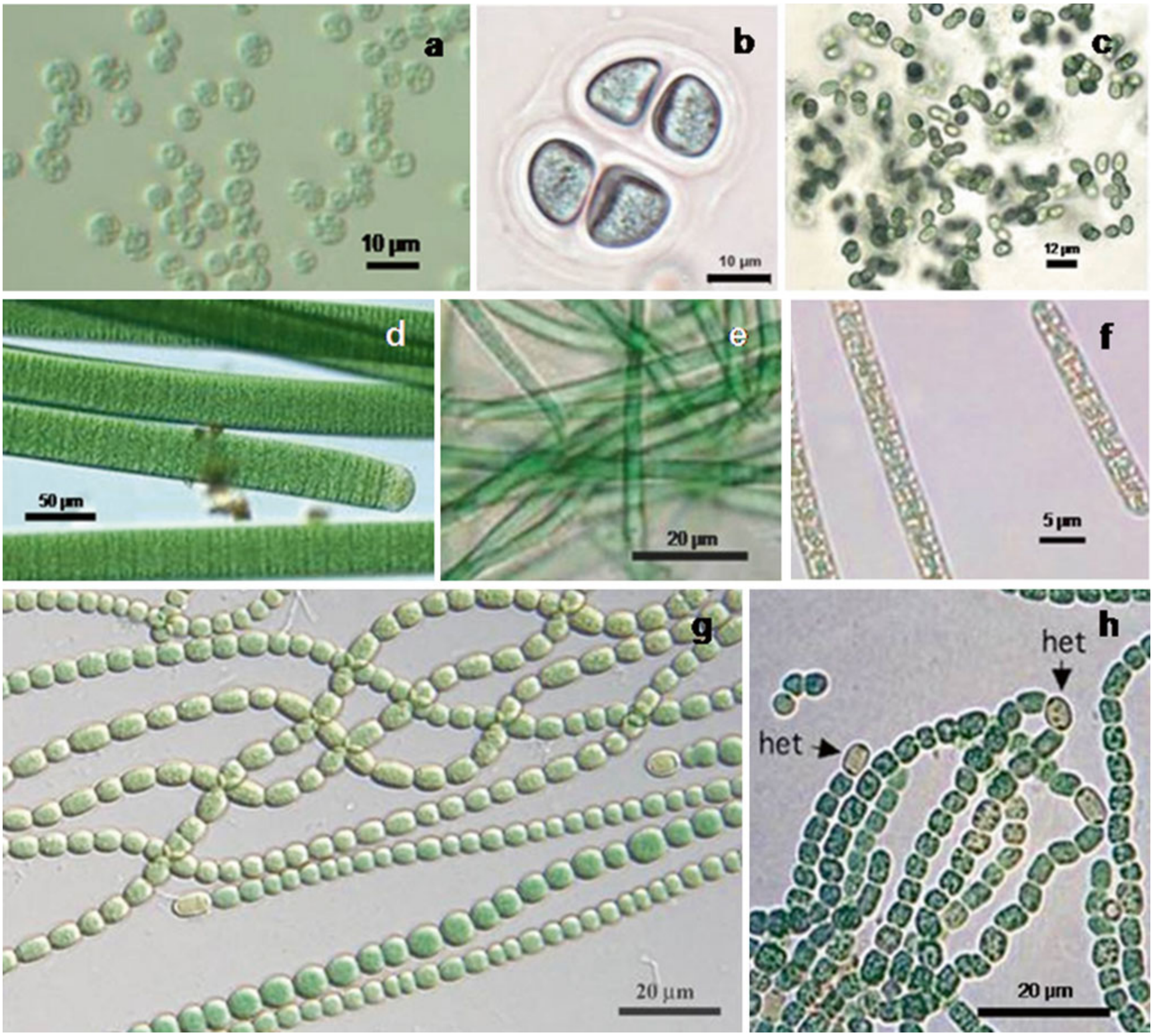


**Fig. 8.2** Photomicrographs of commonly occurring cyanobacterial genera in eutrophic water bodies (**a**) *Microcystis* sp. (**b**) *Chroococcus* sp. (**c**) *Gloeocapsa* sp. (**d**) *Oscillatoria* sp. (**e**) *Phormidium* sp. (**f**) *Planktothrix* sp. (**g**) *Anabaena* sp. (**h**) *Nostoc* sp.

## 8.3 Role of Cyanobacteria in Wastewater Treatment

The dominance and diversity of cyanobacterial genera in different aquatic bodies illustrates the tolerance of this group to a wide variety of contaminants. This has generated an interest in scientific community to explore their potential in wastewater treatment.

### 8.3.1 Water Quality Improvement

Table 8.3 is a compilation of reports on role of cyanobacteria in water quality improvement which indicates that various cyanobacterial species are efficient in improving the quality of different types of wastewater. El-Bestawy (2008) studied the role of *Anabaena variabilis*, *Anabaena oryzae* and *Tolypothrix ceylonica* in the improvement of water quality in

**Table 8.3** Improvement of water quality by cyanobacteria

| Type of cyanobacteria/consortia | Type of wastewater | Parameters | % removal | Time duration | References |
|---|---|---|---|---|---|
| *Anabaena variabilis A. oryzae* | Domestic-industrial wastewater | BOD | 89.29 | 7 days | El-Bestawy (2008) |
| | | COD | 73.68 | | |
| *Spirulina platensis* | Anaerobically treated swine wastewater | BOD | 45 | 12 days | Cheunbarn and Peerapornpisal (2010) |
| | | COD | 23 | | |
| *S. platensis* | Wastewater diluted with standard growth medium | COD | 81.02 | 28 days | Magro et al. (2012) |
| *S. fusiformis* | Retan chrome liquor | TS | 17–22.6 | | Pandi et al. (2009) |
| | | TDS | 18–22.5 | | |
| | | TSS | 15–23 | | |
| | | BOD | 73.8–78.9 | | |
| | | COD | 82.4–88.5 | | |
| *Nostoc muscorum* | Distillery effluent | BOD | 53.5 | 30 days | Ganapathy et al. (2011) |
| | | COD | 68.5 | | |
| *Phormidium tenue* | Paper mill effluent | Salinity | 14.5 | 20 days | Nagasathya and Thajuddin (2008) |
| | | BOD | 17.6 | | |
| | | COD | 45.2 | | |
| *Oscillatoria annae* (with coir pith) | Tannery effluent | Salinity | 54.5 | 15 days | Shankar et al. (2013) |
| | | BOD | 62.7 | | |
| Consortium of cyanobacterium with green algae and bacteria | Industrial wastewater | Phenols | 85 | NA | Safonova et al. (2004) |
| | | Anionic surface active substances | 73 | | |
| | | Oil spill | 96 | | |
| | | BOD | 97 | | |
| | | COD | 51 | | |
| Cyanobacteria dominated consortia | Sewage wastewater | BOD | 99 | 14 days | Renuka et al. (2013b) |
| | | COD | 87 | | |

domestic-industrial wastewater. Highest reduction in BOD and TDS (89.29 % and 38.84 %, respectively) was recorded with *Anabaena variabilis*, while highest reduction in COD (73.68 %) with *Anabaena oryzae* and total suspended solids (TSS) (64.37 %) with *Tolypothrix ceylonica* was found in 7 days. The growth and wastewater treatment potential of *Spirulina platensis* was investigated in anaerobically treated swine wastewater by Cheunbarn and Peerapornpisal (2010). Maximum reduction of 45 % and 23 % in BOD and COD was observed on 12th day with 10 % dilution with $NaHCO_3$ and $NaNO_3$ at 8.0 g/L and 1.5 g/L, respectively. In another study, *S. platensis* (Leb 52 Strain) removed 81.02 % of COD from wastewater diluted 12.5 % with Zarrouk medium in 28 days (Magro et al. 2012). Pandi et al. (2009) studied the water quality improvement in Retan chrome liquor by *Spirulina fusiformis*. The cyanobacterium was able to remove 17–22.6, 18–22.5, 15–23, 73.8–78.9, 82.4–88.5 and 93–99 % of TS, TDS, TSS, BOD, COD and Cr (VI) in retan chrome liquor with varying Cr concentrations 100–300 ppm. Another cyanobacterium, *Nostoc muscorum* was able to reduce BOD and COD up to 53.5 and 68.5 % respectively from distilleries effluent in 30 days (Ganapathy et al. 2011). Nagasathya and Thajuddin (2008) studied the wastewater treatment potential and water quality improvement of paper mill effluent by *Phormidium tenue*, which removed 14.5, 17.6 and 45.26 % of salinity, BOD and COD, respectively, in 20 days. Shankar et al. (2013) reported that *Oscillatoria annae* removed 36.4 % salinity and BOD from tannery effluent in 15 days (Table 8.3). However, *Oscillatoria annae* with coir pith proved more efficient in reducing salinity and BOD by 54.5 and 62.7 % (Shankar et al. 2013). An integrated system of *Bacillus* sp. immobilised chemo autotrophic activated carbon oxidation (CAACO) and algal batch reactor removed 98, 95, 93, 86 and 100 % of BOD, COD, TOC, volatile fatty acid (VFA) and sulphide respectively from tannery effluent after 30 days (Sekaran et al. 2013). There are reports where researchers have employed consortia of different microorganisms, including cyanobacteria to treated contaminated wastewater (Safonova et al. 2004; Bernal et al. 2008; Renuka et al. 2013b). The consortium of microalgae containing green algae, cyanobacteria (*Chlorella* spp., *Scenedesmus* sp., *Stichococcus* spp. *Phormidium* sp.) and bacteria (*Rhodococcus* sp. and *Kibdelosporangium aridum*) removed 85, 73, 96, 97 and 51 % of phenols, anionic surface active substances, oil spill, BOD and COD respectively from industrial wastewater (Safonova et al. 2004). Bernal et al. (2008)

studied the improvement of water quality of dairy sewage using mixture of native microalgae including cyanobacteria inhabiting the wastewater treatment plant. They observed that the mixture of native microalgae was able to remove 69 % of dissolved organic carbon, 88 % of COD, 88.6 % of TSS, 91.4 % of turbidity, 97.3 % of BOD and 99.9 % of faecal coliforms from dairy sewage wastewater. Recently, Renuka and co-workers (2013b) reported 99 and 89 % reduction in COD and BOD of sewage wastewater by treatment with a cyanobacteria dominated consortium of native strains (Species of *Phormidium*, *Limnothrix*, *Anabaena*, *Westiellopsis*, *Fischerella* and *Spirogyra*) (Table 8.3).

### 8.3.2 Nutrient Removal

Cyanobacteria require higher amount of nutrients (N, P) for their growth, therefore, wastewaters rich in nutrients can be used as growth media for their cultivation and indirectly they sequester these nutrients from wastewater, thereby help in the remediation. Canizares-Villanueva et al. (1994) reported that *Phormidium* sp. was able to remove 48, 30, 100 and 63 % of $PO_4$–P, $NO_3$–N, $NH_4$–N and total P from anaerobically treated swine wastewater. However, 95 and 62 % removal of $NH_4$–N and $PO_4$–P, respectively, was reported by *Phormidium* spp. inoculated on wastewater mixed with swine manure (Pouliot et al. 1989). In another report, *Phormidium* sp. removed 99 % of N and P from secondary treated wastewater in 7 days and 4 days, respectively (Su et al. 2012). Kamilya et al. (2006) evaluated the nutrient removal potential of *Spirulina platensis* and *Nostoc muscorum* from fish culture effluent. They observed that *N. muscorum* was able to remove 83.6, 44.2, 14.17 and 41.79 % of $NH_4$–N, $NO_2$–N, $NO_3$–N and $PO_4$–P respectively from the effluent, while, 92.4, 48.7, 50.39 and 47.76 % removal of $NH_4$–N, $NO_2$–N, $NO_3$–N and $PO_4$–P, respectively, was obtained with *S. platensis* in 7 days (Table 8.4). Apart from monocultures, Silva-Benavides and Torzillo (2011) utilised co-culture of *Planktothrix* sp. and *Chlorella* sp. to phytoremediate municipal wastewater. They observed that this co-culture of *Planktothrix* sp. and *Chlorella* sp. could remove 100 and 88 % of $PO_4$–P and total N from municipal wastewater. Recently, a microalgal consortium of filamentous strains (species of *Phormidium*, *Limnothrix*, *Anabaena*, *Westiellopsis*, *Fischerella* and *Spirogyra*) has been reported to remove 90, 100 and 97 % of $NH_4$–N, $NO_3$–N and $PO_4$–P, respectively, from sewage wastewater. The above studies indicate that cyanobacterial members can efficiently remove nutrients (mainly N and P) effectively from various types of wastewaters.

### 8.3.3 Heavy Metal Removal

Heavy metals are among the most dangerous substances in the environment because of their high level of durability and harmful effects on living organisms. The disturbance of aquatic ecosystems elicited by heavy metal pollution from industrial and domestic sources has severe consequences, including the loss of biological diversity, as well as increased bioaccumulation and magnification of toxicants in the food chain (He et al. 1998). Microalgae are efficient in absorbing heavy metals from wastewaters in actively growing cultures (Table 8.5). Many studies are focused on heavy metal removal under lab conditions; however, the reports on heavy metal removal from natural systems are scarce and need attention. Dwivedi et al. (2010) investigated the potential of green algae and blue green microalgae for accumulation of metals (Cr, Cu, Fe, Mn, Ni and Zn) and metalloid (As). The maximum accumulation of Cr was shown by *Phormidium bohneri* followed by *Oscillatoria tenuis*, *Chlamydomonas angulosa*, *Ulothrix tenuissima* and *Oscillatoria nigra*. Blue green algae represented the dominant community where Cr concentration was higher. The removal of Cr (VI) of 60.9 and up to 99 % was reported by *Spirulina platensis* and *S. fusiformis,* respectively, in different types of wastewater (Pandi et al. 2009; Magro et al. 2012). El-Bestawy (2008) studied the metal removal efficiency of *Anabaena variabilis*, *Anabaena oryzae* and *Tolypothrix ceylonica*. Highest Zn and Cu removal of 86.12 and 94.63 % was recorded in the culture of *Tolypothrix ceylonica* grown in domestic-industrial wastewater. *O. quadripunctulata* removed 37, 32.1, 34.6 and 20.3 %, and 50, 32.1, 34.6 and 33.3 % of Cu, Zn, Pb and Co from sewage wastewater and petrochemical effluent, respectively (Ajayan et al. 2011). El-Sheekh et al. (2005) observed that *Nostoc muscorum*, *Anabaena subcylindrica* and mixed culture of *N. muscorum* and *A. subcylindrica* significantly removed heavy metals from sewage wastewater, Verta Company and salt and soda company wastewater. *Nostoc muscorum* and *Anabaena subcylindrica* individually were able to remove 64.4, 22.2, 84.6 and 64.1 % and 33.3 %, 33.3 %, 86.2 % and 40 % of Cu, Co, Pb and Mn, respectively from sterilised sewage wastewater. However, the mixed culture of *N. muscorum* and *A. subcylindrica* removed 75 %, 11.8 %, 100 % and 61.5 % of Cu, Co, Pb and Mn from sewage wastewater. Since, the initial concentration of heavy metals were different in all the sewage wastewater used, hindering generalisations on the degree of removal or quantitative estimates or comparisons (El-Sheekh et al. 2005).

A basic generalisation regarding the reports on wastewater treatment (Tables 8.3, 8.4 and 8.5), reveals that cyanobacteria vary in their potential, in terms of improvement of water quality parameters, nutrient sequestration and heavy metal removal. This observed variation could be due to their difference in genetic constitution of different cyanobacteria, difference in composition and constitution of wastewaters, and experimental and environmental set up. Although *Microcystis*

**Table 8.4** Nutrient removal potential of different cyanobacteria alone or in consortia

| Type of cyanobacteria/consortia | Wastewater used | Parameter studied | % Removal | Time duration | References |
|---|---|---|---|---|---|
| *Nostoc muscorum* *Spirulina platensis* | Fish culture effluent | $NH_4$–N | 83.6 | 7 days | Kamilya et al. (2006) |
| | | $NO_2$–N | 44.2 | | |
| | | $NO_3$–N | 14.176 | | |
| | | $PO_4$–P | 41.79 | | |
| | | $NH_4$–N | 92.4 | | |
| | | $NO_2$–N | 48.7 | | |
| | | $NO_3$–N | 50.39 | | |
| | | $PO_4$–P | 47.76 | | |
| *Phormidium* sp. | Anaerobically treated swine wastewater | $PO_4$–P | 48 | 4 days | Canizares-Villanueva et al. (1994) |
| | | $NO_3$–N | 30 | | |
| | | $NH_4$–N | 100 | | |
| | | Total P | 63 | | |
| *Phormidium* spp. | Swine manure mixed wastewater | $NH_4$–N | 95 | 1 day | Pouliot et al. (1989) |
| | | $PO_4$–P | 62 | | |
| *Phormidium* sp. | Secondary treated wastewater | N | 99 | 7 day | Su et al. (2012) |
| | | P | 99 | 4 days | |
| Co-culture of *Planktothrix* sp. and *Chlorella* sp. | Municipal wastewater | $PO_4$–P | 100 | 4 days | Silva-Benavides and Torzillo (2011) |
| | | Total N | 80 | 4 days | |
| Cyanobacteria dominated microalgal consortium | Sewage wastewater | $NH_4$–N | 90 | 14 days | Renuka et al. (2013b) |
| | | $NO_3$–N | 100 | | |
| | | $PO_4$–P | 97 | | |

**Table 8.5** Removal of heavy metals by microalgae

| Medium | Microalgae | Heavy metal | Percent removal | References |
|---|---|---|---|---|
| Tannery effluent | *Oscillatoria tenuis* | Cr | NA | Dwivedi et al. (2010) |
| *Spirulina platensis* | Wastewater diluted with standard growth medium | Cr (VI) | 60.9 | Magro et al. (2012) |
| *Spirulina fusiformis* | Retan chrome liquor | Cr (VI) | 93–99 | Pandi et al. (2009) |
| *Nostoc* sp. | Wastewater | Cr (VI) | NA | Colica et al. (2010) |
| Sewage petrochemical effluent | *Oscillatoria quadripunctulata* | Cu | 37 | Ajayan et al. (2011) |
| | | Co | 20.3 | |
| | | Pb | 34.6 | |
| | | Zn | 32.1 | |
| | | Cu | 50 | |
| | | Co | 33.3 | |
| | | Pb | 34.6 | |
| | | Zn | 32.1 | |
| Sewage wastewater | *Nostoc muscorum* | Cu | 64.4 | El-Sheekh et al. (2005) |
| | | Co | 22.2 | |
| | | Pb | 84.6 | |
| | | Mn | 64.1 | |
| Sewage wastewater | *Anabaena subcylindrica* | Cu | 33.3 | El-Sheekh et al. (2005) |
| | | Co | 33.3 | |
| | | Pb | 86.2 | |
| | | Mn | 40.0 | |
| Sewage wastewater | Mixed culture of *Nostoc muscorum* and *Anabaena subcylindrica* | Cu | 75 | El-Sheekh et al. (2005) |
| | | Co | 11.8 | |
| | | Pb | 100 | |
| | | Mn | 61.5 | |

was the dominant cyanobacterial genus (Tables 8.1 and 8.2), reports on its application in wastewater treatment are not available. This could be due to release of toxin in the aquatic ecosystem by this cyanobacterium, and its tendency to form algal bloom. Such type of reasons may be responsible for researchers to become biased during the selection of cyanobacteria for bioremediation.

## 8.4 Limitations and Constraints

Although the idea of exploitation of photosynthetic organisms for wastewater treatment originated long back in the late 1950s, this technology has still to go a long way before it can be actually commercially accepted. The main limitations are:

*Inconsistent composition of wastewater at different sites*: One of the major limitations is the variation in both composition and concentration of constituents present in all types of wastewaters i.e. domestic wastewater from one contaminated site differs in its physico-chemical composition from other contaminated site. Therefore, any selected promising strain of cyanobacteria may or may not perform and treat wastewater with the same efficiency as recommended by the researcher/scientists/technologists based on another site.

*Variation in indigenous flora and fauna of contaminated site*: There is huge variation in the native micro flora inhabiting a particular locality (Lopez-Archilla et al. 2004; Mishra and Das 2011). Therefore, the treatment potential of selected stains varies and depends on their ability to compete and establish in the containment sites.

*Dependency of cyanobacterial strains on environmental conditions*: Being prokaryotic and autotrophic organisms, the phytoremediation potential of cyanobacteria is directly influenced by the environmental factors that affect their growth (Muller 1994). Hence, environmental factors such as-light, temperature, pH etc. can indirectly affect the treatment efficiency of selected promising strains, which in turn will be variable at different geographical locations.

## 8.5 Conclusions

Available reports clearly indicate that cyanobacteria are an important part of aquatic ecosystem and are dominant in most contaminated habitats. This is attributed to their inherent potential to survive in such eutrophic and adverse habitats and resilience to environmental extremes. Reports on their wastewater treatment potential reflect that cyanobacteria are efficient in treating various wastewaters in terms of nutrient removal, water quality improvement as well as heavy metal removal. However, these areas still needs more in depth understanding. The indigenous differences in the characteristics and composition of various wastewaters are the main factors responsible for varied response of cyanobacteria in terms of their remediation potential. Therefore, more strains of cyanobacteria need to be screened for their bioremediation potential which not only easily establish themselves in these habitats, but also show a relatively consistent performance on scale-up of such technologies.

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## References

Ajayan KV, Selvaraju M, Thirugnanamoorthy K (2011) Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and petrochemical effluent. Pak J Biol Sci 14:805–811

Akoijam C, Singh AK (2011) Molecular typing and distribution of filamentous heterocystous cyanobacteria isolated from two distinctly located regions in North-Eastern India. World J Microbiol Biotechnol 27:2187–2194

Aneesh C, Manilal VB (2013) Continuous presence of colony forming *Microcystis* and *Scenedesmus* in fresh water lake and ponds. Bot Res Int 6:07–12

Bernal CB, Vazquez G, Quintal IB et al (2008) Microalgal dynamics in batch reactors for municipal wastewater treatment containing dairy sewage water. Water Air Soil Pollut 190:259–270

Beversdorf LJ, Miller TR, McMahon KD (2013) The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. PLoS One 8:e56103. doi:10.1371/journal.pone.0056103

Caldwell DH (1946) Sewage oxidation ponds: design. Sewage Work J 18:433–458

Canizares-Villanueva RO, Ramos A, Corona AI et al (1994) *Phormidium* treatment of anaerobically treated swine wastewater. Water Res 28:1891–1895

Central Pollution Control Board (2009) Status of water supply and wastewater generation and treatment in class-I cities and class-II towns of India—a report by CPCB (2009). http://www.indiawaterportal.org

Cheunbarn S, Peerapornpisal Y (2010) Cultivation of *Spirulina platensis* using anaerobically swine wastewater treatment effluent. Int J Agric Biol 12:586–590

Colica G, Mecarozzi PC, De Philippis R (2010) Treatment of Cr(VI) containing waste water with exopolysaccharide-producing cyanobacteria in pilot flow through and batch systems. Appl Microbiol Biotechnol 87:1953–1961

Dadheech PK, Glockner G, Casper P et al (2013) Cyanobacterial diversity in the hot spring, pelagic and benthic habitats of a tropical soda lake. FEMS Microbiol Ecol 85:389–401

Dwivedi S, Srivastava S, Mishra S et al (2010) Characterization of native microalgal strains for their chromium bioaccumulation potential: phytoplankton response in polluted habitats. J Hazard Mater 173:95–101

Egun NK (2010) Effect of channelling wastewater into water bodies: a case study of the Orogodo river in Agbor, Delta State. J Hum Ecol 31:47–52

El-Bestawy E (2008) Treatment of mixed domestic-industrial wastewater using cyanobacteria. J Ind Microbiol Biotechnol 35:1503–1516

El-Sheekh MM, El-Shouny WA, Osman MEH et al (2005) Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol 19:357–365

Ganapathy SG, Baskaran R, Mohan PM (2011) Microbial diversity and bioremediation of distilleries effluent. J Res Biol 3:153–162

Han X, Wong YS, Wong MH, Tam NFY (2007) Biosorption and bioreduction of Cr (VI) by a microalgal isolate, *Chlorella miniata*. J Hazard Mater 146:65–72

He M, Wang Z, Tang H (1998) The chemical, toxicological and ecological studies in assessing the heavy metal pollution in Le AN river, China. Water Res 32:510–518

Jagtap MN, Dama LB, Shaik KA et al (2012) Isolation of phytoplanktons from three urban lakes of Solapur city, Maharashtra state, India. DAV Int J Sci 1:68–71

Kamilya D, Sarkar S, Maiti TK et al (2006) Growth and nutrient removal rates of *Spirulina platensis* and *Nostoc muscorum* in fish culture effluent: a laboratory-scale study. Aqua Res 37:1594–1597

Lin S, Shen J, Liu Y et al (2011) Molecular evaluation on the distribution, diversity, and toxicity of *Microcystis* (Cyanobacteria) species from Lake Ulungur—a mesotrophic brackish desert lake in Xinjiang, China. Environ Monit Assess 175:139–150

Lopez-Archilla AI, Lopez-Garcıa DMP, Guerrero C (2004) Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. Extremophiles 8:109–115

Magana-Arachchi D, Wanigatunge R, Liyanage M (2011) Molecular characterization of cyanobacterial diversity in Lake Gregory, Sri Lanka. Chinese J Oceanol Limnol 29:898–904

Magro CD, Deon MC, De Rossi A et al (2012) Chromium (VI) biosorption and removal of chemical oxygen demand by *Spirulina platensis* from wastewater supplemented culture medium. J Environ Sci Health A Tox Hazard Subst Environ Eng 47:1818–1824

Maske SS, Sangolkar LN, Chakrabati T (2010) Temporal variation in density and diversity of cyanobacteria and cyanotoxins in lakes at Nagpur (Maharashtra state), India. Environ Monit Assess 169:299–308

Miller TR, McMahon KD (2011) Genetic diversity of cyanobacteria in four eutrophic lakes. FEMS Microbiol Ecol 78:336–348

Mishra AK, Das MK (2011) Cyanobacterial diversity of heavy metal contaminated rice-fields and wetlands of Sambalpur District, Odisha. Biohelica 2:78–84

Mukherjee B, Nivedita M, Mukherjee D (2010) Plankton diversity and dynamics in a polluted eutrophic lake, Ranchi. J Environ Biol 31:827–839

Muller U (1994) Seasonal development of epiphytic algae on *Phragmites australis* (Cav.) Trin. Ex Sten. on a eutrophic lake. Archiv für Hydrobiologie 129:273–292

Munoz R, Guieysse B (2008) Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815

Nagasathya A, Thajuddin N (2008) Decolourization of paper mill effluent using hypersaline cyanobacterium. Res J Environ Sci 2:408–414

Oswald WJ, Gotaas HB (1955) Photosynthesis in sewage treatment. Trans Am Soc Civ Eng 122:73–105

Pandey J, Pandey U (2002) Cyanobacterial flora and the physicochemical environment of six tropical fresh water lakes of Udaipur, India. J Environ Sci 14:54–62

Pandi M, Shashirekhab V, Swamy M (2009) Bioabsorption of chromium from retan chrome liquor by cyanobacteria. Microbiol Res 164:420–428

Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 102:17–25

Pouliot Y, Buelna G, Racine C et al (1989) Culture of cyanobacteria for tertiary wastewater treatment and biomass production. Biol Waste 29:81–91

Rawat I, Kumar RR, Mutanda T, Bux F (2011) Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energ 88:3411–3424

Renuka N, Sood A, Ratha SK, Prasanna R, Ahluwalia AS (2013a) Nutrient sequestration, biomass production by microalgae and phytoremediation of sewage water. Int J Phytoremediation 15:789–800

Renuka N, Sood A, Ratha SK, Prasanna R, Ahluwalia AS (2013b) Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. J Appl Phycol 25: 1529–1537

Rinta-Kanto JM, Wilhelm SW (2006) Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. Appl Environ Microbiol 72:5083–5085

Safonova E, Kvitko KV, Lankevitch MI et al (2004) Biotreatment of industrial wastewater by selected algal bacterial consortia. Eng Life Sci 4:347–353

Sekaran G, Karthikeyan S, Nagalakshmi C et al (2013) Integrated *Bacillus* sp. immobilized cell reactor and *Synechocystis* sp. algal reactor for the treatment of tannery wastewater. Environ Sci Pollut Res 20:281–291

Shankar AM, Henciya S, Malliga P (2013) Bioremediation of tannery effluent using fresh water cyanobacterium *Oscillatoria annae* with coir pith. Int J Environ Sci 3:1881–1890

Sharma R, Sharma V, Sharma MS et al (2011) Studies on limnological characteristic, planktonic diversity and fishes (species) in Lake Pichhola, Udaipur, Rajasthan (India). Univ J Environ Res Technol 1:274–285

Shi L, Cai Y, Chen Z et al (2010) Diversity and abundance of aerobic anoxygenic phototrophic bacteria in two cyanobacterial bloom-forming lakes in China. Ann Limnol Int J Lim 46:233–239

Sidhu MA, Ahluwalia AS (2011) Water quality and cyanobacterial diversity in Lower Western Himachal Lakes. Vegetos 24:165–170

Silva-Benavides AM, Torzillo G (2011) Nitrogen and phosphorous removal through laboratory batch cultures of microalgae *Chlorella vulgaris* and cyanobacterium *Planktothrix isothrix* grown as monoalgal and as co-cultures. J Appl Phycol 24:267–276

Sood A, Uniyal PK, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, *Azolla*. Ambio 41:122–137

Su Y, Mennerich A, Urban B (2012) Comparison of nutrient removal capacity and biomass settleability of four high-potential microalgal species. Bioresour Technol 124:157–162

Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl Environ Microbiol 69:5157–5169

Tiwari A, Chauhan SVS (2006) Seasonal phytoplanktonic diversity of Kitham lake, Agra. J Environ Biol 27:35–38

Vareli K, Pilidis G, Mavrogiorgou MC, Briasoulisbc E, Sainis I (2009) Molecular characterization of cyanobacterial diversity and yearly fluctuations of microcystin loads in a suburban Mediterranean Lake (Lake Pamvotis, Greece). J Environ Monit 11:1506–1512

Wei Q, Hu Z, Li G, Xiao B, Sun H, Tao M (2008) Removing nitrogen and phosphorus from simulated wastewater using algal biofilm technique. Front Environ Sci Engin China 2:446–451

Yang XE, Wu X, Hao HL, Zl H (2008) Mechanisms and assessment of water eutrophication. J Zhejiang Univ Sci B 9:197–209

Ye W, Liu X, Tan J et al (2009) Diversity and dynamics of microcystin—producing cyanobacteria in China's third largest lake, Lake Taihu. Harmful Algae 8:637–644

Zwart G, Kamst-van AMP, van der WI, Hagen F, Hoogveld HL, Gons HJ (2005) Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. Environ Microbiol 7:365–377

# 9 Phytoremediation Using Rhizobia

Clarisse Brígido and Bernard R. Glick

## 9.1 Rhizobia and Stress of Different Types

The Earth's population is expected to reach 9.1 billion by 2050 and, as a consequence, unprecedented increases in crop productivity will be needed in order to feed all of the world's people (FAO 2009). Furthermore, the climate changes and anthropogenic activities, such as urban development, road construction, industrial processes, mining, and inadequate agricultural practices, are resulting in the eutrophication and pollution of soils and fresh water resources, soil degradation, loss of soil fertility, and desertification (McLauchlan 2006; Spiertz 2010; Gordon et al. 2010). These factors will exacerbate the need for increases in crop production that must be achieved despite a significant deterioration of considerable prime agricultural land and likely utilizing large areas of land now considered marginal.

Plant growth is often limited under stressful conditions, and as a consequence, plant growth is invariably lower than it would be in their absence (Glick et al. 2007a). In general, plant growth may be inhibited or limited by different types of biotic or abiotic stresses, such as extremes of temperature, flooding, drought, heavy metals and other organic compounds, salinity and by the presence of phytopathogens (bacteria, fungi, or viruses) (Abeles et al. 1992). In this context, it is a challenge to increase the productivity of marginal soils through low-input sustainable agricultural practices.

Some soil bacteria, called plant growth-promoting bacteria or PGPB (Bashan and Holguin 1998), can help plants to grow by alleviating or avoiding the negative effects of both biotic and abiotic stresses on plant growth and their use has been suggested as a promising approach (Kloepper et al. 1989; Frommel et al. 1991; Glick 1995, 2012). PGPB can simply be defined as root colonizing bacteria that exert beneficial effects on plant development by direct mechanisms, indirect mechanisms, or a combination of the two (Glick 1995; Gupta et al. 2000). The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick et al. 1999). On the other hand, indirect promotion of plant growth occurs when PGPB decrease or prevent the deleterious effects of phytopathogenic microorganisms on plants by a variety of different mechanisms (Glick 1995, 2012; Glick et al. 1999; Cartieaux et al. 2003). PGPB may fix atmospheric nitrogen (N) and supply it to plants; synthesize and secrete siderophores which can solubilize and sequester iron from the soil and provide it to plant cells; synthesize different phytohormones, including auxins, cytokinins, and gibberellins; solubilize minerals such as phosphorus which then become more readily available for plant growth; and may synthesize an enzyme that can modulate plant ethylene levels (Brown 1974; Kloepper et al. 1989; Glick 1995, 2012; Patten and Glick 1996, 2002; Glick et al. 1999). These soil bacteria are extremely important in agricultural terms since when they are present in the plant rhizosphere they act as an environmentally friendly and less expensive means of improving soil health and promoting plant growth.

Within the PGPB group, there are soil bacteria, collectively known as rhizobia, able to form root nodules and fix atmospheric N in association with legumes. The relationship between rhizobia and legume plants has been studied for over 100 years. Historically, symbiotic rhizobia–legume associations were studied extensively, from physiological, biochemical, and molecular biological perspectives, as a classic example of mutualistic associations between two organisms. Currently, rhizobia include 13 genera with 98 species of α- and β-proteobacteria (Weir 2012), with a continuing

C. Brígido, Ph.D. (✉)
ICAAM-Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal
e-mail: ccb@uevora.pt

B.R. Glick (✉)
Department of Biology, University of Waterloo, Waterloo, ON, Canada, N21 3G1
e-mail: glick@sciborg.uwaterloo.ca

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increase in this number as new rhizobia are discovered. Rhizobia are studied largely due to their ability to efficiently fix atmospheric N and their highly efficient symbiotic association with legumes. Hence, the use of rhizobial inoculants is considered an important component of sustainable agriculture. Furthermore, the use of rhizobial inoculants is remarkably inexpensive compared to the use of N-chemical fertilizers and therefore important for sustainable agricultural practices in legume production in developing countries (Zahran 2006) where grain legumes are cultivated on a very large scale (Graham and Vance 2003). In addition, the use of efficient strains in the symbiotic rhizobium–legume association not only benefits the legume plants but also increases the soil fertility with fixed N, thereby enhancing crop productivity for subsequent cultivation of non-legumes.

The interaction between a legume and a rhizobium is highly regulated, and a great deal of communication occurs between the two organisms as the symbiosis is being established. Generally speaking, two main developmental processes are required for the formation of symbiotic N-fixing nodules: bacterial infection and nodule organogenesis (Gage 2004; Oldroyd and Downie 2008). These processes must be coordinated in both a spatial and a temporal manner to ensure nodule formation at the site of bacterial infection (Oldroyd and Downie 2008). However, these processes are very sensitive to several environmental stresses, such as fluctuations in pH, temperature, nutrient availability, and water deficiency which greatly influence their growth, survival and metabolic activity as well as their ability to enter into symbiotic associations. For example, early events in the symbiosis process such as molecular signaling, rhizobial attachment, root hair curling, infection thread formation, and nodule initiation, are particularly sensitive to high temperatures, salinity, acidity, heavy metals, and other environmental stresses (Graham et al. 1982; Zhang and Smith 1996; Ibekwe et al. 1997; Hungria and Stacey 1997; Hungria and Vargas 2000; Zheng et al. 2005). Moreover, during the infection process rhizobia also have to deal with adverse conditions within the host cells and with the plant's innate immunity which may interfere with the symbiosis (Soto et al. 2009). Nevertheless, some rhizobial strains have evolved or acquired various mechanisms in addition to its ability to fix atmospheric N, which allow them to counteract the negative effects associated with the environmental stresses, thereby contributing to the growth of leguminous plants under stressful conditions.

As a result, it should be possible to utilize some of the PGPB traits found in rhizobia to facilitate the use of rhizobia in phytoremediation protocols. Phytoremediation is the use of plants to remediate polluted soils, a sustainable and economical technology that is currently receiving considerable global attention (Glick 2010). However, among other considerations, the success of phytoremediation of metals relies on a plant's ability to tolerate the accumulation of high concentrations of metals, while yielding a large plant biomass (Grčman et al. 2001). Therefore, plant–microbe associations have been the objective of particular attention due to the potential of microorganisms to facilitate plant growth in the presence of metal stress or their effect on metal mobilization/immobilization, consequently enhancing metal uptake and plant growth. From this perspective, the use of the legume–rhizobium symbiosis as a tool for bioremediation of both metals (Sriprang et al. 2002, 2003; Pastor et al. 2003; Dary et al. 2010) and some organic compounds (Doty et al. 2003) is of great interest. Rhizobia can enhance phytoremediation through nitrogen fixation or by production of plant growth-promoting factors, and this can lead to increased soil fertility and to metal extraction or stabilization (Carrasco et al. 2005; Zheng et al. 2005; Ike et al. 2007; Pajuelo et al. 2008b; Dary et al. 2010).

The intrinsic potential of rhizobia to express high-level tolerance toward toxic metals along with their ability to transform atmospheric N into a usable form of N makes them one of the most important organisms in agronomic practices for legume improvement in soils polluted with metals. Moreover, rhizobia can promote the growth of plants at elevated concentrations of a heavy metal via mechanisms other than improved N nutrition (Reichman 2007). Rhizobia also may facilitate the growth and yield of legumes by other mechanisms, such as synthesis of siderophores and phytohormones (Boiero et al. 2007; Wani et al. 2007d; Avis et al. 2008), solubilization of inorganic phosphate (Khan et al. 2007; Ahmad et al. 2008), synthesis of ACC deaminase to lower ethylene levels (Tittabutr et al. 2008; Duan et al. 2009), and depression of plant diseases (Khan et al. 2002; Chandra et al. 2007). Thus, the potential use of rhizobia as plant growth-promoting rhizobacteria for the remediation of metal contaminated sites is a promising strategy for the improvement of legumes in metal contaminated soils. The mechanisms found in some rhizobia strains and their potential to enhance phytoremediation in legume plants is discussed in the following sections.

## 9.2 Rhizobial Mechanisms for Overcoming Stress

### 9.2.1 Auxins

Multiple mechanisms in rhizobia assisting development and growth of legumes may play an important role to help plants overcoming either biotic or abiotic stresses. Rhizobia, like other soil bacteria, produce a number of secondary metabolites that, at low concentrations can activate the stress response system in plants leading to higher resistance against pathogens as well as other stressors. One of the mechanisms that explain the direct effects of plant growth-promoting bacteria on plants is the production of plant growth regulators including auxins (such as indoleacetic acid or IAA) (Brown 1974; Patten and Glick 1996, 2002).

In plants, auxins play a central role in cell division, elongation, fruit development, and senescence (Phillips et al. 2011). However, plants under stress show a progressive decline in the level of IAA in their root system (Shakirova et al. 2003) and application of additional exogenous auxins supplies a sufficient amount of the hormone for normal plant development and growth in saline conditions (Kabar 1987; Gulnaz et al. 1999), contributing in the alleviation of the adverse effects of salt stress (Gul et al. 2000; Khan et al. 2004; Egamberdieva 2009). Thus, regulation of the auxin levels in plant roots may be a strategy to help plants to overcome stress.

In earlier works, it was suggested that root-colonizing bacteria that produce phytohormones, when bound to a developing seedling may act as a mechanism for plant growth stimulation (Frankenberger and Arshad 1995). These microorganisms when associated with various plants synthesize and release auxin as secondary metabolites because of the rich supplies of substrates exuded from the roots compared with non-rhizospheric soils (Kampert et al. 1975; Shahab et al. 2009). Today, it has been estimated that more than 80 % of the soil bacteria are able to produce auxins, especially IAA, indolebutyric acid, or similar compounds derived from tryptophan metabolism (Solano et al. 2008). IAA, one of the most studied phytohormones-produced by bacteria, works as a reciprocal signaling molecule in plant–microbe interactions (Ahmed and Hasnain 2010). Moreover, the majority of root associated bacteria that display beneficial effects on plant growth produce IAA (Hayat et al. 2010).

Many rhizobial strains are reported to produce auxins in variable amounts (Costacurta and Vanderleyden 1995; Frankenberger and Arshad 1995; Mehnaz and Lazarovits 2006; Vargas et al. 2009). It was also suggested that rhizobia are able to produce IAA via different pathways (see Spaepen and Vanderleyden 2011 for review the different pathways). In a study focused on interactions between rhizobacteria and the orchid *Dendrobium moschatum* revealed that strains belonging to the genus *Rhizobium* were among the most active IAA producers (Tsavkelova et al. 2007). It was also documented that nodulated plants contain higher concentrations of IAA compared to the non-nodulated ones (Hirsch and Fang 1994; Ghosh and Basu 2006) probably due to the high concentration of IAA produced by rhizobium in the nodules (Spaepen et al. 2007). Moreover, plant cells take up some of the IAA that is secreted by the bacteria and, together with the endogenous plant IAA, can stimulate plant cell proliferation and elongation (Glick et al. 2007b). It was also reported that IAA produced by rhizobia is transported to other parts of the plant and might be involved in several stages of the symbiotic relationship (Wheeler et al. 1979; Badenochjones et al. 1983; Hunter 1989). In the symbiotic rhizobium–legume association, production of IAA by rhizobia may play a fundamental role in nodulation and competitive ability. For example, Boiero et al. (2007) showed that IAA-synthesizing rhizobia are more effective at plant nodulation than IAA negative mutants. Ali et al. (2008) also observed that IAA significantly affected the length of mung bean, fresh and dry mass of roots and shoots, the number of nodules, and the nitrogenase activity. Also in *Rhizobium leguminosarum* bv. *viciae*, the introduction and overexpression of the indole-3-acetamide (IAM) biosynthetic pathway resulted in *Vicia hirsuta* root nodules containing up to 60-fold more IAA than nodules invoked by the wild-type strain as well as its nitrogen fixation capacity increased (Camerini et al. 2008). Interestingly, the augment of the IAA production may contribute to the increase of stress tolerance. For instance, the inoculation of *Medicago truncatula* plants with an *Ensifer meliloti* strain overexpressing the IAM biosynthetic pathway showed higher IAA content in nodules and roots and were more resistant to salt stress (Bianco and Defez 2009). In addition, inoculation of *M. truncatula* with the IAA-overproducing strain resulted in better plant growth under phosphorus deficiency because of the release of organic acids by the bacterium (Bianco and Defez 2010).

In non-legumes, IAA produced by rhizobia may stimulate plant root system, increasing its size and weight, branching number and the surface area in contact with soil, resulting in the development of more expansive root architecture (Dazzo and Yanni 2006). Inoculation with auxin-producing bacteria may also result in high plant IAA levels and the stimulation of adventitious root formation (Mayak et al. 1999; Solano et al. 2008). Plants with a more extensive root system have an increased ability to take up nutrients uptake from the soil (Mañero et al. 1996). However, bacterial IAA can also inhibit primary root growth (Schlindwein et al. 2008). In fact, IAA exerts a stimulatory effect on plant growth when it is within a specific concentration range, outside that range, plant growth is inhibit or unaffected. Additionally, exogenous IAA may also induce resistance in plants against some soil-borne diseases. For example, Fernández-Falcón et al. (2003) suggested that the exogenous application of IAA to banana plants induces resistance to Panama disease and that the resistance is more effective when performed using low doses and frequent applications of IAA. Similar result was obtained by Sharaf and Farrag (2004) where application of exogenous IAA reduced the infection rate of tomato plants by *Fusarium oxysporum lycopersici*. Interestingly, a low level of IAA was sufficient to stimulate rooting system development as well as to be helpful to plant mineral and nutrient uptake and bacterial colonization (Biswas et al. 2000). Altogether, the positive effects that IAA-producing rhizobia demonstrated on the success of their symbiosis may be an advantage to counteract the negative effects of stress on the both nodulation and nitrogen fixation processes.

### 9.2.2 Cytokinins

Cytokinins, like auxins, play an important role regulating various aspects of plant growth and development (Ferguson and Lessenger 2006; Frugier et al. 2008; Hussain and Hasnain 2011). Cytokinins are involved in the regulation of metabolite transport, cell division, and chloroplast differentiation; induce stem morphogenesis and retard leaf senescence; and in roots, cytokinins control the functioning of the aboveground organs (Kulaeva and Kusnetsov 2002; Romanov 2009). Plants grown under salt stress decrease the supply of cytokinin from root to shoot and also the recovery of diffusible auxin from maize coleoptile tips (Itai et al. 1968). In other studies the exogenous application of plant growth regulators, including cytokinins, produced some benefit in alleviating the adverse effects of salt stress and they also improve germination, growth, fruit setting, fresh vegetable and seed yields, and yield quality (Dhingra and Varghese 1985; Khan and Weber 1986; Gul et al. 2000). A number of bacterial genera from plant rhizosphere, including both phytopathogenic and plant growth-promoting bacteria, have been reported to produce cytokinins (de Salamone et al. 2001; Tsavkelova et al. 2006; Pertry et al. 2009), In contrast to auxins production, PGPB able to produce cytokinins are less common, possibly because there is no simple method available to detect and quantify cytokinins; thus, there are only a limited number of reports of bacterial cytokinins affecting plant growth. In rhizobia, the production of cytokinin, however, should be a more common trait, since this hormone is involved in nodule development, as it is required to initiate the cortical cell divisions necessary to form a root nodule, and may also mediate rhizobial infection in legumes (Frugier et al. 2008). To date, several studies indicate the involvement of cytokinin in the development of the symbiosis between legume plants and nodule bacteria. Oldroyd (2007) reported that cytokinin production in plants is enhanced by rhizobia through regulation of the expression of Nod factor pathway, acting as a mechanism to coordinate epidermal and cortical responses during nodule formation. However, the activation of the cell cycle by cytokinins, which lead to cortical-cell division, is not fully understood.

Treatment of plants with exogenous cytokinins induces expression of early nodulin genes related to nodulation (Dehio and Debruijn 1992; Bauer et al. 1996; Fang and Hirsch 1998; Ferguson and Mathesius 2003) and the control of a pre-infection stage, recognition, is determined by highly specific plant Nod signals, possibly, in combination with cytokinins (Oldroyd 2007). Heckmann et al. (2011) verified that exogenous cytokinin induced formation of discrete and easily visible nodule primordial in *Lotus japonicum* roots, suggesting that cytokinins may partially replace Nod factors (Bauer et al. 1996). Some researchers have noted a direct relationship between cytokinins concentration and the process of nodulation (Pavlova and Lutova 2000; Akimova and Sokolova 2012). These results demonstrate that cytokinins aren't only involved in nodule formation but also in rhizobial infection via local and systemic mechanisms (Frugier et al. 2008; Heckmann et al. 2011). Taken together, these data demonstrate that cytokinins play an important role in symbiotic relations; they may act as a secondary signal that is synthesized in epidermal cells perceiving the Nod factor and is then translocated to underlying cortex cells (Murray et al. 2007; Oldroyd 2007; Tirichine et al. 2007; Frugier et al. 2008). In addition, Nod factors are known as the initial communication signals between rhizobia and leguminous plants, however under stressed conditions the production of Nod factors may be stimulated or suppressed which may affect the rhizobium–legumes symbioses. For example, the production of Nod factors by *Rhizobium leguminosarum* bv. *Trifolii* have been found to be disrupted by pH, temperature, and both P and N concentration (McKay and Djordjevic 1993). Thus, cytokinins produced by rhizobia may be an advantage or an alternative pathway to counteract the negative effects caused by the different types of stresses on Nod factors biosynthesis, which is an essential step for the successful establishment of a symbiotic relationship between both partners.

### 9.2.3 Ethylene

A well-established mechanism that promotes plant growth and development is the modulation of the ethylene levels in plant tissues. In fact, the efficacy of a large number of PGPB in promoting growth and productivity of plants, especially under stress conditions, via lowering plant ethylene levels has been demonstrated (Burd et al. 2000; Glick 2005; Safronova et al. 2006; Shaharoona et al. 2006b). Ethylene is a gaseous plant hormone produced endogenously by almost all plants that plays a key role in inducing multifarious physiological changes in plants aspects of fruit ripening, seed germination, tissue differentiation, the formation of root and shoot primordia, lateral bud development, leaf abscission, flowering wilting, and the response of plants to both biotic and abiotic stresses (Abeles et al. 1992). Under stress conditions, the endogenous production of ethylene is substantially accelerated which adversely affects the growth of a plant (Abeles et al. 1992). Therefore, for normal growth and development regulation of ethylene production in plant tissues is essential (Safronova et al. 2006).

Two naturally occurring mechanisms that can modulate plant ethylene levels are the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and the production of the ACC synthase enzyme inhibitor rhizobitoxine. Some bacteria are able to decrease the ethylene levels in plant root (and shoot) tissue mediated by the bacterial enzyme ACC deaminase, through the cleavage of ACC (the immediate precursor of ethylene in plants) to ammonia and α-ketobutyrate, both of which are readily metabolized by the bacteria. Glick et al. (1998) proposed a model that explains the role of ACC deaminase in plant growth promotion. Briefly, PGPB that express the enzyme ACC deaminase bind to either the seed coat or root of a developing plant. In this way, these bacteria act as a sink for ACC, lowering ethylene levels in plant tissues, and thereby increasing the growth of plant roots and shoots and reducing the inhibitory effects of ethylene synthesis (Glick et al. 1998; Glick 2004). On the other hand, rhizobitoxine, an enol-ether amino acid (enzyme inhibitor), inhibits the enzyme ACC synthase, one of the key enzymes in the ethylene biosynthetic pathway, and thus decreases the ethylene levels in plants (Yuhashi et al. 2000). Therefore, the use of bacteria, which possess one of these mechanisms, can help in sustaining plant growth and development, especially under stress conditions.

In case of leguminous plants, ethylene is also known for its negative role in nodulation (Ma et al. 2002; Middleton et al. 2007), as it inhibits the formation and functioning of nodules (Duodu et al. 1999; Nandwal et al. 2007; Ding and Oldroyd 2009). Ethylene may also be involved in several phases of symbiosis, including the initial response to bacterial Nod factors, nodule development, senescence, and abscission (Csukasi et al. 2009; Patrick et al. 2009). Although not all legumes respond similarly, addition of exogenous ethylene to most nodulating plants reduces the frequency of nodule primordia formation (Nukui et al. 2000; Oldroyd et al. 2001). Ethylene controls the epidermal responses during the nodulation process and thus negatively regulates multiple epidermal responses in order to inhibit rhizobial infection (Nukui et al. 2004; Sugawara et al. 2006). Ethylene is also involved in the development of infection threads, especially the infection thread initiation and elongation (Gage 2004). In the presence of ethylene, the number of infected root hairs does not change; however, many infection threads are aborted and the epidermis or outer cortex and nodule primordia does not form (Lee and Larue 1992). This leads to a reduction in infection as well as in the number of nodules in legumes. It was shown that endogenous ethylene interferes with nodulation in legumes (Prayitno et al. 2006). Ethylene production significantly increases in roots infected by *Rhizobium* or *Bradyrhizobium* and decreases the number of nodules that form on the infected plants (Gonzalez-Rizzo et al. 2006; Middleton et al. 2007). Due to the importance of the nodulation process, it is essential to regulate the ethylene level in the plant roots in order to achieve a successful symbiotic association (Lohar et al. 2009). By inhibiting ACC synthase and reducing the production of ethylene, rhizobitoxine is responsible for sustained plant growth, development and productivity even under unfavorable conditions. For example, *Bradyrhizobium elkanii* is known to suppress ethylene biosynthesis in the host plant *Mactroptilium atropurpureum* and enhance nodulation by producing rhizobitoxine (Yuhashi et al. 2000; Yasuta et al. 2001). Similar to the rhizobitoxine effect, several bacteria including both rhizobia and free-living bacteria containing ACC deaminase that are able to lower ethylene synthesis by degrading its precursor ACC have also been reported to promote nodulation in leguminous plants (Ma et al. 2004; Shaharoona et al. 2006a; Saleem et al. 2007; Nascimento et al. 2012a). Furthermore, it has been reported that ACC deaminase-producing rhizobial cells reduce ethylene concentrations in the infection threads and increase the persistence of infection threads by suppressing the defense signals in the plant cells (Ma et al. 2004). Therefore, ACC deaminase activity or rhizobitoxine production may be helpful in sustain plant growth and development by reducing stress-induced ethylene production and thereby increase the nitrogen supply for legume plants due to a more effective nodulation. This may have particularly interest when plants are growing under stressful conditions and when ethylene may reach to levels that may inhibit nodulation. For instance, several rhizobial species including *Mesorhizobium*, *Sinorhizobium*, and *Rhizobium* species were reported to possess ACC deaminase (Sullivan et al. 2002; Ma et al. 2003a; Di Gregorio et al. 2006), which in some cases was shown to assist plant survival and metal uptake.

Moreover, inoculation of various plant species with those bacteria possessing ACC deaminase activity lead to increased root growth and/or enhanced formation of lateral roots and root hairs. For instance, strains of *R. leguminosarum* bv. *viciae* and *M. loti* expressing ACC deaminase increased the number of lateral roots in *Arabidopsis thaliana* (Contesto et al. 2008). In addition, an augment of the root system was observed in chickpea plants when inoculated with an ACC deaminase-transformed *M. ciceri* strain LMS-1 either in control or stress conditions (Nascimento et al. 2012a, c). In addition, it is well established that changes in root morphology are common adaptation mechanisms of plants exposed to environmental stresses (Potters et al. 2007), such as extremes temperature, high salinity, drought, and nutrient deficiency, a process in which phytohormones are known to play a key role (Spaepen et al. 2007). Furthermore, phytohormones, such as auxins and ethylene, play a role in plant responses to heavy-metals (Hong-Bo et al. 2010). Therefore, production of ACC deaminase and IAA is likely to be an important and efficient way for rhizobia to manipulate their plant hosts especially under either biotic or abiotic stresses.

### 9.2.4 Siderophores

Bacterial activities that facilitate the uptake of nutrients by plants can facilitate plant growth under stressful conditions. These activities include the production of siderophores and the solubilization of phosphates.

Iron, an element essential for growth, is mostly unavailable for direct assimilation by plants, because it is mainly present in soil in a hard-to-solubilize mineral form, $Fe^{3+}$. This form is also unavailable to bacteria which, in order to obtain iron for their growth and development, release low-molecular weight iron-binding molecules, or siderophores (Hilder and Kong 2010). Siderophore-producing bacteria can promote plant growth either directly by improving plant iron nutrition, or indirectly by inhibiting the growth of pathogens in the rhizosphere by limiting their iron availability (Glick 1995; Solano et al. 2008; Ma et al. 2011a). Most rhizobial biofertilizer strains are poor rhizospheric colonizers due to their inability to compete with the indigenous soil microflora for nutrients, the major one being iron, in the iron-limited soil environment (Verma and Long 1983). Some free-living rhizobia and bradyrhizobia not only produce and import their own siderophores but also benefit from utilization of heterologous siderophores (produced by other microorganisms) present in the soil. For example, *Bradyrhizobium japonicum* strain 61A152, a citrate producer (Guerinot et al. 1990), is able to utilize iron bound to hydroxamate-type siderophores like ferrichrome and rhodotorulic acid, produced by soil fungi (Plessner et al. 1993). Utilization of heterologous siderophores is considered to be an important mechanism to attain iron sufficiency as well as in the suppression of plant pathogens. In fact, there are several reports of the synthesis of siderophores and its subsequent inhibition of pathogen growth in the rhizosphere by siderophore-producing rhizobia (Sessitsch et al. 2002; Chandra et al. 2007; Ahemad and Khan 2010). It was also reported that soil bacteria were induced to synthesize siderophores in heavy metal-contaminated soils because of Fe deficiency (Imsande 1998; Glick 2003). Furthermore, the siderophores produced by soil bacteria, including rhizobia (Rajkumar and Freitas 2008; Wani et al. 2007d, 2008a, b), are used as metal chelating agents that regulate the availability of iron and in turns help plants to alleviate the toxicity of metals. Although plants are also able to produce siderophores, these siderophores bind to iron with much lower affinity compared to the ones produced by bacteria (Glick 2003). Therefore, microbial siderophores play an important role in sequestering metals, as reported by Braud et al. (2009), where the production of pyoverdin and pyochelin by *Pseudomonas aeruginosa* increased the concentrations of bioavailable Cr and Pb in the rhizosphere, thus making them available for maize uptake. Likewise, Tank and Saraf (2009) observed that the inoculation with Ni resistant-siderophore producing *Pseudomonas* increased the plant growth and reduced Ni uptake in chickpea plants. Considering all these data, it can be hypothesized that by inoculating the plants with siderophore-producing microbes, it should be possible to improve plant growth in metal-contaminated soils.

### 9.2.5 Phosphorus

Phosphorus (P) is one of the major mineral nutrients required by plants whose deficiency is often limiting for crop production. In nature, P is found in a variety of organic and inorganic forms that are very poorly soluble. It is one of the less soluble elements in the natural environment, with less than 5 % of the total soil P content being available to plants (Dobbelaere et al. 2003). Furthermore, in acid soils and in mining or metal-polluted areas, most of P present in soil is immobilized and thus unavailable for plants (Rodriguez and Fraga 1999; Shilev et al. 2001). Many soil microorganisms can solubilize mineral P, generally via the production of organic acids (Zaidi et al. 2009), so it is thought that solubilization of P is more efficient in basic soils than in naturally acid soils (Solano et al. 2008). It was estimated that these microorganisms constitute about 20–40 % of the cultivable population of soil microorganisms and that a significant proportion of them can be isolated from rhizosphere soil (Chabot et al. 1993).

According to Rodríguez and Fraga (1999), the genus *Rhizobium* is one of the major P solubilizers. Like *Rhizobium* species, other rhizobia also possess this trait, suggesting that this trait is a common feature among rhizobia. For example, isolates belonging to the genera *Bradyrhizobium*, *Mesorhizobium*, *Ensifer*, and *Rhizobium* all have the ability to solubilize inorganic and organic P under in vitro conditions (Alikhani et al. 2006). However, these rhizobial isolates differ in their P-solubilizing ability. In this study, *R. leguminosarum* bv. *viciae* was the most prominent P solubilizer, followed by *M. ciceri*, *M. mediterraneum*, *E. meliloti*, and *R. leguminosarum* bv. *phaseoli*. Interestingly, none of the 70 strains of *Bradyrhizobium* tested were able to solubilize inorganic P, which confirms previous studies (Antoun et al. 1998). Also, the two species nodulating chickpea, *M. ciceri* and *M. mediterraneum*, are known as good phosphate solubilizers (Rivas et al. 2006). The results of in vitro tests for P solubilization are, however, not always related to effects in vivo, which makes the screening for PGP activity difficult. On the other hand, the results obtained in vitro are sometimes similar to the results obtained following plant inoculation. For example, the most efficient P solubilizer strain, PECA21, selected among several rhizobia belonging to different genera, as expected improved the P content of chickpea and barley plants as well as their dry matter, N, K, Ca, and Mg content (Peix et al. 2001).

In addition, it was also suggested that co-inoculation with P-solubilizing bacteria and rhizobia may enhance phosphate availability and thus improve the plant growth. For example, Taurian et al. (2013) verified that co-inoculation with a native P-solubilizing strain *Pantoea* sp. J49 and *Bradyrhizobium* resulted in promotion of peanut plant growth mostly probably due to PGPB's ability to solubilize phosphate.

Similarly, co-inoculation with *Rhizobium* and *Bacillus* sp. enhanced the availability of phosphate and exerted positive effects on the growth and yield of wheat (Akhtar et al. 2013). Furthermore, co-inoculation with *Pseudomonas* solubilizing-P and rhizobia resulted in some positive adaptative responses of maize plants under salinity (Bano and Fatima 2009). Another example of the role of P solubilization was observed by Wani et al. (2007c) where the high P solubilization (via pH reduction) by *Bacillus* sp. PSB1 strain resulted in the mobilization of large amounts of both Pb and Zn. Altogether, solubilization of phosphate through soil bacteria may be useful to help plants in P-acquisition and thus promoting plant growth and vigor, especially in soils where P is scarce or limited due to an imbalance with other nutrients, as in the case of hydrocarbon contaminated soils (Hall et al. 2011).

## 9.3 Lowering Ethylene Levels in Stressed Plants

As mentioned above, ethylene levels in plant tissues increase as part of a plant's response to different types of stresses, such as extreme temperatures, water stress, ultraviolet light, insect damage, disease, and mechanical wounding, all of which may cause some stress symptoms as well as inducing defense responses in the plant. In addition, ethylene has been reported as an autoregulator of the nodulation process (Oldroyd and Downie 2008). It is well established that high concentrations of ACC or ethylene in root tissues negatively affect the nodulation process in legume plants (Ma et al. 2004; Middleton et al. 2007). On the other hand, inhibitors of ethylene synthesis or its physiological activity enhances nodulation (Tirichine et al. 2006). These studies clearly demonstrate that ethylene acts as a negative regulator of nodulation, and reduction in ethylene concentration has a stimulatory effect on the formation and development of nodules in legumes (Ding and Oldroyd 2009). Thus, a potential target for normal or extreme root growth is to regulate or limit the biosynthesis of ethylene. This can be achieved by the use of soil microbes with the ability to reduce ethylene levels in plant roots, through different mechanisms. In rhizobia, two alternative mechanisms to lower ethylene levels in plant roots have been identified: (1) inhibition of the enzyme ACC synthase or (2) cleavage of ACC into ammonia and α-ketobutyrate.

### 9.3.1 Rhizobitoxine

Rhizobitoxine, an enol-ether amino acid [2-amino-4-(2-amino-3-hydroxypropoxy)-trans-3-butenoic acid] is a naturally occurring structural analog of the commonly used ethylene inhibitor aminoethoxyvinylglycine (AVG). In recent years, rhizobitoxine has been implicated in inducing a positive effect on the legume–rhizobium symbiosis although when it was first discovered it was regarded as a phytotoxin. Rhizobitoxine blocks ethylene synthesis in two ways; first, it inhibits β-cystathionase necessary for methionine biosynthesis (Sugawara et al. 2006) and second, it inhibits the enzyme ACC synthase in the ethylene biosynthesis pathway (Yasuta et al. 1999; Yuhashi et al. 2000; Sugawara et al. 2006; Tittabutr et al. 2008). By inhibiting ACC synthase, rhizobitoxine reduces the production of ethylene in plant roots.

In the genus *Bradyrhizobium*, slow-growing *Bradyrhizobium* strains generally produce rhizobitoxine (Minamisawa et al. 1997). Interestingly, among bradyrhizobia able to produce rhizobitoxine, *B. elkanii* accumulates rhizobitoxine in cultures and in nodules, while *B. japonicum* does not (Kuykendall et al. 1992). Rhizobial spp. which produce rhizobitoxine (Owens et al. 1972; Ratcliff and Denison 2009), have been found to be relatively more effective in enhancing nodulation and competitiveness for nodule formation (Duodu et al. 1999; Okazaki et al. 2003, 2004; Sugawara et al. 2006). For example, enhanced nodulation and competitiveness of *B. elkanii* variants producing rhizobitoxine has been established in *Amphicarpaea edgeworthii* and *Vigna radiata* (Parker and Peters 2001) and in *Macroptilium atropurpureum* (Yuhashi et al. 2000). Rhizobitoxine not only promotes nodulation but also benefits rhizobia living inside nodules by allowing more rhizobial reproduction or by enhancing the synthesis of poly-3-hydroxybutyrate to support lateral reproduction (Ruan and Peters 1992; Ratcliff et al. 2008). However, the effect of rhizobitoxine on nodulation has been contradictory and is both legume- and rhizobia-dependent. For example, nodulation of *Glycine max* is generally not sensitive to ethylene (Xie et al. 1996; Schmidt et al. 1999), while nodulation of *Vigna radiata* is sensitive (Duodu et al. 1999). Other reports have shown that there is not a significant difference in nodule number between plants inoculated with *B. elkanii* USDA61 and plants inoculated with rhizobitoxine-deficient mutants during nodulation of *G. max*, *G. soja*, *V. unguiculata*, and *M. atropurpureum* (Xiong and Fuhrmann 1996). It is possible that variances in the performance of rhizobitoxine may be due to the differences in the abilities of the legume genotypes and rhizobial strains forming symbiosis with their host plant. In contrast, the rhizobitoxine producing strain BS KT-24 is considered to exhibit better survival and nodulation protection besides competitiveness for pigeon pea and other legumes grown under abiotic stress (Kanika et al. 2010). From this perspective, rhizobitoxine is thereby responsible for sustained plant growth, development, and productivity even under unfavorable conditions. Rhizobitoxine production by rhizobium is of interest for its application in the development of rhizobial inoculants as a successful rhizobial inoculant not only has to be a superior nitrogen fixer but also has to possess greater competitiveness when compared to indigenous strains. Overall, rhizobitoxine-producing strains exhibit better survival, nodulation, and competitiveness for legumes grown under abiotic stress.

### 9.3.2 ACC Deaminase

ACC deaminase (EC 4.1.99.1) is a multimeric sulfhdryl enzyme with a monomeric subunit molecular mass of approximately 35–42 kDa. This enzyme can cleave ACC into ammonia and α-ketobutyrate (Honma and Shimomura 1978). Moreover, it is widespread among a varied range of bacterial and fungal strains, and may also be found in some plants (Glick et al. 2007a; McDonnell et al. 2009). In some strains, the ACC deaminase (encoded by *acdS* gene) has been isolated and characterized. A wide range in the level of ACC deaminase is found within different bacteria. For example, rhizobia that express the enzyme ACC deaminase typically exhibit only a low level of enzyme activity compared with free-living plant growth-promoting bacteria (i.e., 10- to 30-fold less than free-living bacteria). This suggests that there may be at least two types of ACC deaminase-producing bacteria (Glick and Stearns 2011). There are free-living bacteria that bind relatively nonspecifically to plant roots and have a high level of ACC deaminase activity, protecting plants from different stresses by lowering ethylene levels throughout the plant. Alternatively, rhizobia bind tightly to the roots of specific plants and have a low level of enzyme activity that facilitates nodulation by locally lowering ethylene levels (Ma et al. 2003b; Okazaki et al. 2004). Despite the difference in the level of ACC deaminase activity, the ACC deaminase activity in rhizobial strains is sufficient to facilitate the nodulation process in the host plants; however, it is generally insufficient to decrease the high levels of ethylene formed in plant roots due to various environmental stresses. Even within rhizobial strains, which have been identified as expressing ACC deaminase activity under free-living conditions, the extent of ACC deaminase activity in different strains of rhizobia varies greatly (Duan et al. 2009).

Genes encoding ACC deaminase have been reported in many rhizobial species (Kaneko et al. 2000, 2002; Sullivan et al. 2002; Ma et al. 2003a, 2004; Nukui et al. 2004; Contesto et al. 2008; Duan et al. 2009; Farajzadeh et al. 2010; Nascimento et al. 2012b). Although the presence of the *acdS* gene in several rhizobia strains, not all strains display enzyme activity when it is induced by ACC, suggesting diverse types of regulation or requires different elements for induction. Presently two different modes of regulation of the *acdS* gene have been hypothesized. One, and apparently the most common among rhizobacteria, is through the leucine-responsive regulatory protein-like gene (lrpL) that is present in several *Rhizobium* spp. strains (Ma et al. 2003b, 2004). Other, and particularly reported only in strains belonging to the genus *Mesorhizobium*, is through the transcriptional regulation of the NifA2 protein (Nukui et al. 2006) exclusively under symbiotic conditions (Uchiumi et al. 2004; Nukui et al. 2006; Nascimento et al. 2012b). Nevertheless, in both cases the ACC deaminase knockout mutant strains showed a decreased ability to nodulate its host plant when compared to its respective wild-type strain (Ma et al. 2003b; Uchiumi et al. 2004), indicating that the presence of such gene improves symbiotic efficiency and increases nodulation in legumes.

Furthermore, an improvement of both nodulation efficiency and bacterial competiveness was obtained in *Lotus* spp. plants when they were inoculated with strain *M. loti* MAFF303099 that had been genetically transformed to constitutively express an exogenous copy of the ACC deaminase gene (Conforte et al. 2010). Similarly, expression of an exogenous *acdS* gene in either *E. meliloti* strains or *Rhizobium* sp. strain TAL1145 increased their nodulation abilities in alfalfa and *Leucaena leucocephala*, respectively (Ma et al. 2004; Tittabutr et al. 2008), suggesting that modulation of the ethylene levels in root tissues through ACC deaminase is an effective strategy to increase nodulation and competitiveness of the bacterium. Another strategy to increase nodulation is the use of a combination of rhizobial strains and ACC deaminase-containing rhizobacteria. For example, Remans et al. (2007) used specific PGPB mutant strains for co-inoculation along with rhizobia and observed that PGPB ACC deaminase activity played an important role in enhancing nodulation in common beans. Further, Shaharoona et al. (2006a) reported that co-inoculation with a PGPB carrying ACC deaminase activity and *Bradyrhizobium japonicum* resulted in up to 48 % better nodulation in mung bean plants compared with *B. japonicum* alone. Besides co-inoculation with ACC deaminase-containing PGPB and rhizobia promotes nodulation, it was also reported that by adjusting ethylene levels, an improvement of plant growth and yield was obtained in different plants even when under stress conditions. For example, co-inoculation of plants with rhizobia and ACC deaminase-containing rhizobacteria strains enhanced nodulation and plant growth (Dey et al. 2004) even under stress conditions (Ahmad et al. 2011). Similar results were obtained with chickpea and lentil plants when inoculated with a consortium of rhizobia and rhizospheric bacteria with high ACC deaminase activity (Shahzad et al. 2010; Zahir et al. 2011). Furthermore, plants inoculated with bacteria containing ACC deaminase have been found resistant to the harmful effects of stress ethylene, generated under undesirable environments, including in the presence of metals (e.g., Grichko and Glick 2001; Mayak et al. 2004; Reed and Glick 2005; Cheng et al. 2007; Hao et al. 2007; Bonfante and Anca 2009; Belimov et al. 2009). Taking all these results into account, it is possible to conclude that PGPB containing ACC deaminase can be employed to improve the resistance of plants to environmental stresses by lowering the content of stress-induced ethylene in plants. Furthermore, rhizobia expressing ACC deaminase naturally or through genetically engineering, or in co-inoculation with ACC deaminase-containing rhizobacteria are more competitive and increase nodulation in legumes, and consequently contribute to plant growth and development.

## 9.4 Phytoremediation with Rhizobia

### 9.4.1 Examples

At low concentrations, many metals and metalloids can serve as important components in life processes, often involved in important enzyme functions. However, above certain threshold concentrations, these metals can become toxic and induce morphological and physiological changes in microbial communities (Frostegard et al. 1996), to nitrogen fixers (Chaudri et al. 2000; Pereira et al. 2006a; Paudyal et al. 2007) and to the growth and development of various agronomic crops (Bose and Bhattacharyya 2008; Liu et al. 2009) including legumes (Wani et al. 2007a, 2008a). It was reported that the presence of metals in high concentration in soils has substantial deleterious effects on both survival and nitrogen-fixing efficiency of symbiotic rhizobia (Alexander et al. 1999; Broos et al. 2004; Younis 2007). For example, it was observed that there was a reduction in the population of *Rhizobium leguminosarum* bv. *trifolii* able to form a symbiosis with white clover (*Trifolium repens* L.) grown in soil polluted with metals (McGrath et al. 1988). In other studies, nitrogen-fixing rhizobia could survive in metal contaminated soils but failed to fix N with clover plants (Giller et al. 1989; Hirsch et al. 1993). In addition, nitrogen fixation decayed significantly in white clover (Broos et al. 2004), chickpea (Wani et al. 2007a), greengram (Wani et al. 2007b), pea (Wani et al. 2008c), and lentil (Wani et al. 2008d) when grown in soils treated with metals. Nevertheless, reports of changes in rhizobial populations due to high concentration of metals as well as effects of metals on legume plants are conflicting (Wani et al. 2008a, b). For instance, Paudyal et al. (2007) revealed that aluminum, even in small concentrations had negative effects on rhizobial growth, while other studies only observed inhibition of rhizobial strains multiplication at elevated Al concentrations (Wood and Cooper 1988; Chaudri et al. 1993; Broos et al. 2004). In addition, following metals uptake by plants and translocation to various organs, they can directly interact with cellular components and disrupt metabolic activities causing cellular injury or even plant death. For example, cadmium has an adverse effect on legume nodule metabolism even at low concentrations (Pereira et al. 2006b; Younis 2007), and it inhibits nitrogenase activity as well as affects legume metabolic activities (Balestrasse et al. 2004; Bibi and Hussain 2005; Noriega et al. 2007). Although toxic levels of metals are known to reduce the formation of root nodules in legumes as well as the nitrogen fixation efficiency, once symbiosis is established, metals may accumulate in nodules and therefore be an alternative and less expensive method to remove metals from the soil.

Because of the importance of legumes and their associated rhizobial bacteria as components of the biogeochemical cycles in agricultural and natural ecosystems, it is important to use both symbiotic partners with some degree of tolerance to metals to achieve a successful symbiotic interaction in metal contaminated soils. Of the two symbiotically interacting partners, rhizobia in particular is reported to tolerate higher levels of metals (Wani et al. 2009) and hence could help to remediate heavy metal polluted soils besides providing a good system to understand metal–microbe interactions (Ike et al. 2007). Since nitrogen fixation is considered to be the most important trait of rhizobia compared to other microbes used in metal phytoremediation, metal tolerance ensures rhizobia survival and formation of effective legume, and therefore improved plant growth under such conditions.

Legumes have been found to be the dominant portion of the plant species that survive in long-term metal-contaminated soils (Del Rio et al. 2002). Some legume plants, including species of the genera *Vicia*, *Cytisus*, *Astragalus*, *Lupinus* are known to grow on soils polluted by relatively high concentrations of metals (Prasad and Freitas 2003). For instance, legumes have been identified as naturally occurring pioneer species on As contaminated sites (Carrasco et al. 2005; Reichman 2007) and free-living rhizobia are commonly found in soils with high As (Carrasco et al. 2005). Although root nodulation of inoculated legumes grown in As contaminated soil is generally significantly reduced or absent (Carrasco et al. 2005; Mench et al. 2006), the rhizobium–legume interaction has been used to remediate soils contaminated with As and other metals (Pajuelo et al. 2008a; Mandal et al. 2008). Alfalfa (*Medicago sativa* L.), the most widely grown perennial legume in the world, is a deep-rooted perennial species that may have strong potential for the remediation of a number of organic contaminants (Muratova et al. 2003; Chekol et al. 2004), mainly due to its ability to grow and uptake heavy metals in low pH soils (Peralta-Videa et al. 2002, 2004). For example, results using the symbiotic alfalfa–rhizobium association suggest that this relationship can stimulate the rhizosphere microflora to degrade polycyclic aromatic hydrocarbons (PAH) and its application may be a promising bioremediation strategy for aged PAH-contaminated soils (Teng et al. 2011). In spite of some legumes being tolerant to various metals, most of them fall into the category of metal excluders that accumulate only very low concentrations of metals in shoots and an almost undetectable amount in grains (Wani et al. 2008a). In fact, plants used to clean up metal polluted soils should exhibit two basic properties: (1) they must be able to take up and accumulate high concentration of metals, and (2) they must be able to produce a large biomass.

Rhizobia, particularly those with both increase metal resistance and plant growth-promoting abilities are of great interest for their ability to help in the phytoremediation of metals by increasing plant growth and development. To begin with, several reports indicate that rhizobial strains belonging to different genera including *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Ensifer*, and *Rhizobium* are metal resistant when they are isolated from metal contaminated soils (Figueira et al. 2005; Zheng et al. 2005; Chaintreuil et al. 2007; Wani et al. 2008a, 2009; Mandal et al. 2008; Pajuelo et al. 2008a; Vidal et al. 2009). In fact, metal tolerant rhizobial strains can effectively nodulate their host plant and increase the metal levels in the plant (Martensson and Witter 1990; Obbard and Jones 1993). More recently, it has been shown that a *Ensifer meliloti* strain with high tolerance to As is able to form effective symbiosis with *Medicago sativa* (Pajuelo et al. 2008a). In other studies, rhizobial species isolated from nodules of greengram (*Bradyrhizobium* spp.), lentil (*Rhizobium* spp.), chickpea (*Mesorhizobium* spp.), and pea (*Rhizobium* spp.) have shown greater tolerance to one or more metals and, when tested in a greenhouse environment, substantially increased the growth, symbiotic properties, and nutrient uptake of inoculated legumes grown in metal treated soils (Wani et al. 2008a, b, d, 2009). Furthermore, a substantial reduction in metal uptake by various plant organs was also observed in the inoculated legumes, which in turn decreased the metal toxicity and consequently improved the overall performance of legumes in metal contaminated soils. In addition, in *Mimosa pudica*, the uptake of Pb, Cu, and Cd was enhanced in inoculated plants with *Cupriavidus taiwanensis* compared to non-inoculated plants (Chen et al. 2008) revealing the efficacy of using nodulated plants to remove metals. Altogether, nodules could serve as metal buffer areas which provide plants with an extra place to stock metals and reduce the risk of direct exposure of the plant to metals, suggesting that in addition to fixing nitrogen, the rhizobial strains could also assist plant growth via adsorption and tolerance to metals (Mamaril et al. 1997). In addition, Wani and Khan (2013) isolated a rhizobium strain RL9 possessing not only high tolerance to several heavy metals but also plant growth-promoting traits, such as production of IAA and siderophores. It was observed that lentil plants inoculated with this strain and grown in the presence of nickel had higher growth, nodulation, chlorophyll, leghemoglobin, nitrogen content, seed protein, and yield compared to plants grown in the absence of bioinoculant. Also, strain RL9 decreased the nickel uptake in lentil plants compared to plants grown in the absence of bioinoculant. Overall, it appears those strains that nodulate their hosts may increase metal accumulation in root nodules, while those that remain in the rhizosphere may reduce metal toxicity in the rhizosphere through different mechanisms. For example, it was suggested that released exopolysaccharides (EPS) could bind to a variety of metals, such as Mn(II), and consequently serve as a bioremediation tool, as reported for zinc uptake by hyperaccumulator plant *Thlaspi caerulescens* (Lopareva and Goncharova 2007). The EPS is considered one of the factors involved in protection of rhizobial species from stressed environments (Lopareva and Goncharova 2007).

In some cases, increased biomass in inoculated plants is also due to plant growth-promoting traits expressed by the bacterial partners during symbiosis. Thus, the potential of rhizobia in metal resistance/reduction and their ability to facilitate legume growth by several mechanisms other than nitrogen fixation in metal-stressed soil make them a suitable choice for cleanup of the metal contaminated sites and hence may further help in reducing problems associated with the legumes when grown in ruined soils.

In a recent study, a screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) revealed that the positive effects of these strains on shoot and root weight and nutrient uptake of maize plants were attributed to IAA production, phosphate solubilization, or even other less known traits that stimulate plant growth (Arruda et al. 2013). Many PGPB, including rhizobia, are reported to possess two or more plant growth-promoting properties; these traits may interact synergistically, e.g., IAA synthesis and ACC deaminase, IAA synthesis and P solubilization or IAA synthesis and siderophore production. However, precisely how this occurs is as yet unclear. Also, the regulation of plant hormone levels, such as either by reducing the high levels of ethylene or by the synthesis of auxin or cytokinins, may result in plant growth promotion, since they are implicated in virtually all aspects of plant growth and development, ranging from seed germination to shoot growth and leaf abscission. In fact, apparently both mechanisms are intimately linked. The bacterial IAA production stimulates the activity of the enzyme ACC deaminase involved in the degradation of the ethylene precursor ACC and in turn the lowering of ethylene levels in plant roots also relieves the suppression of auxin response factor synthesis, which acting together can directly or indirectly increases plant growth (Glick 2005; Lugtenberg and Kamilova 2009). In addition, there is substantial evidence suggesting that rhizobacteria containing ACC deaminase activity protect plants from both biotic and abiotic stresses and dramatically increase plant biomass; which is a desirable parameter for plants to be used for phytoremediation (Burd et al. 1998). It was also reported that ACC deaminase-containing rhizobacterial species can assist nodulation by *Rhizobium* (Belimov et al. 2009) and exhibit an increase in plant biomass as well as longer roots in spite of growth inhibition caused by heavy metals (Di Gregorio et al. 2006; Safronova et al. 2006). In addition, ACC deaminase-containing bacteria, by lowering growth inhibitory ethylene concentrations, could facilitate plant growth in the presence of different metals and organic contaminants (Glick 2003; Glick et al. 2007a; Glick and Stearns 2011), suggesting the importance of the use of bacteria with ACC deaminase activity to facilitate the phytoremediation of metals. All these results suggested that rhizobia with ACC deaminase activity may be valuable for use in phytoremediation due to their enhanced nodulation abilities and positive effects on plant growth. Under stressful conditions, as the case of the presence of high concentration of metals, the IAA overproducing rhizobial strains by increasing the root system of plants grown in metal contaminated soils can also promote nodulation and consequently, to fix N. For example, the production of IAA by *Agrobacterium tumefaciens* CCNWGS0286 decreases in the presence of copper and zinc, but detectable levels of IAA were obtained in the presence of 2.0 mM $Zn^{2+}$. Moreover, the legume growth was higher when inoculated with the wild-type strain than when inoculated *A. tumefaciens* mutant strain with lower IAA production even in the presence of $Zn^{2+}$ (Hao et al. 2012). On the other hand, by providing iron to plants, microbial siderophores help plants to alleviate the toxicity of metals, e.g., as reported for As uptake by fern (Wang et al. 2007). For example, Roy and Chakrabartty (2000) evaluated the production of siderophores by a *Rhizobium* sp. influenced by the concentration of $Al^{3+}$. Besides increasing iron availability, rhizobial siderophores also reduced $Al^{3+}$ toxicity to the bacterium through the formation of a complex mechanism. Similarly, Rogers et al. (2001) proved the effectiveness of the hydroxamate siderophore vicibactin produced by *R. leguminosarum* bv. *viciae* in alleviating aluminum toxicity.

As a result of these properties, PGPB when applied to seeds or incorporated into soil reduce the toxicity of heavy metals (Wani et al. 2007c; Cardón et al. 2010) and consequently enhance the growth and yield of plants (Wani et al. 2008d). Therefore, synthesis of IAA and siderophores, ACC deaminase and P-solubilization are promising traits that not only effectively promote plant growth but also are essential to the legume–rhizobia symbioses through effective nodulation and nitrogen fixation. Besides rhizobia ability to fix N, all these processes can act together and therefore may enhance phytoremediation, by facilitate legume grow in metal-stressed soils, which became the basis of using this symbiosis for phytoremediation.

### 9.4.2 Interaction with Mycorrhizae and Bacterial Endophytes

The term "endophytic bacteria" refers to bacteria living within plant tissues in contrast to rhizospheric bacteria living on or around the plant roots. Some endophytes are diazotrophic and can provide fixed nitrogen to the host plant (Reinhold-Hurek and Hurek 1998). Interestingly, a study of the rhizospheric bacteria and shoot endophytes of the nickel hyperaccumulator *Thlaspi goesingense* revealed that the species in the two communities were strikingly different (Idris et al. 2004). Moreover, many reports attest to the role of rhizospheric bacteria in phytoremediation, but bacterial endophytes offer several advantages over rhizospheric bacteria. A rhizospheric population is difficult to control, and competition between microbes often reduces the numbers of the desired strains unless metabolism of the pollutant is selective for the added bacterium. Endophytes, in contrast, live in the internal tissues of the plant, and their population is selected or controlled by the plant. Therefore, the use of endophytes that naturally inhabit the plant would reduce the problem of competition.

Bacterial endophytes possess similar mechanisms to rhizospheric PGPB (Becerra-Castro et al. 2011; He et al. 2013), but can establish a more intimate association with plants and in turn they may promote plant growth and development in a higher extent than the rhizospheric bacteria. For instance, Fürnkranz et al. (2012) reported bacterial endophytes suitable as inoculants for plant growth promotion, biocontrol, and enhancing stress tolerance in Styrian oil pumpkins. The bacterial endophytes can assist their host plants in improving plant growth through various mechanisms including the production of plant growth-promoting substances such as IAA, siderophores, ACC deaminase, or phosphate solubilization (Ryan et al. 2008). Likewise to the benefits of these features shown by PGPB in promotion of plant growth under different environmental stress conditions, bacterial endophytes possessing these plant growth-promoting traits may have an extraordinary role in the facilitation of phytoremediation. Furthermore, the bacterial endophytes seem to be more tolerant to higher metals concentrations than the rhizospheric isolates. In fact, several studies showing the high resistance to diverse metals of bacterial endophytes as well as their ability to help plants to overcome the negative effects of metals toxicity makes them one of the most promising agents to facilitate phytoremediation (Luo et al. 2011; Li et al. 2012). For example, some bacterial endophytes were found to be able to produce IAA to improve host plant growth in polluted soils and enhanced the phytoremediation (Sheng et al. 2008; Chen et al. 2010; Zhang et al. 2011). Moreover, certain bacterial endophytes have also been shown to alter metal availability to the plant by producing siderophores and organic acids (Saravanan et al. 2007; Long et al. 2011). It was also reported that siderophores produced by *Streptomyces tendae* F4 significantly enhanced uptake of Cd by sunflower plant (Dimkpa et al. 2009). Zhang et al. (2011) have confirmed that an ACC deaminase-producing endophytic bacteria and Pb-resistant conferred metal tolerance onto plants by lowering the synthesis of metal-induced stress ethylene and promoted the growth of rape. Similar results were obtained by Ma et al. (2011b) with ACC deaminase producing bacterial endophytes inoculated in *Allysusm serpyllifolium* and *B. juncea* grown under Ni stress. Also, the bacterial endophyte *Pantoea* sp. Jp3-3 enhanced Cu tolerance in guinea grass (Huo et al. 2012).

It was reported that co-inoculation of rhizobia and bacterial endophytes suppressed *Phytophthora* root rot in chickpea plants, suggesting that dual-inoculation with these microorganisms may act as biocontrol agents and therefore an alternative to the use of pesticides (Misk and Franco 2011). So, it is possible that bacterial endophytes used in combination with rhizobia may also assist in phytoremediation of metals. Considering the bacterial endophytes' abilities to tolerate and transform toxic to less toxic forms of metals, it is possible that a plant–microbe partnership including symbiotic nitrogen fixers and some endophytic bacteria may be an alternative option to traditional methods of phytoremediation (Pajuelo et al. 2008b; Rajkumar et al. 2009).

Another beneficial association is the one that occurs between plants and arbuscular mycorrhizal (AM) fungi, which plays a major role in terrestrial environments and in the sustainability of agro-ecosystems. Several processes with regard to plant stress resistance and the provision of mineral nutrients are related to AM fungi (Fester and Sawers 2011). Today, it has been estimated that 80 % of all plant species can be colonized by AM fungi (Öpik et al. 2006). In this sense, the majority of legumes form symbiotic associations with both P acquiring AM fungi and atmospheric N-fixing rhizobia, which are of agronomic and ecological importance (Vance 2001; Scheublin and van der Heijden 2006). The tripartite symbiosis between the host plant, AM fungi, and N-fixing bacteria can affect the uptake of N by the host plant. In such symbiotic association, N and P are supplied by the micro-symbionts to the host plant. Accordingly, the association between each micro-symbiont is affected by the interaction effects between the host plant and the micro-symbionts as well as by the interactions effects between both micro-symbionts. The synergistic benefits between AM fungi and rhizobia on plant growth and development have been reported in several legume plants (Azcón et al. 1991; Andrade et al. 2004; Ahmed et al. 2006; Yasmeen et al. 2012). Several reports revealed that dual-inoculation improves plant growth compared to single and non-inoculation (Gong et al. 2012). For example, plant height, leaf number, pod number, plant biomass, and shoot and root P concentration increased significantly as a consequence of mycorrhizal infection (Ahmed et al. 2006). Furthermore, a study conducted by Azcón et al. (1991) revealed that most of the combinations (different AM fungal species with *Ensifer meliloti*) increased the concentration and/or content of N in *Medicago sativa* shoots but effectiveness was dependent on the AM fungal species. These data clearly indicate that the best performance on promotion of plants growth depends upon the consortium chosen. In addition, AM fungi are able to alleviate the effects of different stresses on plant growth and yield production by significantly increasing the uptake of water and nutrients including N by the host plant (Miransari et al. 2007, 2008, 2009a, b; Daei et al. 2009). For example, Andrade et al. (2010) recently assessed the influence of AMF on the growth of coffee seedlings under Cu and Zn stress and found that mycorrhizal coffee seedlings grew faster, exhibited improved mineral nutrition (P and K) and had higher yields than non-mycorrhizal seedlings. In addition, it was also observed that higher P accumulation in plant tissues through AM fungi colonization led to reduction in internal Mn toxicity through ATP-dependent sequestration of Mn or formation of low-solubility P-Mn complexes (Nogueira et al. 2007). While AM fungi can often colonize plant roots in metal contaminated soil (Jamal et al. 2002; Vivas et al. 2003, 2006; Vogel-Mikus et al. 2005), their effects on metal uptake by plants are conflicting. In slightly metal contaminated soils, most studies show that AM fungi increased shoot uptake of metals (Weissenhorn et al. 1995), while in severely contaminated soil, AM fungi typically reduce shoot metal concentration and protect plants against the harmful effects of metals (Malcova et al. 2003). On the other hand, AM fungi increased As uptake in the hyperaccumulating fern *Pteris vittata* (Trotta et al. 2006) via the phosphate uptake system (Wang et al. 2002). Thus, the benefits of mycorrhizae may be associated with metal tolerance, and also with metal plant nutrition. In degraded and contaminated soils that are often poor in nutrients and with low water holding capacity, mycorrhizae formation would be of great importance. In addition, AM fungi may also reduce metal availability and toxicity to the plant host by the precipitation of metal oxalates in their intercellular spaces. For example, González-Guerrero et al. (2008) reported that AM fungi accumulated heavy metals in their vacuoles leading to a reduction of the toxic effects of heavy metals in plants. Also the "dilution effect" has been pointed out as a mechanism to reduce the metal phytotoxicity in plants by the AM fungi, which consist in AM fungi ability to promote plant growth through an enhancement of nutrient acquisition together with the reduction of metals concentrations in the aboveground tissues (Chen et al. 2007; Orlowska et al. 2011).

Considering dual inoculations, some reports indicate that co-inoculation with rhizobia and AM fungi may directly inhibit pathogen growth and reproduction and activate the plant's defense system by increasing pathogen defense-related gene expression (Gao et al. 2012). In addition, benefits of nitrogen-fixing bacteria and AM fungi consortia in the protection of host plants against the detrimental effects of metals have been reported (Andrade et al. 2004; Ahmed et al. 2006; Al-Garni 2006). Therefore, rhizobia–AM fungi–legume tripartite symbiosis could be a new approach to increase the metal tolerance of legumes. Also, a consortium constituted by ACC deaminase-containing *Bacillus subtilis*, *Ensifer meliloti*, and *Rhizophagus irregularis* revealed higher mycorrhizal colonization and rhizobial nodulation in *Trigonella foenum-graecum* plants grown under drought stress, resulting in improved nutrient uptake and plant growth (Barnawal et al. 2013). Thus, ACC deaminase by alleviating the negative effects caused by drought stress contributes to help simultaneously mycorrhizal colonization and rhizobia nodulation. It is possible that inoculation with microbial consortia where the mode of action of each inoculant, such as the ability to endure metal stress as well as the potential to promote plant growth through several plant growth-promoting traits, may act synergistically in metal-contaminated soils, leading to a successful phytoremediation process.

## 9.5 Conclusions

Rhizobia have been widely studied due to their role as legume symbionts. More recent research has suggested that rhizobia can be beneficial to crops through their action as PGPB for both non-legumes and host legume plants. Several strains of rhizobia resistant to various metalloids and metals have been isolated from polluted soils. Some of these bacteria are fully able to nodulate legumes and fix nitrogen even in the presence of moderate metal concentrations. Due to their multiplicity of biological activities, rhizobia are an ideal inoculant for raising the productivity of legumes in metal-contaminated soils. Also, inoculation of legumes with bacterial consortia resistant to heavy metals (including rhizobia, mycorrhiza, bacterial endophytes, and other PGPB) has proved to be a promising and cost-effective technology for metal phytoremediation, allowing the re-vegetation of metal-contaminated areas with moderate levels of pollution. Despite the high expectations in the use of rhizobia or consortia with other soil microorganisms, the interactions between plant and rhizobia and that between rhizobia and other soil microorganisms in the presence of metals are not fully understood. Therefore, it is required an extra effort to understand how these synergistic interactions occur as well as how to combine the major advantages of each inoculant present in the consortia in order to (1) obtain consistency in the results in the field and (2) maximize the legume phytoremediation process.

## References


Abeles F, Morgan P, Saltveit M (1992) Ethylene in plant biology. Academic, New York

Ahemad M, Khan MS (2010) Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum*. Crop Prot 29:325–329

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 57:578–589

Ahmed A, Hasnain S (2010) Auxin-producing *Bacillus* sp.: auxin quantification and effect on the growth of *Solanum tuberosum*. Pure Appl Chem 82:313–319

Ahmed FRS, Killham K, Alexander I (2006) Influences of arbuscular mycorrhizal fungus *Glomus mosseae* on growth and nutrition of lentil irrigated with arsenic contaminated water. Plant Soil 283:33–41

Akhtar N, Arshad I, Shakir MA, Qureshi MA, Sehrish J, Ali L (2013) Co-inoculation with *Rhizobium* and *Bacillus* sp. to improve the phosphorus availability and yield of wheat (*Triticum aestivum* L.). J Anim Plant Sci 23:190–197

Akimova GP, Sokolova MG (2012) Cytokinin content during early stages of legume-rhizobial symbiosis and effect of hypothermia. Russ J Plant Physiol 59:656–661

Alexander E, Pham D, Steck TR (1999) The viable but nonculturable condition is induced by copper in *Agrobacterium tumefaciens* and *Rhizobium leguminosarum*. Appl Environ Microbiol 65:3754–3756

Al-Garni SMS (2006) Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium. Afri J Biotechnol 5:133–142

Ali B, Hayat S, Hasan SA, Ahmad A (2008) IAA and 4-Cl-IAA increases the growth and nitrogen fixation in mung bean. Commun Soil Sci Plant Anal 39:2695–2705

Alikhani HA, Saleh-Rastin N, Antoun H (2006) Phosphate solubilization activity of rhizobia native to Iranian soils. Plant Soil 287:35–41

Andrade SAL, Abreu CA, de Abreu MF, Silveira APD (2004) Influence of lead additions on arbuscular mycorrhiza and *Rhizobium* symbioses under soybean plants. Appl Soil Ecol 26:123–131

Andrade S, Silveira A, Mazzafera P (2010) Arbuscular mycorrhiza alters metal uptake and the physiological response of *Coffea arabica* seedlings to increasing Zn and Cu concentrations in soil. Sci Total Environ 208:5381–5391

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Arruda L, Beneduzi A, Martins A, Lisboa B, Lopes C, Bertolo F, Passaglia LMP, Vargas LK (2013) Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Appl Soil Ecol 63:15–22

Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

Azcón R, Rubio R, Barea JM (1991) Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, $N_2$-fixation ($N^{15}$) and nutrition of *Medicago sativa* L. New Phytol 117:399–404

Badenochjones J, Rolfe BG, Letham DS (1983) Phytohormones, *Rhizobium* mutants, and nodulation in legumes. 3. Auxin metabolism in effective and ineffective pea root nodules. Plant Physiol 73:347–352

Balestrasse KB, Gallego SM, Tomaro ML (2004) Cadmium-induced senescence in nodules of soybean (*Glycine max* L.) plants. Plant Soil 262:373–381

Bano A, Fatima M (2009) Salt tolerance in Zea mays (L). Salt tolerance in *Zea mays* (L). following inoculation with *Rhizobium* and *Pseudomonas*. Biol Fert Soils 45:405–413

Barnawal D, Maji D, Bharti N, Chanotiya C, Kalra A (2013) ACC deaminase-containing *Bacillus subtilis* reduces stress ethylene-induced damage and improves mycorrhizal colonization and rhizobial nodulation in *Trigonella foenum-graecum* under drought stress. J Plant Growth Regul. doi:10.1007/s00344-013-9347-3

Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (Plant growth-promoting bacteria) and PGPB. Soil Biol Biochem 30:1225–1228

Bauer P, Ratet P, Crespi MD, Schultze M, Kondorosi A (1996) Nod factors and cytokinins induce similar cortical cell division, amyloplast deposition and MsEnod12A expression patterns in alfalfa roots. Plant J 10:91–105

Becerra-Castro C, Susan Kidd P, Prieto-Fernandez A, Weyens N, Acea M-J, Vangronsveld J (2011) Endophytic and rhizoplane bacteria associated with *Cytisus striatus* growing on hexachlorocyclohexane-contaminated soil: isolation and characterisation. Plant Soil 340:413–433

Belimov AA, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 181:413–423

Bianco C, Defez R (2009) *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. J Exp Bot 60:3097–3107

Bianco C, Defez R (2010) Improvement of phosphate solubilization and medicago plant yield by an indole-3-acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76:4626–4632

Bibi M, Hussain M (2005) Effect of copper and lead on photosynthesis and plant pigments in black gram *Vigna mungo* (L.) Hepper. Bull Environ Contam Toxicol 74:1126–1133

Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci Soc Am J 64:1644–1650

Boiero L, Perrig D, Masciarelli O, Penna C, Cassan F, Luna V (2007) Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl Microbiol Biotechnol 74:874–880

Bonfante P, Anca I-A (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol 63:363–383

Bose S, Bhattacharyya AK (2008) Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. Chemosphere 70:1264–1272

Braud A, Jézéquel K, Bazot S, Lebeau T (2009) Enhanced phytoextraction of an agricultural Cr, Hg- and Pb-contaminated soil by bioaugmentation with siderophore producing bacteria. Chemosphere 74:280–286

Broos K, Uyttebroek M, Mertens J, Smolders E (2004) A survey of symbiotic nitrogen fixation by white clover grown on metal contaminated soils. Soil Biol Biochem 36:633–640

Brown JP (1974) Reaction of 1,2-benzodithiole-3-thione with ethylene diamines and trimethylene-diamines. J Chem Soc Perkin Trans 1:869–870

Burd GI, Dixon DG, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Camerini S, Senatore B, Lonardo E, Imperlini E, Bianco C, Moschetti G, Rotino GL, Campion B, Defez R (2008) Introduction of a novel pathway for IAA biosynthesis to rhizobia alters vetch root nodule development. Arch Microbiol 190:67–77

Cardón DL, Montes Villafan S, Rodriguez Tovar A, Perez Jimenez S, Guerrero Zuniga LA, Amezcua Allieri MA, Perez NO, Rodriguez Dorantes A (2010) Growth response and heavy metals tolerance of *Axonopus affinis*, inoculated with plant growth-promoting rhizobacteria. Afr J Biotechnol 9:8772–8782

Carrasco JA, Armario P, Pajuelo E, Burgos A, Caviedes MA, Lopez R, Chamber MA, Palomares AJ (2005) Isolation and characterisation of symbiotically effective *Rhizobium* resistant to arsenic and heavy metals after the toxic spill at the *Aznalcollar* pyrite mine. Soil Biol Biochem 37:1131–1140

Cartieaux F, Thibaud MC, Zimmerli L, Lessard P, Sarrobert C, David P, Gerbaud A, Robaglia C, Somerville S, Nussaume L (2003) Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. Plant J 36:177–188

Chabot R, Antoun H, Cescas MP (1993) Growth-stimulation for corn and cos lettuce using phosphorus-solubilizing microorganisms. Can J Microbiol 39:941–947

Chaintreuil C, Rigault F, Moulin L, Jaffre T, Fardoux J, Giraud E, Dreyfus B, Bailly X (2007) Nickel resistance determinants in *Bradyrhizobium* strains from nodules of the endemic New Caledonia legume *Serianthes calycina*. Appl Environ Microbiol 73:8018–8022

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Braz J Microbiol 38:124–130

Chaudri AM, McGrath SP, Giller KE, Rietz E, Sauerbeck DR (1993) Enumeration of indigenous *Rhizobium leguminosarum* biovar *Trifolii* in soils previously treated with metal-contaminated sewage sludge. Soil Biol Biochem 25:301–309

Chaudri AM, Lawlor K, Preston S, Paton GI, Killham K, McGrath SP (2000) Response of a rhizobium based luminescence biosensor to Zn and Cu in soil solutions from sewage sludge treated soils. Soil Biol Biochem 32:383–388

Chekol T, Vough LR, Chaney RL (2004) Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. Environ Int 30:799–804

Chen BD, Zhu YG, Duan J, Xiao XY, Smith S (2007) Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environ Pollut 147:374–380

Chen W-M, Wu C-H, James EK, Chang J-S (2008) Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*. J Hazard Mater 151:364–371

Chen L, Luo S, Xiao X, Guo H, Chen J, Wan Y, Li B, Xu T, Xi Q, Rao C, Liu C, Zheng G (2010) Application of plant growth-promoting endophytes (PGPE) isolated from *Solanum nigrum* L. for phytoextraction of Cd-polluted soils. Appl Soil Ecol 46:383–389

Cheng Z, Park E, Glick BR (2007) 1-aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918

Conforte VP, Echeverria M, Sanchez C, Ugalde RA, Menendez AB, Lepek VC (2010) Engineered ACC deaminase-expressing free-living cells of *Mesorhizobium loti* show increased nodulation efficiency and competitiveness on Lotus spp. J Gen Appl Microbiol 56:331–338

Contesto C, Desbrosses G, Lefoulon C, Bena G, Borel F, Galland M, Gamet L, Varoquaux F, Touraine B (2008) Effects of rhizobacterial ACC deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. Plant Sci 175:178–189

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18

Csukasi F, Merchante C, Valpuesta V (2009) Modification of plant hormone levels and signaling as a tool in plant biotechnology. Biotechnol J 4:1293–1304

Daei G, Ardekani MR, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

Dary M, Chamber-Perez MA, Palomares AJ, Pajuelo E (2010) "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177:323–330

Dazzo F, Yanni Y (2006) The natural rhizobium-cereal crop association as an example of plant-bacterial interaction. In: Uphoff N, Ball A, Fernandes E et al (eds) Biological approaches to sustainable soil systems. CRC Press, Boca Raton, pp 109–127

de Salamone IEG, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47:404–411

Dehio C, Debruijn FJ (1992) The early nodulin gene SRENOD2 from *Sesbania rostrata* is inducible by cytokinin. Plant J 2:117–128

Del Rio M, Font R, Almela C, Velez D, Montoro R, Bailon AD (2002) Heavy metals and arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the Aznalcollar mine. J Biotechnol 98:125–137

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394

Dhingra HR, Varghese TM (1985) Effect of growth-regulators on the In vitro germination and tube growth of maize (*Zea mays* L) pollen from plants raised under sodium-chloride salinity. New Phytol 100:563–569

Di Gregorio S, Lampis S, Malorgio F, Petruzzelli G, Pezzarossa B, Vallini G (2006) *Brassica juncea* can improve selenite and selenate abatement in selenium contaminated soils through the aid of its rhizospheric bacterial population. Plant Soil 285:233–244

Dimkpa C, Merten D, Svatoš A, Büchel G, Kothe E (2009) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41:154–162

Ding Y, Oldroyd GED (2009) Positioning the nodule, the hormone dictum. Plant Signal Behav 4:89–93

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Doty SL, Shang TQ, Wilson AM, Moore AL, Newman LA, Strand SE, Gordon MP (2003) Metabolism of the soil and groundwater contaminants, ethylene dibromide and trichloroethylene, by the tropical leguminous tree, *Leucaena leucocephala*. Water Res 37:441–449

Duan J, Muller KM, Charles TC, Vesely S, Glick BR (2009) 1-aminocyclopropane-1-carboxylate (ACC) deaminase genes in rhizobia from southern Saskatchewan. Microb Ecol 57:423–436

Duodu S, Bhuvaneswari TV, Stokkermans TJW, Peters NK (1999) A positive role for rhizobitoxine in *Rhizobium*-legume symbiosis. Mol Plant Microbe Interact 12:1082–1089

Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31:861–864

Fang YW, Hirsch AM (1998) Studying early nodulin gene ENOD40 expression and induction by nodulation factor and cytokinin in transgenic alfalfa. Plant Physiol 116:53–68

FAO (2009) How to feed the World in 2050. Food and Agriculture Organization of the United Nations, Rome, Italy

Farajzadeh D, Aliasgharzad N, Bashir NS, Yakhchali B (2010) Cloning and characterization of a plasmid encoded ACC deaminase from an indigenous *Pseudomonas fluorescens* FY32. Curr Microbiol 61:37–43

Ferguson F, Lessenger J (2006) Plant growth regulators. In: Lessenger J (ed) Agricultural medicine. Springer, New York, pp 156–166

Ferguson BJ, Mathesius U (2003) Signaling interactions during nodule development. J Plant Growth Regul 22:47–72

Fernandez-Falcon M, Borges AA, Borges-Perez A (2003) Induced resistance to *Fusarium* wilt of banana by exogenous applications of indoleacetic acid. Phytoprotection 84:149–153

Fester T, Sawers R (2011) Progress and challenges in agricultural applications of arbuscular mycorrhizal fungi. Crit Rev Plant Sci 30:459–470

Figueira E, Lima IG, Pereira SIA (2005) Cadmium tolerance plasticity in *Rhizobium leguminosarum* bv. *viciae*: glutathione as a detoxifying agent. Can J Microbiol 51:7–14

Frankenberger JWT, Arshad M (1995) Microbial synthesis of auxins. In: Frankenberger WT, Arshad M (eds) Phytohormones in soils. Marcel Dekker Inc, New York, pp 35–71

Frommel MI, Nowak J, Lazarovits G (1991) Growth enhancement and developmental modifications of in vitro grown potato (*Solanum tuberosum* ssp *Tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp. Plant Physiol 96:928–936

Frostegard A, Tunlid A, Baath E (1996) Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol Biochem 28:55–63

Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K (2008) Cytokinin: secret agent of symbiosis. Trends Plant Sci 13:115–120

Fürnkranz M, Adam E, Müller H, Grube M, Huss H, Winkler J, Berg G (2012) Promotion of growth, health and stress tolerance of Styrian oil pumpkins by bacterial endophytes. Eur J Plant Pathol 134:509–519

Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 68:280–300

Gao X, Lu X, Wu M, Zhang H, Pan R, Tian J, Li S, Liao H (2012) Co-inoculation with rhizobia and AMF inhibited soybean red crown rot: from field study to plant defense-related gene expression analysis. PLoS One 7(3):e33977

Ghosh S, Basu PS (2006) Production and metabolism of indole-acetic-acid in roots and root nodules of *Phaseolus mungo*. Microbiol Res 161:362–366

Giller KE, McGrath SP, Hirsch PR (1989) Absence of nitrogen fixation in clover grown on soil subject to long-term contamination with heavy-metals is due to survival of only ineffective *Rhizobium*. Soil Biol Biochem 21:841–848

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR (2004) Changes in plant growth and development by rhizosphere bacteria that modify plant ethylene levels. In: Nicola S, Nowak J, Vavrina CS (eds) Issues and advances in transplant production and stand establishment research. Acta Hortic, 26th International horticultural congress; international society horticultural science, Toronto, 631:265–273

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:15. Article ID 963401

Glick BR, Stearns JC (2011) Making phytoremediation work better: maximizing a plant's growth potential in the midst of adversity. Int J Phytoremediation 13:4–16

Glick BR, Penrose DM, Li JP (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, UK

Glick BR, Cheng Z, Czarny J, Duan J (2007a) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007b) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

Gong M, Tang M, Chen H, Zhang Q, Xu H, Zheng C (2012) Effects of *Glomus mosseae* and rhizobium on the growth of black locust seedlings and the quality of weathered soft rock soils in the Loess Plateau, China. Ann Microbiol 62:1579–1586

González-Guerrero M, Melville LH, Ferrol N, Lott JN, Azcón-Aguilar C, Peterson RL (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Can J Microbiol 54:103–110

Gonzalez-Rizzo S, Crespi M, Frugier F (2006) The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. Plant Cell 18:2680–2693

Gordon LJ, Finlayson CM, Falkenmark M (2010) Managing water in agriculture for food production and other ecosystem services. Agr Water Manag 97:512–519

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Graham PH, Viteri SE, Mackie F, Vargas AT, Palacios A (1982) Variation in acid soil tolerance among strains of *Rhizobium phaseoli*. Field Crops Res 5:121–128

Grčman H, Velikonja-Bolta S, Vodnik D, Kos B, Lestan D (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil 235:105–114

Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17

Guerinot ML, Meidl EJ, Plessner O (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. J Bacteriol 172:3298–3303

Gul B, Khan MA, Weber DJ (2000) Alleviation of salinity and dark-enforced dormancy in *Allenrolfea occidentalis* seeds under various thermoperiods. Aust J Bot 48:745–752

Gulnaz A, Iqbal J, Azam F (1999) Seed treatment with growth regulators and crop productivity. II. Response of critical growth stages of wheat (*Triticum aestivum* L.) under salinity stress. Cereal Res Commun 27:419–426

Gupta A, Gopal M, Tilak KV (2000) Mechanism of plant growth promotion by rhizobacteria. Indian J Exp Biol 38:856–862

Hall J, Soole K, Bentham R (2011) Hydrocarbon phytoremediation in the family Fabacea—a review. Int J Phytoremediation 13(4): 317–332

Hao Y, Charles TC, Glick BR (2007) ACC deaminase from plant growth-promoting bacteria affects crown gall development. Can J Microbiol 53:1291–1299

Hao X, Xie P, Johnstone L, Miller SJ, Rensing C, Wei G (2012) Genome sequence and mutational analysis of plant growth-promoting bacterium *Agrobacterium tumefaciens* CCNWGS0286 isolated from a zinc-lead mine tailing. Appl Environ Microbiol 78:5384–5394

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

He H, Ye Z, Yang D, Yan J, Xiao L, Zhong T, Yuan M, Cai X, Fang Z, Jing Y (2013) Characterization of endophytic *Rahnella* sp JN6 from *Polygonum pubescens* and its potential in promoting growth and Cd, Pb, Zn uptake by *Brassica napus*. Chemosphere 90:1960–1965

Heckmann AB, Sandal N, Bek AS, Madsen LH, Jurkiewicz A, Nielsen MW, Tirichine L, Stougaard J (2011) Cytokinin induction of root nodule primordia in *Lotus japonicus* is regulated by a mechanism operating in the root cortex. Mol Plant Microbe Interact 24: 1385–1395

Hilder RC, Kong X (2010) Chemistry and biology of siderophores. Mol Plant Microbe Interact 4:5–13

Hirsch AM, Fang YW (1994) Plant hormones and nodulation—what's the connection. Plant Mol Biol 26:5–9

Hirsch PR, Jones MJ, McGrath SP, Giller KE (1993) Heavy-metals from past applications of sewage-sludge decrease the genetic diversity of *Rhizobium leguminosarum* biovar *Trifolii* populations. Soil Biol Biochem 25:1485–1490

Hong-Bo S, Li-Ye C, Cheng-Jiang R, Hua L, Dong-Gang G, Wei-Xiang L (2010) Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils. Crit Rev Biotechnol 30:23–30

Honma M, Shimomura T (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agric Biol Chem 42:1825–1831

Hungria M, Stacey G (1997) Molecular signals exchanged between host plants and rhizobia: basic aspects and potential application in agriculture. Soil Biol Biochem 29:819–830

Hungria M, Vargas MAT (2000) Environmental factors affecting $N_2$ fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res 65:151–164

Hunter WJ (1989) Indole-3-acetic-acid production by bacteroids from soybean root-nodules. Physiol Plant 76:31–36

Huo W, Zhuang C-H, Cao Y, Pu M, Yao H, Lou L-Q, Cai Q-S (2012) Paclobutrazol and plant-growth promoting bacterial endophyte *Pantoea* sp enhance copper tolerance of guinea grass (*Panicum maximum*) in hydroponic culture. Acta Physiol Plant 34:139–150

Hussain A, Hasnain S (2011) Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. World J Microbiol Biotechnol 27:2645–2654

Ibekwe AM, Angle JS, Chaney RL, vanBerkum P (1997) Enumeration and $N_2$ fixation potential of *Rhizobium leguminosarum* biovar trifolii grown in soil with varying pH values and heavy metal concentrations. Agric Ecosyst Environ 61:103–111

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl Environ Microbiol 70:2667–2677

Ike A, Sriprang R, Ono H, Murooka Y, Yamashita M (2007) Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. Chemosphere 66:1670–1676

Imsande J (1998) Iron, sulfur, and chlorophyll deficiencies: a need for an integrative approach in plant physiology. Physiol Plant 103:139–144

Itai C, Richmond A, Vaadia Y (1968) Role of root cytokinins during water and salinity stress. Isr J Bot 17:187–195

Jamal A, Ayub N, Usman M, Khan AG (2002) Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil. Int J Phytoremediation 4:205–221

Kabar K (1987) Alleviation of salinity stress by plant growth regulators on seed-germination. J Plant Physiol 128:179–183

Kampert M, Strzelczyk E, Pokojska A (1975) Production of auxins by bacteria isolated from roots of pine seedlings (*Pinus silvestris* L). Acta Microbiol Pol 7:135–143

Kaneko T, Nakamura Y, Sato S, Asamizu E, Kato T et al (2000) Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. DNA Res 7:331–338

Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T et al (2002) Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res 9:189–197

Kanika, Dogra T, Lata (2010) Biochemical and molecular characterization of a rhizobitoxine-producing *Bradyrhizobium* from pigeon pea plants. Folia Microbiol 55:233–238

Khan MA, Weber DJ (1986) Factors influencing seed-germination in *Salicornia pacifica* var utahensis. Am J Bot 73:1163–1167

Khan MS, Zaidi A, Aamil M (2002) Biocontrol of fungal pathogens by the use of plant growth promoting rhizobacteria and nitrogen fixing microorganisms. Indian J Bot Soc 81:255–263

Khan MA, Gul B, Weber DJ (2004) Action of plant growth regulators and salinity on seed germination of *Ceratoides lanata*. Can J Bot 82:37–42

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27:29–43

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–44

Kulaeva ON, Kusnetsov VV (2002) Recent advances and horizons of the cytokinin studying. Russ J Plant Physiol 49:561–574

Kuykendall LD, Saxena B, Devine TE, Udell SE (1992) Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can J Microbiol 38:501–505

Lee KH, Larue TA (1992) Exogenous ethylene inhibits nodulation of *Pisum sativum* cv *Sparkle*. Plant Physiol 100:1759–1763

Li H, Wei D, Shen M, Zhou Z (2012) Endophytes and their role in phytoremediation. Fungal Divers 54:11–18

Liu WX, Liu JW, Wu MZ, Li Y, Zhao Y, Li SR (2009) Accumulation and translocation of toxic heavy metals in winter wheat (Triticum *aestivum L.)* growing in agricultural soil of Zhengzhou, China. Bull Environ Contam Toxicol 82:343–347

Lohar D, Stiller J, Kam J, Stacey G, Gresshoff PM (2009) Ethylene insensitivity conferred by a mutated Arabidopsis ethylene receptor gene alters nodulation in transgenic Lotus japonicus. Ann Bot 104:277–285

Long X, Chen X, Chen Y, Woon-Chung WJ, Wei Z, Wu Q (2011) Isolation and characterization endophytic bacteria from hyperaccumulator *Sedum alfredii* Hance and their potential to promote phytoextraction of zinc polluted soil. World J Microbiol Biotechnol 27:1197–1207

Lopareva A, Goncharova N (2007) Plant-microbe symbiosis for bioremediation of heavy metal contaminated soil: perspectives for Belarus. In: Hull RN et al (eds) Strategies to enhance environmental security in transition countries. Springer, Netherlands, pp 401–414

Lugtenberg B, Kamilova F (2009) Plant growth-promoting rhizobacteria, Annu Rev Microbiol, 63:541–556

Luo S, Chen L, Chen J, Xiao X, Xu T, Wan Y, Rao C, Liu C, Liu Y, Lai C, Zeng G (2011) Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator *Solanum nigrum* L. and their potential use for phytoremediation. Chemosphere 85:1130–1138

Ma WB, Penrose DM, Glick BR (2002) Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Can J Microbiol 48:947–954

Ma WB, Guinel FC, Glick BR (2003a) *Rhizobium leguminosarum* biovar *viciae* 1-aminocyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. Appl Environ Microbiol 69:4396–4402

Ma WB, Sebestianova SB, Sebestian J, Burd GI, Guinel FC, Glick BR (2003b) Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in Rhizobium spp. Anton Leeuw Int J G 83:285–291

Ma WB, Charles TC, Glick BR (2004) PV expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in *Sinorhizobium meliloti* increases its ability to nodulate alfalfa. Appl Environ Microbiol 70:5891–5897

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011a) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Ma Y, Rajkumar M, Luo Y, Freitas H (2011b) Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. J Hazard Mater 196:230–237

Malcova R, Vosatka M, Gryndler M (2003) Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L. Appl Soil Ecol 23:55–67

Mamaril JC, Paner ET, Alpante BM (1997) Biosorption and desorption studies of chromium (III) by free and immobilized *Rhizobium* (BJV r 12) cell biomass. Biodegradation 8(4):275–285

Mandal SM, Pati BR, Das AK, Ghosh AK (2008) Characterization of a symbiotically effective rhizobium resistant to arsenic: isolated from the root nodules of *Vigna mungo* (L.) Hepper grown in an arsenic-contaminated field. J Gen Appl Microbiol 54:93–99

Mañero FJG, Acero N, Lucas JA, Probanza A (1996) The influence of native rhizobacteria on European alder (Alnus glutinosa (L) Gaertn) growth. 2. Characterisation and biological assays of metabolites from growth promoting and growth inhibiting bacteria. Plant Soil 182:67–74

Martensson AM, Witter E (1990) Influence of various soil amendments on nitrogen fixing soil microorganisms in a long-term field experiment, with special reference to sewage-sludge. Soil Biol Biochem 22:977–982

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18:49–53

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166(2):525–530

McDonnell L, Plett JM, Andersson-Gunneras S, Kozela C, Dugardeyn J, Van Der Straeten D, Glick BR, Sundberg B, Regan S (2009) Ethylene levels are regulated by a plant encoded 1-aminocyclopropane-1-carboxylic acid deaminase. Physiol Plant 136:94–109

McGrath SP, Brookes PC, Giller KE (1988) Effects of potentially toxic metals in soil derived from pasta applications of sewage-sludge on nitrogen fixation by *Trifolium repens* L. Soil Biol Biochem 20:415–424

McKay IA, Djordjevic MA (1993) Production and excretion of nod metabolites by *Rhizobium leguminosarum* bv. *trifolii* are disrupted by the same environmental factors that reduce nodulation in the field. Appl Environ Microbiol 59:3385–3392

McLauchlan K (2006) The nature and longevity of agricultural impacts on soil carbon and nutrients: a review. Ecosystems 9:1364–1382

Mehnaz S, Lazarovits G (2006) Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. Microb Ecol 51(3):326–335

Mench M, Vangronsveld J, Beckx C, Ruttens A (2006) Progress in assisted natural remediation of an arsenic contaminated agricultural soil. Environ Pollut 144:51–61

Middleton PH, Jakab J, Penmetsa RV, Starker CG, Doll J et al (2007) An ERF transcription factor in *Medicago truncatula* that is essential for nod factor signal transduction. Plant Cell 19:1221–1234

Minamisawa K, Onodera S, Tanimura Y, Kobayashi N, Yuhashi KI, Kubota M (1997) Preferential nodulation of *Glycine max*, *Glycine soja* and *Macroptilium atropurpureum* by two *Bradyrhizobium* species *japonicum* and *elkanii*. FEMS Microbiol Ecol 24:49–56

Miransari M, Bahrami HA, Rejali F, Malakouti MJ, Torabi H (2007) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. Soil Biol Biochem 39:2014–2026

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2008) Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biol Biochem 40:1197–1206

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2009a) Effects of arbuscular mycorrhiza, soil sterilization, and soil compaction on wheat (*Triticum aestivum* L.) nutrients uptake. Soil Tillage Res 104:48–55

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2009b) Effects of soil compaction and arbuscular mycorrhiza on corn (*Zea mays* L.) nutrient uptake. Soil Tillage Res 103:282–290

Misk A, Franco C (2011) Biocontrol of chickpea root rot using endophytic actinobacteria. Biocontrol 56:811–822

Muratova AY, Turkovskaya OV, Hubner T, Kuschk P (2003) Studies of the efficacy of alfalfa and reed in the phytoremediation of hydrocarbon-polluted soil. Appl Biochem Microbiol 39:599–605

Murray JD, Karas BJ, Sato S, Tabata S, Amyot L, Szczyglowski K (2007) A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. Science 315:101–104

Nandwal AS, Kukreja S, Kumar N, Sharma PK, Jain M, Mann A, Singh S (2007) Plant water, status, ethylene evolution, N(2)-fixing efficiency, antioxidant activity and lipid peroxidation in *Cicer arietinum* L. nodules as affected by short-term salinization and desalinization. J Plant Physiol 164:1161–1169

Nascimento F, Brígido C, Alho L, Glick BR, Oliveira S (2012a) Enhanced chickpea growth-promotion ability of a *Mesorhizobium* strain expressing an exogenous ACC deaminase gene. Plant Soil 353:221–230

Nascimento FX, Brígido C, Glick BR, Oliveira S (2012b) ACC deaminase genes are conserved among *Mesorhizobium* species able to nodulate the same host plant. FEMS Microbiol Lett 336:26–37

Nascimento FX, Brígido C, Glick BR, Oliveira S, Alho L (2012c) *Mesorhizobium ciceri* LMS-1 expressing an exogenous 1-aminocyclopropane-1-carboxylate (ACC) deaminase increases its nodulation abilities and chickpea plant resistance to soil constraints. Lett Appl Microbiol 55:15–21

Nogueira MA, Nehls U, Hampp R, Poralla K, Cardoso EJBN (2007) Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant Soil 298:273–284

Noriega GO, Balestrasse KB, Batlle A, Tomaro ML (2007) Cadmium induced oxidative stress in soybean plants also by the accumulation of delta-aminolevulinic acid. Biometals 20:841–851

Nukui N, Ezura H, Yuhashi KI, Yasuta T, Minamisawa K (2000) Effects of ethylene precursor and inhibitors for ethylene biosynthesis and perception on nodulation in *Lotus japonicus* and *Macroptilium atropurpureum*. Plant Cell Physiol 41:893–897

Nukui N, Ezura H, Minamisawa K (2004) Transgenic *Lotus japonicus* with an ethylene receptor gene Cm-ERS1/H70A enhances formation of infection threads and nodule primordia. Plant Cell Physiol 45:427–435

Nukui N, Minamisawa K, Ayabe S-I, Aoki T (2006) Expression of the 1-aminocyclopropane-1-carboxylic acid deaminase gene requires symbiotic nitrogen-fixing regulator gene nifA2 in *Mesorhizobium loti* MAFF303099. Appl Environ Microbiol 72:4964–4969

Obbard JP, Jones KC (1993) The effect of heavy-metals on dinitrogen fixation by rhizobium white clover in a range of long-term sewage-sludge amended and metal-contaminated soils. Environ Pollut 79:105–112

Okazaki S, Yuhashi KI, Minamisawa K (2003) Quantitative and time-course evaluation of nodulation competitiveness of rhizobitoxine-producing *Bradyrhizobium elkanii*. FEMS Microbiol Ecol 45:155–160

Okazaki S, Sugawara M, Minamisawa K (2004) *Bradyrhizobium elkanii* rtxC gene is required for expression of symbiotic phenotypes in the final step of rhizobitoxine biosynthesis. Appl Environ Microbiol 70:535–541

Oldroyd GED (2007) Nodules and hormones. Science 315(5808): 52–53

Oldroyd GED, Downie JM (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546

Oldroyd GED, Engstrom EM, Long SR (2001) Ethylene inhibits the nod factor signal transduction pathway of *Medicago truncatula*. Plant Cell 13:1835–1849

Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J Ecol 94:778–790

Orlowska E, Przybyłowicz W, Orlowski D, Turnau K, M-P J (2011) The effect of mycorrhiza on the growth and elemental composition of Ni-hyperaccumulating plant *Berkheya coddii* Roessler. Environ Pollut 159:3730–3738

Owens LD, Williams T, Thompson JF, Pitcher RG (1972) Structure of rhizobitoxine, an antimetabolic ether amino-acid from *Rhizobium japonicum*. J Chem Soc Chem Commun 12:714

Pajuelo E, Dary M, Palomares AJ, Rodriguez-Llorente ID, Carrasco JA, Chamber MA (2008a) Biorhizoremediation of heavy metals toxicity using Rhizobium-legume symbioses. In: Dakota FD, Chimphango ABM, Valentine AJ, Elmerich C, Newton WE (eds) Biological nitrogen fixation: towards poverty alleviation through sustainable agriculture, vol 42. Springer, Netherlands, pp 101–104

Pajuelo E, Rodriguez-Llorente ID, Dary M, Palomares AJ (2008b) Toxic effects of arsenic on *Sinorhizobium-Medicago sativa* symbiotic interaction. Environ Pollut 154:203–211

Parker MA, Peters NK (2001) Rhizobitoxine production and symbiotic compatibility of Bradyrhizobium from Asian and North American lineages of Amphicarpaea. Can J Microbiol 47:889–894

Pastor J, Hernandez AJ, Prieto N, Fernandez-Pascual M (2003) Accumulating behaviour of *Lupinus albus* L. growing in a normal and a decalcified calcic luvisol polluted with Zn. J Plant Physiol 160:1457–1465

Patrick B, Antonin L, Servane L-L, Deleu C, Le Deunff E (2009) Ethylene modifies architecture of root system in response to stomatal opening and water allocation changes between root and shoot. Plant Signal Behav 4:44–46

Patten CL, Glick BR (1996) Bacterial biosynthesis on indole-3-acetic acid. Can J Microbiol 42:207–220

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Paudyal SP, Aryal RR, Chauhan SVS, Maheshwari DK (2007) Effects of heavy metals on growth of rhizobium strains and symbiotic efficiency of two species of tropical legumes. Sci World 5:27–32

Pavlova ZB, Lutova LA (2000) Nodulation as a model for studying differentiation in higher plants. Russ J Genet 36:975–988

Peix A, Rivas-Boyero AA, Mateos PF, Rodriguez-Barrueco C, Martinez-Molina E, Velazquez E (2001) Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. Soil Biol Biochem 33:103–110

Peralta-Videa JR, Gardea-Torresdey JL, Gomez E, Tiemann KJ, Parsons JG, Carrillo G (2002) Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environ Pollut 119:291–301

Peralta-Videa JR, de la Rosa G, Gonzalez JH, Gardea-Torresdey JL (2004) Effects of the growth stage on the heavy metal tolerance of alfalfa plants. Adv Environ Res 8:679–685

Pereira SIA, Lima AIG, de Figueira EMPA (2006a) Screening possible mechanisms mediating cadmium resistance in *Rhizobium leguminosarum* bv. viciae isolated from contaminated Portuguese soils. Microb Ecol 52:176–186

Pereira SIA, Lima AIG, Figueira EMAP (2006b) Heavy metal toxicity in Rhizobium leguminosarum biovar viciae isolated from soils subjected to different sources of heavy-metal contamination: effects on protein expression. Appl Soil Ecol 33:286–293

Pertry I, Vaclavikova K, Depuydt S, Galuszka P, Spichal L, Temmerman W, Stes E, Schmuelling T, Kakimoto T, Van Montagu MCE, Strnad M, Holsters M, Tarkowski P, Vereecke D (2009) Identification of *Rhodococcus fascians* cytokinins and their modus operandi to reshape the plant. Proc Natl Acad Sci U S A 106:929–934

Phillips KA, Skirpan AL, Liu X, Christensen A, Slewinski TL, Hudson C, Barazesh S, Cohen JD, Malcomber S, McSteen P (2011) Vanishing tassel2 encodes a grass-specific tryptophan aminotransferase required for vegetative and reproductive development in maize. Plant Cell 23:550–566

Plessner O, Klapatch T, Guerinot ML (1993) Siderophore utilization by *Bradyrhizobium japonicum*. Appl Environ Microbiol 59:1688–1690

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MAK (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12:98–105

Prasad MNV, Freitas HMD (2003) Metal hyperaccumulation in plants—biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:285–321

Prayitno J, Rolfe BG, Mathesius U (2006) The ethylene-insensitive sickle mutant of *Medicago truncatula* shows altered auxin transport regulation during nodulation. Plant Physiol 142:168–180

Rajkumar M, Freitas H (2008) Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99(9):3491–3498

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160

Ratcliff WC, Denison RF (2009) Rhizobitoxine producers gain more poly-3-hydroxybutyrate in symbiosis than do competing rhizobia, but reduce plant growth. ISME J 3:870–872

Ratcliff WC, Kadam SV, Denison RF (2008) Poly-3-hydroxybutyrate (PHB) supports survival and reproduction in starving rhizobia. FEMS Microbiol Ecol 65:391–399

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069

Reichman SM (2007) The potential use of the legume-rhizobium symbiosis for the remediation of arsenic contaminated sites. Soil Biol Biochem 39:2587–2593

Reinhold-Hurek B, Hurek T (1998) Interactions of gramineous plants with Azoarcus spp. and other diazotrophs: identification, localization, and perspectives to study their function. Crit Rev Plant Sci 17:29–54

Remans R, Croonenborghs A, Gutierrez RT, Michiels J, Vanderleyden J (2007) Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition. Eur J Plant Pathol 119:341–351

Rivas R, Peix A, Mateos PF, Trujillo ME, Martinez-Molina E, Velazquez E (2006) Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. Plant Soil 287:23–33

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rogers NJ, Carson KC, Glenn AR, Dilworth MJ, Hughes MN, Poole RK (2001) Alleviation of aluminum toxicity to *Rhizobium leguminosarum* bv. viciae by the hydroxamate siderophore vicibactin. Biometals 14(1):59–66

Romanov GA (2009) How do cytokinins affect the cell? Russ J Plant Physiol 56:268–290

Roy N, Chakrabartty PK (2000) Effect of aluminum on the production of siderophore by *Rhizobium* sp (*Cicer arietinum*). Curr Microbiol 41:5–10

Ruan XA, Peters NK (1992) Isolation and characterization of rhizobitoxine mutants of *Bradyrhizobium japonicum*. J Bacteriol 174: 3467–3473

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Saravanan VS, Madhaiyan M, Thangaraju M (2007) Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere 66:1794–1798

Scheublin TR, van der Heijden MGA (2006) Arbuscular mycorrhizal fungi colonize nonfixing root nodules of several legume species. New Phytol 172:732–738

Schlindwein G, Vargas LK, Lisboa BB, Azambuja AC, Granada CE, Gabiatti NC, Prates F, Stumpf R (2008) Influence of rhizobial inoculation on seedling vigor and germination of lettuce. Cienc Rural 38:658–664

Schmidt JS, Harper JE, Hoffman TK, Bent AF (1999) Regulation of soybean nodulation independent of ethylene signaling. Plant Physiol 119:951–959

Sessitsch A, Howieson JG, Perret X, Antoun H, Martinez-Romero E (2002) Advances in rhizobium research. Crit Rev Plant Sci 21:323–378

Shahab S, Ahmed N, Khan NS (2009) Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. Afr J Agric Res 4:1312–1316

Shaharoona B, Arshad M, Zahir ZA (2006a) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Lett Appl Microbiol 42:155–159

Shaharoona B, Bibi R, Arshad M, Zahir ZA, Zia Ul H (2006b) 1-Aminocylopropane-1-carboxylate (ACC)deaminase rhizobacteria extenuates ACC-induced classical triple response in etiolated pea seedlings. Pak J Bot 38:1491–1499

Shahzad SM, Khalid A, Arshad M, Tahir J, Mahmood T (2010) Improving nodulation, growth and yield of Cicer arietinum L. through bacterial ACC-deaminase induced changes in root architecture. Eur J Soil Biol 46:342–347

Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci 164:317–322

Sharaf EF, Farrag AA (2004) Induced resistance in tomato plants by IAA against *Fusarium oxysporum lycopersici*. Pol J Microbiol 53:111–116

Sheng X, Chen X, He L (2008) Characteristics of an endophytic pyrene-degrading bacterium of *Enterobacter* sp. 12J1 from *Allium macrosteman* Bunge. Int Biodeterior Biodegrad 62:88–95

Shilev SI, Ruso J, Puig A, Benlloch M, Jorrin J, Sancho E (2001) Rhizospheric bacteria promote sunflower (*Helianthus annuus* L.) plant growth and tolerance to heavy metals. Minerva Biotechnol 13:37–39

Solano B, Maicas J, Mañero FJG (2008) Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). In: Ahmad I, Pichtel J, Hayat S (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. Wiley, Weinhein, Germany, pp 41–52

Soto MJ, Domínguez-Ferreras A, Perez-Mendoza D, Sanjuan J, Olivares J (2009) Mutualism versus pathogenesis: the give-and-take in plant-bacteria interactions. Cell Microbiol 11:381–388

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3(4)

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Spiertz JHJ (2010) Nitrogen, sustainable agriculture and food security. A review. Agron Sustain Dev 30:43–55

Sriprang R, Hayashi M, Yamashita M, Ono H, Saeki K, Murooka Y (2002) A novel bioremediation system for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia. J Biotechnol 99:279–293

Sriprang R, Hayashi M, Ono H, Takagi M, Hirata K, Murooka Y (2003) Enhanced accumulation of $Cd^{2+}$ by a *Mesorhizobium* sp transformed with a gene from Arabidopsis thaliana coding for phytochelatin synthase. Appl Environ Microbiol 69:1791–1796

Sugawara M, Okazaki S, Nukui N, Ezura H, Mitsui H, Minamisawa K (2006) Rhizobitoxine modulates plant-microbe interactions by ethylene inhibition. Biotechnol Adv 24:382–388

Sullivan JT, Trzebiatowski JR, Cruickshank RW, Gouzy J, Brown SD et al (2002) Comparative sequence analysis of the symbiosis island of *Mesorhizobium loti* strain R7A. J Bacteriol 184:3086–3095

Tank N, Saraf M (2009) Enhancement of plant growth and decontamination of nickel-spiked soil using PGPR. J Basic Microbiol 49:195–204

Taurian T, Soledad AM, Luduena LM, Angelini JG, Munoz V, Valetti L, Fabra A (2013) Effects of single and co-inoculation with native phosphate solubilising strain *Pantoea* sp J49 and the symbiotic nitrogen fixing bacterium *Bradyrhizobium* sp SEMIA 6144 on peanut (*Arachis hypogaea* L.) growth. Symbiosis 59:77–85

Teng Y, Shen Y, Luo Y, Sun X, Sun M, Fu D, Li Z, Christie P (2011) Influence of *Rhizobium meliloti* on phytoremediation of polycyclic aromatic hydrocarbons by alfalfa in an aged contaminated soil. J Hazard Mater 186:1271–1276

Tirichine L, James EK, Sandal N, Stougaard J (2006) Spontaneous root-nodule formation in the model legume *Lotus japonicus*: a novel class of mutants nodulates in the absence of rhizobia. Mol Plant Microbe Interact 19:373–382

Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 315:104–107

Tittabutr P, Awaya JD, Li QX, Borthakur D (2008) The cloned 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene from *Sinorhizobium* sp strain BL3 in *Rhizobium* sp strain TAL1145 promotes nodulation and growth of *Leucaena leucocephala*. Syst Appl Microbiol 31:141–150

Trotta A, Falaschi P, Cornara L, Minganti V, Fusconi A, Drava G, Berta G (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. Chemosphere 65:74–81

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: a review. Appl Biochem Microbiol 42:117–126

Tsavkelova EA, Cherdyntseva TA, Klimova SY, Shestakov AI, Botina SG, Netrusov AI (2007) Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. Arch Microbiol 188: 655–664

Uchiumi T, Ohwada T, Itakura M, Mitsui H, Nukui N et al (2004) Expression islands clustered on the symbiosis island of the *Mesorhizobium loti* genome. J Bacteriol 186:2439–2448

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397

Vargas LK, Lisboa BB, Schlindwein G, Granada CE, Giongo A, Beneduzi A, Pereira Passaglia LM (2009) Occurrence of plant growth-promoting traits in clover-nodulating rhizobia strains isolated from different soils in Rio Grande do Sul state. Rev Bras Cienc Solo 33(5):1227–12357

Verma DPS, Long S (1983) The molecular biology of rhizobium-legume symbiosis. Int Rev Cytol Suppl 14:211–245

Vidal C, Chantreuil C, Berge O, Maure L, Escarre J, Bena G, Brunel B, Cleyet-Marel J-C (2009) *Mesorhizobium metallidurans* sp nov., a metal-resistant symbiont of *Anthyllis vulneraria* growing on metallicolous soil in Languedoc, France. Int J Syst Evol Microbiol 59:850–855

Vivas A, Voros A, Biro B, Barea JM, Ruiz-Lozano JM, Azcon R (2003) Beneficial effects of indigenous Cd-tolerant and Cd-sensitive *Glomus mosseae* associated with a Cd-adapted strain of *Brevibacillus* sp in improving plant tolerance to Cd contamination. Appl Soil Ecol 24:177–186

Vivas A, Biro B, Nemeth T, Barea JM, Azcon R (2006) Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. Soil Biol Biochem 38:2694–2704

Vogel-Mikus K, Drobne D, Regvar M (2005) Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ Pollut 133:233–242

Wang JR, Zhao FJ, Meharg AA, Raab A, Feldmann J, McGrath SP (2002) Mechanisms of arsenic hyperaccumulation in Pteris vittata. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiol 130:1552–1561

Wang HB, Wong MH, Lan CY, Baker AJM, Qin YR, Shu WS, Chen GZ, Ye ZH (2007) Uptake and accumulation of arsenic by 11 Pteris taxa from southern China. Environ Pollut 145:225–233

Wani PA, Khan MS (2013) Nickel detoxification and plant growth promotion by multi metal resistant plant growth promoting Rhizobium species RL9. Bull Environ Contam Toxicol 91:117–124

Wani PA, Khan MS, Zaidi A (2007a) Impact of heavy metal toxicity on plant growth, symbiosis, seed yield and nitrogen and metal uptake in chickpea. Aust J Exp Agr 47:712–720

Wani PA, Khan MS, Zaidi A (2007b) Cadmium, chromium and copper in greengram plants. Agron Sustain Dev 27:145–153

Wani PA, Khan MS, Zaidi A (2007c) Chromium reduction, plant growth-promoting potentials, and metal solubilizatrion by *Bacillus* sp. isolated from alluvial soil. Curr Microbiol 54:237–243

Wani PA, Khan MS, Zaidi A (2007d) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45

Wani PA, Khan MS, Zaidi A (2008a) Effects of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. Bull Environ Contam Toxicol 81:152–158

Wani PA, Khan MS, Zaidi A (2008b) Chromium-reducing and plant growth-promoting *Mesorhizobium* improves chickpea growth in chromium-amended soil. Biotechnol Lett 30:159–163

Wani PA, Khan MS, Zaidi A (2008c) Impact of zinc-tolerant plant growth-promoting rhizobacteria on lentil grown in zinc-amended soil. Agron Sustain Dev 28:449–455

Wani PA, Khan S, Zaidi A (2008d) Effect of metal-tolerant plant growth-promoting Rhizobium on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol 55:33–42

Wani PA, Zaidi A, Khan MS (2009) Chromium reducing and plant growth-promoting potential of *Mesorhizobium* species under chromium stress. Bioremediation J 13:121–129

Weir (2012) The current taxonomy of rhizobia. NZ Rhizobia website. http://www.rhizobia.co.nz/taxonomy/rhizobia. Accessed 10 April 2012

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza 5:245–251

Wheeler CT, Henson IE, McLaughlin ME (1979) Hormones in plants bearing actinomycete nodules. Bot Gaz 140:S52–S57

Wood M, Cooper JE (1988) Acidity, aluminum and multiplication of *Rhizobium trifolii* possible mechanisms of aluminum toxicity. Soil Biol Biochem 20:95–99

Xie ZP, Staehelin C, Wiemken A, Boller T (1996) Ethylene responsiveness of soybean cultivars characterized by leaf senescence, chitinase induction and nodulation. J Plant Physiol 149:690–694

Xiong K, Fuhrmann JJ (1996) Soybean response to nodulation by wild-type and an isogenic *Bradyrhizobium elkanii* mutant lacking rhizobitoxine production. Crop Sci 36:1267–1271

Yasmeen T, Hameed S, Tariq M, Ali S (2012) Significance of arbuscular mycorrhizal and bacterial symbionts in a tripartite association with *Vigna radiata*. Acta Physiol Plant 34:1519–1528

Yasuta T, Satoh S, Minamisawa K (1999) New assay for rhizobitoxine based on inhibition of 1-aminocyclopropane-1-carboxylate synthase. Appl Environ Microbiol 65:849–852

Yasuta T, Okazaki S, Mitsui H, Yuhashi KI, Ezura H, Minamisawa K (2001) DNA sequence and mutational analysis of rhizobitoxine biosynthesis genes in *Bradyrhizobium elkanii*. Appl Environ Microbiol 67:4999–5009

Younis M (2007) Responses of *Lablab purpureus*-rhizobium symbiosis to heavy metals in pot and field experiments. World J Agr Sci 3:111–122

Yuhashi KI, Ichikawa N, Ezura H, Akao S, Minakawa Y, Nukui N, Yasuta T, Minamisawa K (2000) Rhizobitoxine production by *Bradyrhizobium elkanii* enhances nodulation and competitiveness on *Macroptilium atropurpureum*. Appl Environ Microbiol 66:2658–2663

Zahir ZA, Zafar-ul-Hye M, Sajjad S, Naveed M (2011) Comparative effectiveness of *Pseudomonas* and *Serratia* sp containing ACC-deaminase for coinoculation with *Rhizobium leguminosarum* to improve growth, nodulation, and yield of lentil. Biol Fert Soils 47:457–465

Zahran HH (2006) Nitrogen (N2) fixation in vegetable legumes: biotechnological perspectives. In: Ray RC, Ward OP (eds) Microbial biotechnology in horticulture, vol 1. Science Publishers, Inc, Enfield, USA, pp 49–82

Zaidi A, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Zhang F, Smith DL (1996) Genistein accumulation in soybean (*Glycine max* L Merr) root systems under suboptimal root zone temperatures. J Exp Bot 47:785–792

Zhang Y, He L, Chen Z, Zhang W, Wang Q, Qian M, Sheng X (2011) Characterization of lead-resistant and ACC deaminase-producing endophytic bacteria and their potential in promoting lead accumulation of rape. J Hazard Mater 186:1720–1725

Zheng ZW, Fang W, Lee HY, Yang ZY (2005) Responses of *Azorhizobium caulinodans* to cadmium stress. FEMS Microbiol Ecol 54:455–461

# 10 Phytoremediation of Heavy Metals: The Use of Green Approaches to Clean the Environment

N.P. Singh and Anita Rani Santal

## 10.1 Introduction

The rapid development of industrialization results in overall environmental contamination with persistent organic and inorganic wastes (Chaudhry et al. 1998). Among these, heavy metals are playing a vital role in polluting the environment. Heavy metals are present in soils as natural components or as a result of human activity, for example mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, severe agricultural practices, and sludge dumping pollute the soil with large quantities of toxic metals (Seaward and Richardson 1990; Förstner 1995; Kumar et al. 1995; Srivastava 2007). A list of sources causing heavy metal pollution is shown in Table 10.1. These heavy metals have a relatively high density and are toxic or poisonous at low concentrations (Lenntech 2004; Duruibe et al. 2007). Heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). Industries such as mining, petroleum, coal, and garbage burning create heavy metal pollution in the environment, which cannot be easily degraded or destroyed. In a very small amount they enter our bodies via food, drinking water, and air (Duruibe et al. 2007). As a trace element, some heavy metals are needed in small concentration to maintain the metabolism of the human body (Garbisu and Alkorta 2003). However, at higher concentrations they can lead to poisoning (Alkorta et al. 2004). Heavy metals such as lead and mercury are never desirable in any amount in our body. Elevated levels of mercury can cause various health problems (Clarkson 1992). Mercury is a toxic heavy metal which has no known function in human biochemistry or physiology and does not occur naturally in living organisms (Ferner 2001; Nolan 2003; Young 2005; Duruibe et al. 2007). Monomethylmercury has detrimental effects on brain and the central nervous system in humans. However, fetal and postnatal exposures of this form of mercury resulted in abortion, congenital malformation, and other abnormalities in children. However, cadmium is a bio-persistent heavy metal, which once absorbed by an organism is deposited in the body for many years, as far as over decades for humans. In humans and animals, its excessive exposure leads to renal disfunction, lung diseases, bone defects, etc. (Levine and Muenke 1991; Gilbert-Barness 2010).

Various physicochemical methods have been applied to clean up the heavy metals from the environment but these methods are very expensive and cost-effective. Moreover, these methods when applied to the soil are of high impact but are detrimental to soil texture and fertility (Negri and Hinchman 1996; Chaudhry et al. 1998). Heavy metals are only transformed from one oxidation state or organic complex to another. Microorganisms can be used for the bioremediation of metals as they reduce metals in their detoxification mechanism (Garbisu and Alkorta 2001; Edwards et al. 2013).

Phytoremediation can prove to be an important strategy for the removal of the heavy metal from the environment. It is the study of using green plants for the removal of harmful environmental contaminants. This new technology offers a potentially cost-effective cleanup of contaminated groundwater, terrestrial soil, sediments, sludge, etc. Various studies have been carried out for the removal of the heavy metals from the contaminated soils by using phytoremediation strategies (Table 10.2) The purpose of this chapter is to explore the use of a new technology to remove heavy metals from those environments, where it is concentrated. Its toxicity has been enhanced and its mobility into sensitive organisms increased with the increase in heavy metal pollution in the environment. The present

N.P. Singh, Ph.D.
Centre for Biotechnology, Maharshi Dayanand University, Rohtak-124001, Haryana, India

A.R. Santal, Ph.D. (✉)
Department of Microbiology, Maharshi Dayanand University, Rohtak-124001, Haryana, India
e-mail: anita.gangotra@gmail.com

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**Table 10.1** Heavy metals and their sources of contamination

| Sr. No. | Heavy metals | Sources | References |
|---|---|---|---|
| 1 | As | Timber treatment, paints and pesticides, semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, volcanoes, mining, and smelting | (Bissen and Frimmel 2003; Walsh et al. 1979) |
| 2 | Cu | Timber treatment, fertilizers, fungicides, electroplating industry, smelting and refining, mining, biosolids | (Liu et al. 2005) |
| 3 | Cd | Anthropogenic activities, smelting and refining, fossil fuel burning, application of phosphate fertilizers, sewage sludge | (Alloway 1995; Kabata-Pendias 2001) |
| 4 | Pb | Batteries, metal products, mining and smelting of metalliferous ores, burning of leaded gasoline, municipal sewage, industrial wastes enriched in Pb, paints | (Gisbert et al. 2003; Seaward and Richardson 1990) |
| 5 | Cr | Timber treatment, leather tanning, pesticides and dyes, electroplating industry | (Knox et al. 1999; Gowd et al. 2010) |
| 6 | Hg | Fumigants and fertilizers, volcano eruptions, forest fire, emissions from industries producing caustic soda, coal, peat, and wood burning | (Lindqvist 1991) |
| 7 | Zn | Dyes, paints, timber treatment, fertilizers and mine tailings, electroplating industry, smelting and refining, mining | (Liu et al. 2005) |
| 8 | Ni | Alloys, batteries and mine tailings, volcanic eruptions, land fill, forest fire, bubble bursting, and gas exchange in ocean | (Knox et al. 1999) |
| 9 | Cd, Pb, and As | Over application of fertilizers and pesticides | (Atafar et al. 2010) |
| 10 | Pb | Commercial organic fertilizer | (Wang et al. 2013) |
| 11 | Cd, Cu, Ni, and Zn | Urban and industrial wastewater used in agricultural practices | (Hani and Pazira 2011) |

**Table 10.2** Plants used for phytoremediation of heavy metal contamination

| Sr. No. | Plants used | Contaminants | References |
|---|---|---|---|
| 1 | *Trifolium alexandrinum* | Cd, Pb, Cu, and Zn | (Ali et al. 2012) |
| 2 | *Tithonia diversifolia* and *Helianthus annuus* | Pb and Zn | (Adesodun et al. 2010) |
| 3 | *Thlaspi caerulescens* | Zn, Cd, and Ni | (Assunção et al. 2003) |
| 4 | *Pteris cretica cv Mayii* (Moonlight fern) and *Pteris vittata* (Chinese brake fern) | As | (Baldwin and Butcher 2007) |
| 5 | *Alyssum* and *Thlaspi* | Ni | (Bani et al. 2010) |
| 6 | *Aspalathus linearis* (Rooibos tea) | Aluminum | (Kanu Sheku et al. 2013) |
| 7 | *Helianthus annuus* (sunflower) | Zinc and cadmium | (Marques et al. 2013) |
| 8 | *Pelargonium roseum* | (Ni), cadmium (Cd), or lead (Pb) | (Mahdieh et al. 2013) |
| 9 | *Brassica napus* and *Raphanus Sativus* | Cd, Cr, Cu, Ni, Pb, and Zn | (Marchiol et al. 2004) |
| 10 | *Thlaspi caerulescens* | Cd and Zn | (Perronnet et al. 2003) |
| 11 | *Pteris vittate* L. | Arsenic | (Ma et al. 2001; Tu et al. 2002) |
| 12 | *Solanum nigrum* | Cd | (Chen et al. 2014) |

technology of phytoremediation is centered on plants that have been genetically engineered with bacterial genes. These genetically engineered plants having these genes encode enzymes that catalyze the alteration of heavy metal electrochemical form especially in case of mercury. This new strategy is intended to allow the detoxification and controlled translocation of mercury from locations where it may threaten human health or the integrity of ecosystems. It has been predicted that as the field of genetic engineering advances, engineered organisms will replace mechanical tools for many applications, including in the remediation of environmental pollution. These "clean technologies" will result in reductions to the release of toxic substances so inexorably linked to industrial processes yet so toxic to organisms.

## 10.2 Processes of Phytoremediation

There are five different types of major processes involved in phytoremediation. These include phytoextraction, rhizofiltration, phytovolatilization, phytostabilization, and phytodegradation. A short overview of all these process can be seen in Fig. 10.1.

### 10.2.1 Phytoextraction

Phytoextraction is the use of plants to uptake contaminants into their biomass. In this process plants uptake the contaminants by roots and accumulate in the aerial parts or shoots of

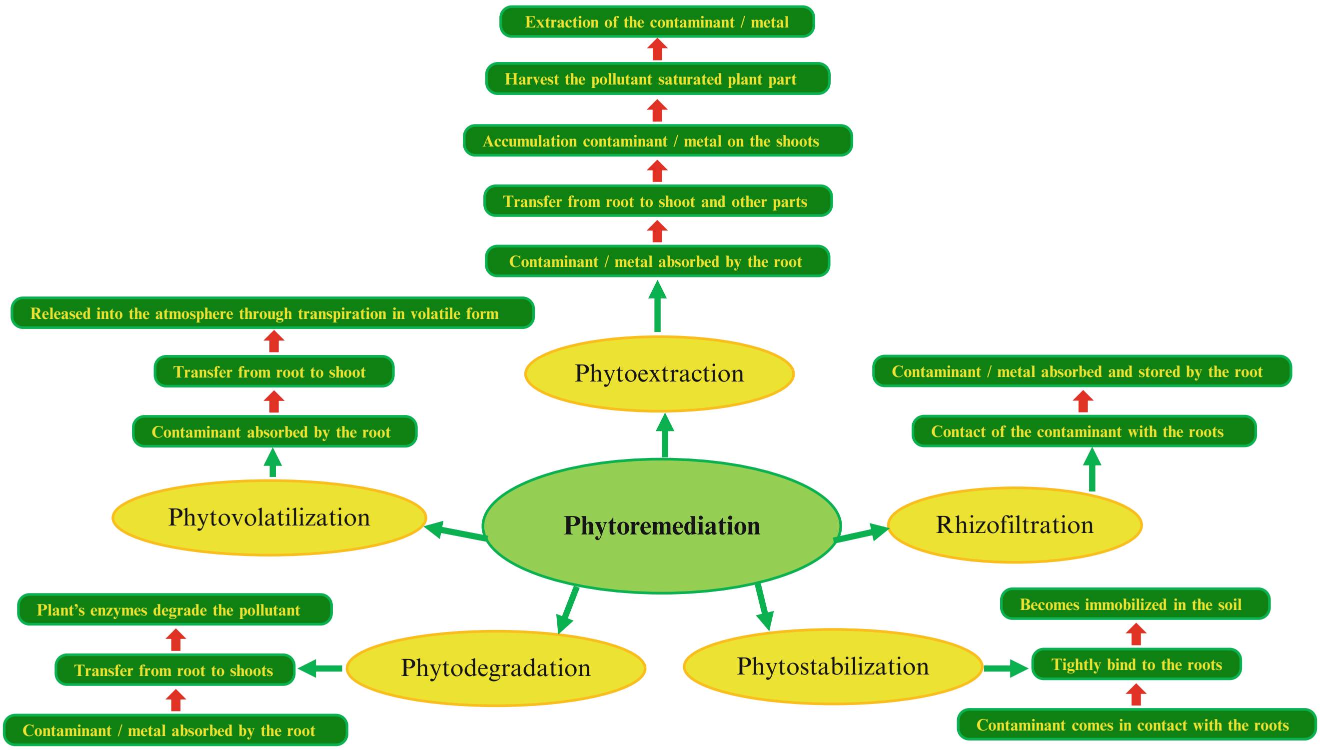


**Fig. 10.1** Different processes of phytoremediation

the plant and finally it is harvested and disposed of (Vishnoi and Srivastava 2007). The plant-based remediation technology is one of the largest technologies to remediate the heavy metal pollution from the environment (Raskin et al. 1997). Phytoextraction can be natural and induced. In natural phytoextraction there is low biomass of hyperaccumulators and it may require decades to reduce the heavy metal concentration in soil, e.g., *Thlaspi caerulescens* (Mahmood 2010). In induced phytoextraction there is high-biomass of hypoaccumulators. Metal hyperaccumulation is triggered through soil amendments that increase the metal phyto availability and translocation from root to shoot e.g., sunflower, ryegrass, and various species of *Brassica* (Salt et al. 1995a). All plant species cannot be used for phytoremediation. Only the hyperaccumulating plants can be used for metal remediation. A plant that is able to take up more metals than normal plants is called a hyperaccumulator, which can absorb more heavy metals that are present in the contaminated soil. This process helps in the reduction of erosion and leaching of the soil. With successive cropping and harvesting, the levels of contaminants in the soil can be reduced (Vandenhove et al. 2001). Various studies emphasized to estimate the metal accumulation capacity of high-biomass plants that can be easily cultivated using agronomic practices. Particularly, on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species. Certain varieties of *Brassica juncea* concentrated toxic heavy metals (Pb, Cu, and Ni) to a level up to several percent of their dried shoot biomass (Kumar et al. 1995). *Zea mays* and *Ambrosia artemisiifilia* were also identified as good accumulators of Pb (Huang and Cunningham 1996; Raskin et al. 1997).

A major setback to the improvement of phytoextraction technology is that the shoot metal accumulation in the hydroponically cultivated plants greatly exceeded the metal accumulation. This phenomenon is explained by the low bioavailability of heavy metals in soils (Cunningham et al. 1995). *Trifolium alexandrinum* effectively extracted the selected heavy metals from the simulated heavy metal-contaminated soil, as evident from the difference of heavy metal concentration values between control and experimental plants. *T. alexandrinum* has many advantages for phytoremediation. It produces considerable biomass and has a relatively short life cycle. It has resistance to prevailing environmental and climatic conditions and above all offers multiple harvests in a single growth period (Ali et al. 2012). Effect of EDTA on phytoremediation has also been studied. The seedling of *Brassica napus* was able to accumulate large quantity of heavy metals in the presence of EDTA. EDTA enhances shoot metals accumulation but does not affect plant growth (Zaier et al. 2010). Phytoextraction can be improved by inoculating some plant-growth beneficial bacterium *Phyllobacterium myrsinacearum* RC6b with plants, e.g., the plant species *Sedum plumbizincicola* affects plant growth and enhances Cd, Zn, and Pb uptake (Ma et al. 2013). They suggested that the metal mobilizing can be improved by using inoculants such as *P. myrsinacearum* RC6b for the multimetal polluted soils. Similarly, the biomass production and shoot Ni concentrations in *Alyssum serpyllifolium* subsp. *malacitanum* was found to be higher when inoculated with two bacterial strains LA44 and SBA82 of *Arthrobacter* than non-inoculated plants (Becerra-Castro et al. 2013). The phytoextraction efficiency can also be affected by fungicidal sprays, soil pH, planting density, and cropping period (Puschenreiter et al. 2013; Simmons et al. 2014).

Roots play a major role in drawing out elements from the soil and deliver to the shoots (Raskin et al. 1997). Scanty information is available on the mechanisms of mobilization, uptake, and transport of most environmentally hazardous heavy metals, such as Pb, Cd, Cu, Zn, U, Sr, and Cs. Before the plant accumulates metals from the environment, it must be mobilized into the environment. Various factors are involved in the mechanism of phytoextraction.

#### 10.2.1.1 Phytoavailability of Metals

The first step of phytoextraction is the phytoavailability of metals in soil. The bioavalability of metals is increased in soil through several means (Ghosh et al. 2011). There are some factors involved by which the plant uptakes the heavy metals from soil (a) quantity factor (the total content of the potentially available metals in soil), (b) the intensity factor (the activity and ionic ratios of metals in the soil solution), and (c) reaction kinetics (the rate of transfer from soil to the liquid phase to the plant roots) (Brümmer et al. 1986). These metals make a complex structure with the soil. Several approaches have been studied and are accomplished in a number of ways. To chelate and solubilize the soil-bound metal some metal-chelating molecules can be secreted into the rhizosphere. Phytosiderophores are iron-chelating compounds, which have also been studied well in plants (Kinnersely 1993). Based on their ability, the phytosiderophores can chelate other heavy metals also other than iron (Meda et al. 2007). These phytosiderophores are released in response to iron deficiency and can mobilize Cu, Zn, and Mn from soil. The Cu toxicity in barley is a signal that activates phytosiderophores release by plant roots, whereas, phytosiderophores release is induced by Cu toxicity which is strongly attenuated by Cd toxicity (Kudo et al. 2013). Metal-chelating proteins called metallothioneins (Robinson et al. 1993) may also function as siderophores in plants. Certain metals induce the synthesis of these proteins in the plant cells. Metallothioneins can tightly bind with zinc, copper, cadmium, mercury, or silver reducing the availability of diffusible forms within the cells and therefore decreasing their toxic potential (Cherian and Goyer 1978). The contribution of phytosiderophores in toxic metal possession by the roots of phytoextracting plants remains largely unexplored. A Ni hyperaccumulator, *Alyssum lesbiacum*, uses histidine, an excellent Ni chelator, to acquire and transport Ni (Kramer et al. 1996). Phytosiderophores such as mugineic acid and avenic acid (which are exuded from roots of graminaceous plants in response to Fe and Zn deficiency) can mobilize Cu, Zn, and Mn (Römheld 1991). The Cd and P phytoavailability of kangkong (*Ipomoea aquatica* Forsk.) with Alfred stonecrop (*Sedum alfredii* Hance) can be induced while inoculated with arbuscular mycorrhizal fungi (Hu et al. 2013).

#### 10.2.1.2 Uptake of Metals by the Roots

The absorption of metals into roots can occur by means of symplastic and apoplastic pathways (Tandy et al. 2006; Lu et al. 2009). In contrast to the apoplastic pathway in which metal ions or metal–chelate complex enters the root through intercellular spaces, the symplastic pathway is an energy-dependent process mediated by specific or generic metal ion carriers or channels. Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants (Crowley et al. 1991). The soil-bound metal ions are reduced by the roots by some enzymes known as reductases bound to the plasma membrane results in the metal availability. For example in case of a pea plant deficient in Fe or Cu, it shows an increased capability to reduce $Fe^{3+}$ and $Cu^{2+}$ and which later on fastens increase in uptake of Cu, Mn, Fe, and Mg from the soil (Welch et al. 1993). The mycorrhizal fungi associated with the roots and root-colonizing bacteria also shows increase in the bioavailability of metals. It is believed that the rhizospheric microorganisms help the plant to uptake the mineral nutrients such as Fe (Crowley et al. 1991), Mn (Barber and Lee 1974), and Cd (Salt et al. 1995b). In a recent report by Lindblom et al. (2014), two rhizosphere fungi *Alternaria seleniiphila* (A1) and *Aspergillus leporis* (AS117) inoculated with selenium (Se) hyperaccumulator *Stanleya pinnata* and non-hyperaccumulator *Stanleya elata* were studied. They concluded that rhizosphere fungi affect the growth and Se and/or S accumulation in these plant species. But some metal ions such as $Ca^{2+}$ and $Mg^{2+}$ that are present at higher concentrations in the soil solution do not require mobilization as they enter the roots through any of the extracellular (apoplastic) or intracellular (symplastic) pathways (Clarkson and Luttge 1989). Recently, a new Mn-hyperaccumulating plant species *Celosia argentea* Linn. has been reported (Liu et al. 2014), which shows higher Mn accumulatation and tolerance level. They found that as the Mn supply level ranged from 2.5 (control) to 400 mg/L, the biomass and the relative growth rate of *C. argentea* were insignificantly changed. In one of the study conducted by Foster and Miklavcic (2014) on the uptake and transport of ions via differentiated root tissues a physical model was proposed. This model indicates both the forced diffusion and convection by the transpiration stream.

The reducing diffusive permeabilities result in altering ion concentration profiles in the pericycle and vascular cylinder regions. However, the increased convective reflectivities affect predominantly ion concentrations in the cortex and endodermis tissues. They concluded that the ion fluxes and accumulation rates are predicted by the self-consistent electric field that arises from ion separation.

#### 10.2.1.3 Transportion from Root to Shoot

In non-hyperaccumulator plants the metal is generally stored within the root cells and is not available for the xylem loading. Whereas, in case of hyperaccumulators roots efficiently transport metals to the shoots, e.g., in case of *Sedum alfredii* ecotype, xylem plays an important role in Cd uptake as compared to the non-hyperaccumulating ecotypes (Lu et al. 2009). The translocation of Cd uptake and Cd phytoextraction has been recently studied by Hu et al. (2013) in another species of *Sedum plumbizincicola*. In this study they found that the rate of Cd uptake was more from roots to the shoots when $NO^{3-}$ treatment was given. For the translocation of metals from roots to shoot via xylem, firstly, they must have to cross the Casparian band on endodermis, which is a water-impervious barrier that blocks the apoplastic flux of metals from the root cortex to the stele. Therefore, to cross this barrier and to reach the xylem, metals must move symplastically. The xylem loading process is mediated by membrane transport proteins (Huang and Van Steveninck 1989; Clemens et al. 2002). However, in metal accumulators, xylem loading as well as translocation to shoot is facilitated by complexing of metal with low-molecular weight chelators (LMWCs), e.g., organic acids (Senden et al. 1995), phytochelatins (Przemeck and Haase 1991), and histidine (Krämer et al. 1996). The metal translocation patterns of important heavy metals such as Cr, Ni, Cu, Cd, and Pb in plant species *Solanum melongena* has recently been studied by Wiseman et al. (2014). They examined tissue patterns of metal (Cr, Ni, Cu, Cd, and Pb) concentrations associated with elemental deposition and soil-to-root and root-to-shoot transfers. They concluded that copper easily translocates to roots in water-logging soils as compared to Cd which has highest soil-to-root and root-to-shoot translocation. Metal chelators and transporters regulate metal homeostasis in plants. Studies have been carried out on HMA2 gene characterization from various plants for their potential application in phytoremediation. The membrane transporter protein helps the plants to become metal-resistant and metal-hyperaccumulator. Whereas, the other gene HMA3 contributes towards metal detoxification by Cd sequestration into the vacuole and the HMA4 gene triggers the process of metal hyperaccumulation. Both the genes HMA2 and HMA4 play an important role during root-to-shoot metal translocation (Park and Ahn 2014). The uptake of gold nanoparticles (AuNPs) followed by translocation and transport into plant cells in case of poplar plants (*Populus deltoides*×*nigra*, DN-34) has been recently studied and found that these gold nanoparticles accumulated in the plasmodesma of the phloem complex in root cells (Zhai et al. 2014).

#### 10.2.1.4 Metal Unloading, Trafficking, and Storage in Leaves

Metal is transported to the apoplast (free diffusional space outside the plasma membrane) of leaves from where it is distributed within the leaf tissue via apoplast or transporters-mediated uptake by symplast (inner side of the plasma membrane in which water (and low-molecular-weight solutes) can freely diffuse) (Mahmood 2010). At any point of the transport pathway metals make a complex with organic ligands and thus the metal converts into a less toxic form (Peers et al. 2005). Metals are sequestered in extracellular or subcellular compartments of the leaves. About 35 % of the Cd taken up by *T. caerulescens* was found in the cell walls and the apoplast in leaves (Cosio et al. 2005), whereas in Ni hyperaccumulator *Thlaspi geosingense*, Ni is sequestered in the cell wall as well as in vacuoles (Krämer et al. 2000; Mahmood 2010). Leaf trichomes may be the major sequestering sites for Cd in *Brassica juncea* (Salt et al. 1995b); for Ni in *Alyssum lesbiacum* (Krämer et al. 1997); and for Zn in *Arabidopsis halleri* (Küpper et al. 2000). Different approaches have been envisaged by Clemens et al. (2002) for engineering the plant metal homeostasis network to increase the metal accumulation in plants. For example, keeping in view the importance of vacuoles as the metal storage organelle, engineering tonoplast transporters in specific cell types might enhance the metal accumulation capability. Alternatively, creation of artificial metal sinks in shoots via expression of the cell wall proteins with high-affinity metal binding sites might be explored to increase the metal demand in shoots thus enhancing the accumulation in leaves (Clemens et al. 2002). Metal translocation can also be affected by fertilizer treatment. While working on cadmium translocation in *Oryza sativa* Sebastian and Prasad (2014) they found that ammonium phosphate–sulfur fertilization affects the shoot growth. Due to fertilizer treatment an increase in photosynthetic pigments was recorded that altered the activity of antioxidant enzymes which ultimately results in steady photosynthetic rate.

The molecular mechanisms for heavy metal adaptation has been well studied in some model plants such as *A. halleri* or *Thlaspi/Noccaea* spp. (Becher et al. 2004; Dräger et al. 2004; Hanikenne et al. 2008; Plessl et al. 2010; van de Mortel et al. 2006). A network of transporters tightly controls uptake into roots, xylem loading, and vacuolar sequestration (Broadley et al. 2007; Verbruggen et al. 2009). Although these transporters are thought to balance the concentration of essential metals such as Zn, they also unselectively transport

**Table 10.3** Plant species used for the rhizofiltration of heavy metal contaminants

| Sr. No. | Contaminants | Plant species | References |
|---|---|---|---|
| 1 | Cd and Pb | *Brassica juncea* | (Qiu et al. 2014) |
| 2 | Sb | *Cynodon dactylon* | (Xue et al. 2014) |
| 3 | Pb | *Oxycaryum cubense* (Poep. & Kunth) Palla | (Alves et al. 2014) |
| 4 | Pb | *Azolla pinnata* | (Thayaparan et al. 2013) |
| 5 | Al, Fe, and Mn | *Pistia stratiotes* L. | (Veselý et al. 2012) |
| 6 | As | *Cynara cardunculus* | (Llugany et al. 2012) |
| 7 | Pb | *Carex pendula* | (Yadav et al. 2011) |
| 8 | Cd | *Setaria italica* (L.) Beauv. | (Chiang et al. 2011) |
| 9 | Cd and Pb | *Pistia stratiotes* L., *Salvinia auriculata* Aubl., *Salvinia minima* Baker, and *Azolla filiculoides* Lam | (Veselý et al. 2011) |
| 10 | Cu, Ni, and Zn | *Eichhornia crassipes* (Mart.) Solms | (Hammad 2011) |
| 11 | Al, Fe, Zn, and Pb | *Typha domingensis* | (Hegazy et al. 2011) |
| 12 | Mn | *Cnidoscolus multilobus*, *Platanus mexicana*, *Solanum diversifolium*, *Asclepius curassavica* L., and *Pluchea sympitifolia* | (Juárez-Santillán et al. 2010) |
| 13 | U (Uranium) | *Helianthus annuus* L. and *Phaseolus vulgaris* L. var. *vulgaris* | (Lee and Yang 2010) |

toxic elements such as Cd (Mendoza-Cozatl et al. 2011; Verbruggen et al. 2009). Inside the cells, metals are chelated with small molecules such as the low molecular weight, cysteine-rich metallothioneins or non-translationally synthesized, glutathione-derived phytochelatins (Cobbett and Goldsbrough 2002). Remarkable similarity in copy number expansion and transcriptional regulation was found for the xylem loading transporter HEAVY METAL ATPASE 4 (HMA4) in *A. halleri* and *N. caerulescens*, indicating parallel evolutionary pathways in these two Brassicaceae species (Hanikenne et al. 2008; Ó Lochlainn et al. 2011). Moreover, HMA4 was recently found to be involved in maintenance of Zn homeostasis also in poplar (Adams et al. 2011). This example of cross-species functionality suggests that well-studied pathways might also act in *S. caprea* metal tolerance.

### 10.2.2 Rhizofiltration

Plant roots absorb or adsorb, concentrate, and precipitate toxic metals from contaminated sites (waste water, surface water). Both terrestrial and aquatic plants show these type of activities (Yadav et al. 2011). In rhizofiltration it is the root system of plants that interacts with the contaminants or polluted site for making that area pollution free (Krishna et al. 2012). It is a potential technique for the removal of wide range of organic and inorganic contaminants, and it also reduces the bioavailability of the contaminant in the food chain. During the rhizofiltration, the contaminant remains on/within the root. The different plant species that have been used for rhizofiltration so far are listed in Table 10.3. These contaminants are to be taken up and translocated into other portions of the plant by the roots, which depends on the contaminant, its concentration, and the plant species. This mechanism is supported by the synthesis of certain chemicals within the roots, which cause heavy metals to rise in plant body. The precipitation of the metals/contaminants on the root surfaces is due to the presence of some internal factors within the soil such as root exudates and pH (Day et al. 2010; Krishna et al. 2012). As the plants absorb metals contaminants from the soil, roots or whole plants are harvested for disposal (Prasad and Freitas 2003). Various exudates such as simple phenolics and other organic acids are released during root decay, which results in change of metal speciation (Ernst 1996). This leads to the increased precipitation of the metals. The organic compounds in the root exudates can stimulate microbial growth in the rhizosphere (Pivetz 2001). Genes plays an important role in the plants to make it efficient for metal accumulation. For example glutathione and organic acids metabolism pathways play a key role in making the plant metal tolerant. Other environmental factors such as light, temperature, and pH also affect metal accumulation efficiency (Rawat et al. 2012). Rhizofiltration can be done in situ i.e. in surface water bodies and ex situ by means of engineered tanks having system of contaminated water and the plants. Both the systems require an understanding of the contaminant speciation and interactions of all contaminants and nutrients (Terry and Banuelos 2000; Akpor et al. 2014). The hydroponically cultivated roots of terrestrial plants are found to be more effective than the normal plant-based systems. For an ideal rhizofiltration mechanism, a plant should have rapidly growing roots that have the ability to remediate toxic metals in soluble form. For example some varieties of sunflower and *B. juncea* have high efficiency for rhizofiltration (Dushenkov et al. 1995). For the improvement of the rhizofiltration, attempts have been made to grow young plant seedlings in aquaculture for removing heavy metals. From the last few years studies have been conducted on the ability of plant roots to tolerate, remove, and degrade pollutants. The roots degrade the contaminants by releasing root exudates and some oxidoreductive enzymes such as peroxidases and laccases (Agostini et al. 2013). Due to the root's environmental compatibility and cost-effectiveness it has great potential to remediate contaminated soils and groundwater. So, research has been carried out to develop genetically engineered roots for the remediation of the polluted sites especially organic pollutants and heavy metals. By using this technology, hairy roots can be produced to increase the phytoremediation efficiency. However, with the help of the rhizospheric bacteria this efficiency can be used to improve more tolerance level to pollutants (Zhou et al. 2013). Recently, Al-Shalabi and Doran (2013) has studied hairy root efficiency for hyperaccumulation of Cd and Ni in plants.

### 10.2.3 Phytovolatilization

It involves the use of plants to uptake the contaminants from the soil and transforming them into volatile form and released into the atmosphere through transpiration (Ghosh and Singh 2005). Plants take up organic and inorganic contaminants with water and pass on to the leaves and volatilize into the atmosphere (Mueller et al. 1999). Mercury is the first metal that has been removed by phytovolatilization. The mercuric ion is transformed into less toxic elemental mercury (Henry 2000). Transgenic technology has been applied by inserting an altered mercuric ion reductase gene (*merA*) into *Arabidopsis thaliana*, for the production of a mercury-resistant transgenic plants that volatilized mercury into the atmosphere (Rugh et al. 1996). Some of the other toxic metals such as Se, As, and Hg can be biomethylated to form volatile molecules and liberated into the atmosphere. Phytovolatilization has also been done by using plant–microbe interactions for the volatilization of Se from soils (Karlson and Frankenberger 1989). *Brassica juncea* has been identified as an efficient plant for removal of Se from soils (Bauelos and Meek 1990). The plant species *Pteris vittata* L. (Chinese Brake fern) has been reported as an arsenic (As) hyperaccumulator that can also accumulate a large amount of Se. Some anti-oxidative enzymes such as catalase, ascorbate peroxidase, and peroxidase contribute towards hyperaccumulation of Se (Feng and Wei 2012). Some chemicals such as organochlorines (OCs), 1,4-dichlorobenzene (DCB), 1,2,4-trichlorobenzene (TCB), and γ-hexachlorocyclohexane (γHCH) are persistent chemicals in the environment. Their uptake depends mostly on their hydrophobicity, solubility, and volatility. The uptake of organochlorines (OCs) has been studied in *Phragmites australis* under hydroponic conditions (San Miguel et al. 2013).

Studies have been carried out also on some other volatile organic compounds (VOCs) such as 1,4-dioxane. It has been found that dioxane (2.5 μg/L) was effectively removed by using phytovolatilization (Ferro et al. 2013).

### 10.2.4 Phytostabilization

Phytostabilization is the process in which plants immobilize the contaminants in the soil or ground water using absorption, adsorption onto the surface of the roots, or by the formation of insoluble compounds. This process reduces the mobility of contaminants and ultimately prevents their migration into the groundwater or into the air (Soudek et al. 2012). It depends on the ability of the roots to limit contaminant's mobility in the soil (Berti and Cunningham 2000). It decreases the amount of water percolation through the soil matrix, forms hazardous leachate. It helps in preventing soil erosion and prevents spreading of toxic metal to other areas. It is not a process of removal of metal contaminants from the sites, but more the stabilization and reduction of the contamination. For an efficient phytostabilization system a plant needs a dense root system (Cunningham and Ow 1996). *Sorghum bicolor* L is one of the plant species which is able to accumulate large quantities of metals in shoots grown in hydroponic conditions. Heavy metals such as Cd and Zn were found to be accumulated primarily in roots. But as the concentration of the metals increased in the solution their transfer to the shoots increased (Soudek et al. 2012). Similarly, in copper-contaminated soil, *Oenothera glazioviana* had high tolerance to copper and shows low upward transportation capacity of copper. Therefore, this plant has a great potential for the phytostabilization of copper from the copper-contaminated soils and a high commercial value without risk to human health (Guo et al. 2014). Other plants such as *Sesbania virgata* have also been reported as excellent phytostabilizers for metals such as copper, zinc, and chromium from the metal-contaminated soils. The main accumulation of heavy metals appeared in plant roots, and more Zn is removed from soils. When supplied in a mixture of Cu and Zn, *Sesbania* plants absorb the highest concentrations of these metals. In contrast, Cr was more absorbed in the individual treatments (Branzini et al. 2012). In one of the study conducted on *B. juncea* by Pérez-Esteban et al. (2013), phytostabilization ability can be enhanced by the addition of manure in the contaminated soil.

### 10.2.5 Phytodegradation

Phytodegradation is the uptake and degradation of contaminants within the plant, or the degradation of contaminants in the soil, ground water, or surface water, by enzymes. This process involves the use of plants with associated microorganisms to degrade organic pollutants, such as 2,4,6-trinitrotoluene (TNT) and polychlorinated biphenyls, herbicides, and pesticides so that they can be converted from toxic form to nontoxic form (Lee 2013; Kukreja and Goutam 2013). Hybrid poplars are capable of degrading trichloroethylene, which is one of the most common pollutants (Newman et al. 1997). Some enzymes such as dehalogenase, peroxidase, nitroreductase, laccase, and nitrilase produced by the plants also helps in degradation of pollutants (Schnoor et al. 1995; Morikawa and Erkin 2003; Boyajian and Carreira 1997). Kagalkar et al. (2011) biodegrades the triphenylmethane dye Malachite Green by using cell suspension cultures of *Blumea malcolmii* Hook. This degradation was occurred due to the induction of enzymes such as laccase, veratryl alcohol oxidase, and DCIP reductase. The textile dye Red RB and Black B has also been achieved by using water hyacinth (*Eichhornia crassipes*) (Muthunarayanan et al. 2011). Plants such as *Hydrilla verticillata* and *Myriophyllum verticillatum* are efficient in degrading chemical contaminants such as bisphenol A(BPA) within the concentration of 1–20 mg/L (Zhang et al. 2011). Recently it has been reported that some chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs), which are present in the terrestrial environment can be degraded by using a water hyacinth (*Eichhornia crassipes*) in combination with some chemicals such as sodium sulfate ($Na_2SO_4$), sodium nitrate ($NaNO_3$), and sodium phosphate ($Na_3PO_4$) (Ukiwe et al. 2013). They resulted that 99.4 % (pH 2.0) of acenaphthrene and 90.4 % (pH 4.0) of acenaphthrene was degraded after using $NaNO_3$ and $Na_2SO_4$ with *E. crassipes*, respectively.

## 10.3 Improvement of Phytoremediation Efficiency of Plants

### 10.3.1 Plant–Microbe Interactions to Enhance the Phytoremediation Efficiency of Plants

As the microbes are the first organisms which come in contact with the contaminated sites therefore they have to develop their own mechanism to grow in such sites and become tolerant to these pollutants. These microbes play an important role in degradation of the complex chemical compounds to the simpler chemicals which can be easily absorbed by the plant systems. Some bacteria have stress-tolerant genes, which make them resistant towards the heavy metals and some bacteria have enzymes such as metal oxidases and reductases to make them tolerant against these contaminants. To improve the phytoremediation efficiency of the plants researchers have made efforts by using the plant and soil–microbe interactions (Table 10.4). They selected biodegradative bacteria, plant growth-promoting bacteria, and other bacterial strains that resist soil pollutants (Wenzel and Jockwer 1999; Glick 2003). As most of the mineral nutrients are taken up by the plants through the rhizosphere where these microbes interacts with the plant root surface (Dakora and Phillips 2002). The root exudates provide source of carbon for the microbes and also take part in direct detoxification by forming chelates with metal ions (Bashan et al. 2008). Rhizosphere has a large quantity of microbes and has high metabolic activity (Anderson et al. 1993). The rate of exudation is increased by the presence of essential microorganisms in the rhizosphere and promoted by the uptake and assimilation of certain nutrients (Gardner et al. 1983). Various plant growth promoting rhizobacteria (PGPR) hydrolyse 1-aminocyclopropane-1-carboxylate (ACC) which is a precursor of the plant hormone ethylene

**Table 10.4** Plant–microbe interaction used for heavy metal phytoremediation

| Sr. No. | Name of metals | Associated plants | Associated microbes | References |
|---|---|---|---|---|
| 1 | Cd, Pb, and Zn | *Brassica napus* | *Enterobacter* sp. and *Klebsiella* sp. | (Jing et al. 2014) |
| 2 | Cd, Zn | Yellow lupine plants | *Rhizobium* sp., *Pseudomonas* sp., *Clavibacter* sp. | (Weyens et al. 2014) |
| 3 | Cd and Pb | *Brassica juncea* | *Enterobacter* sp | Qiu et al. 2014 |
| 4 | Cd, Pb, and Zn | *Brassica napus* | *Enterobacter* sp. and *Klebsiella* sp. | (Jing et al. 2014) |
| 5 | Cd, Zn | *Helianthus annuus* | *Ralstonia eutropha* and *Chrysiobacterium humi* | (Marques et al. 2013) |
| 6 | Multimetal contaminants | *Agrostis capillaris* and *Festuca rubra* | Bacterial consortium | (Langella et al. 2014) |
| 7 | Cd | *Trifolium repens; Solanum nigrum* | Coinoculation of *Brevibacillus* sp. and AM Fungus; *Pseudomonas* sp. Lk9 | (Vivas et al. 2003; Chen et al. 2014) |
| 8 | Cr (VI) | *Cicer arietinum* | *Kocuria flava* | (Singh et al. 2014) |
| 9 | As | *Pteris vittata* | Mycorrhization *Glomus mosseae* or *Gigaspora margarita* | (Trotta et al. 2006) |
| 10 | Ni | *Brassica campestris* | *Kluyvera ascorbata* SUD165 | (Burd et al. 1998) |

**Table 10.5** Various transgenic plants raised by using various gene/genes to improve the heavy metal phytoremediation efficiency

| Sr. No. | Gene | Source of gene | Target plant | Heavy metal | References |
|---|---|---|---|---|---|
| 1 | *gcsgs* | *Enterobacter* sp. | *Brassica juncea* | Cd and Pb | (Qiu et al. 2014) |
| 2 | P450 2E1 | Human | alfalfa plants | Hg | (Zhang et al. 2013) |
| 4 | *ScMTII* | *Saccharomyces cerevisiae* | *N. tabacum* | Cd and Zn | (Daghan et al. 2013) |
| 5 | *ScYCF1* | *Saccharomyces cerevisiae* | *Populus alba* | Cd, Zn, and Pb | (Shim et al. 2013) |
| 6 | *PvACR3* | *Pteris vittata* | *Arabidopsis thaliana* | As | (Chen et al. 2013) |
| 7 | *TaVP1* | *N. tabacum* | *Arabidopsis thaliana* | Cd | (Khoudi et al. 2013) |
| 8 | *YCF1* and AsPCS1 | Garlic and baker's yeast | *Arabidopsis thaliana* | As and Cd | (Guo et al. 2012) |
| 9 | APS1 | *A. thaliana* | *Brassica juncea* | Se and Cd | (Kubachka et al. 2007) |
| 10 | OASTL | *A. thaliana* | *Arabidopsis* | Cd | (Dominguez-Solis et al. 2004) |
| 11 | SMT | *Astragalus bisulcatus* | *Arabidopsis* and *Brassica juncea* | Se | (LeDuc et al. 2004) |
| 12 | gshI and gshII and APSI | *E. coli* | *Arabidopsis thaliana* and *Brassica juncea* | As and Cd | (Bennett et al. 2003) |
| 13 | TaPCS1 | Wheat | *Nicotiana. glauca* | Pb, Cd, Zn, Cu, and Ni | (Gisbert et al. 2003) |
| 14 | HisCUP1 | Yeast | *Nicotiana tabacum* | Cd | (Thomas et al. 2003) |
| 15 | NtCBP4 | *N. tabacum* | *N. tabacum* | Ni, Pb, and Ni | (Sunkar et al. 2000) |

due to the presence of an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Arshad et al. 2007). The application of microbes for metal solubilization from the polluted sites is a potential approach for increasing metal bioavailability to the plants, e.g., some bacterial strains such as *Proteus* sp., *Bacillus* sp., *Clostridium* sp., *Alcaligenes* sp., and *Coccobacillus* sp. have been studied earlier for remediation of cadmium from the environment (Venkatesan et al. 2011). The phytoremediation will be more effective if bacteria can degrade the soil pollutant as well as promote the growth of plants. Recently similar efforts have been done by working on the spinach. In which the plant–microbe interaction in soil contaminated with Cd showed improved the spinach growth and Cd uptake as compared to control (Ali et al. 2013). Similar studies have been carried out by taking some plants like *Alyssum murale, Brassica napus*, and *Thlaspi caerulescens* inoculated with rhizobacteria for the removal of Ni, Cd, Zn, respectively from the contaminated sites (Abou-Shanab et al 2006; Sheng and Xia 2006; Gonzaga et al. 2006).

### 10.3.2 Transgenic Technology to Enhance the Phytoremediation Efficiency of Plants

As the phytoremediation of pollutants is a slow process and accumulation of toxic metabolites also leads to the cycling of these metabolites into the food chain. From the last few decades, work has been carried out to develop transgenic plants to overcome the inbuilt constraints of plant detoxification capabilities. So transgenic technology is the new approach for phytoremediation, which enhances metal uptake, transport, and accumulation as well as plant tolerance capacity to abiotic stresses (Karenlampi et al. 2000). A list of the gene/genes used to raise the transgenic plants is listed in Table 10.5. In this way, *Nicotiana tabacum* was the first transgenic plant that shows the ability to tolerate heavy metal stress. In which the metallothionein gene was taken from a yeast that gives tolerance to cadmium, and *Arabidopsis thaliana* that overexpressed a mercuric ion reductase gene for higher tolerance to mercury (Eapen and D'Souza 2005). Similarly, transgenic alfalfa plants pKHCG co-expressing human CYP2E1 and glutathione S-transferase (GST) genes were developed for the phytoremediation of heavy metals and organic polluted soils. These plants showed tolerance to a mixture of cadmium (Cd) and trichloroethylene (TCE) and metabolized by the introduction of GST and CYP2E1 in combination (Zhang et al. 2013). Earlier, Bañuelos et al. (2005) has developed Indian mustard (*Brassica juncea* (L.) Czern.) lines by introducing overexpressed genes encoding the enzymes adenosine triphosphate sulfurylase (APS), ç-glutamyl-cysteine synthetase (ECS), and glutathione synthetase (GS) to improve their ability to remove selenium (Se). They found that these lines accumulate more Se in their leaves than wild type. Metal tolerance can also be significantly increased by overexpressing some proteins involved in intracellular metal sequestration (Eapen and D'Souza 2005). According to Kiyono et al. (2012) when *Arabidopsis* was introduced with a bacterial *merC* gene from the Tn*21*-encoded *mer* operon resulted in more resistant to cadmium than the wild type and accumulated significantly more cadmium. Similarly, transposon TnMERI1 of *Bacillus megaterium* strain MB1 was used to make the transgenic *Arabidpsis* for the expression of a specific mercuric ion binding protein (MerP) to increase the tolerance and accumulation capacity for mercury, cadmium, and lead (Hsieh et al. 2009).

The root-colonizing bacterium *Pseudomonas fluorescens* has been engineered to express XplA gene to degrade explosive chemicals like Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the rhizosphere (Lorenz et al. 2013). The overexpression of *AsPCS1* and *YCF1* genes in transgenic *Arabidopsis thaliana* leads to increased tolerance and accumulation of heavy metals and metalloids and found to be more tolerant to arsenic and cadmium (Guo et al. 2012). The transgenic white poplar plants plant obtained by the transfer of PsMTa1 gene from *Pisum sativum* for a metallothionein-like protein shows resistance to heavy metal, surviving high concentrations of $CuCl_2$ than the wild type (Balestrazzi et al. 2009). There are some specific genes which are induced by the presence of particular toxic chemicals in the environment are known as "pollutant-responsive elements" (Soleimani et al. 2011). The barley promoter gene HvhsplT in the presence of heavy metals fused to reporter gene was used to make a transformed tobacco plant which could be used as a bioindicator for monitoring heavy metal pollution (Mociardini et al. 1998).

## 10.4 Conclusion

Phytoremediation is a cost-effective technique for the removal of heavy metals from the contaminated soils/sites. During the last two decades a large number of researchers have worked on phytoremediation using plants, microorganisms, plant–microbe interactions, and transgenic plants. Nowadays biotechnology is a powerful tool used in phytoremediation to improve the metal uptake efficiencies of the plants, but it is limited to the lab conditions or at a very small scale. The studies reviewed in this chapter have remarkably contributed towards our knowledge on various phytoremediation strategies. Moreover, the application of transgenic technology and plant-microbe interactions are feasible strategies for the improvement of plants for heavy metal tolerance, their accumulation in the plant parts and also to metabolize the heavy metal pollutants. Hence, it is better to create or find an appropriate plant system for environmental cleanup.

## References

Abou-Shanab RI, Angle JS, Chaney RL (2006) Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils. Soil Biol Biochem 38:2882–2889

Adams JP, Adeli A, Hsu C-Y, Harkess RL, Page GP, dePamphilis CW, Schultz EB, Yuceer C (2011) Poplar maintains zinc homeostasis with heavy metal genes *HMA4* and *PCS1*. J Expt Bot 62:3737–3752

Adesodun JK, Atayese MO, Agbaje T, Osadiaye BA, Mafe O, Soretire AA (2010) Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. Water Air Soil Poll 207:195–201

Agostini E, Talano MA, González PS, Oller ALW, Medina MI (2013) Application of hairy roots for phytoremediation: what makes them an interesting tool for this purpose? Appl Microb Biotech 97(3):1017–1030

Akpor OB, Okolomike UF, Olaolu TD, Aderiye BI (2014) Remediation of polluted wastewater effluents: hydrocarbon removal. Trends Appl Sci Res 9(4):160–173

Ali H, Naseer M, Sajad MA (2012) Phytoremediation of heavy metals by *Trifolium alexandrinum*. Int J Environ Sci 2:1459–1469

Ali T, Mahmood S, Khan MY, Aslam A, Hussain MB, Asghar HN, Akhtar MJ (2013) Phytoremediation of cadmium contaminated soil by auxin assisted bacterial inoculation. Asian J Agric Biol 1(2):79–84

Alkorta I, Hernandez-Allica J, Becerril JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotech 3:71–90

Alloway BJ (1995) Heavy metals in soils. Blackie Academic & Professional, London

Al-Shalabi Z, Doran PM (2013) Metal uptake and nanoparticle synthesis in hairy root cultures. In: Doran PM (ed) Biotechnology of hairy root systems. Springer, Berlin, Heidelberg, pp 135–153

Alves LQ, de Jesus RM, de Almeida AA, Souza VL, Mangabeira PA (2014) Effects of lead on anatomy, ultrastructure and concentration of nutrients in plants *Oxycaryum cubense* (Poep. & Kunth) Palla: a species with phytoremediator potential in contaminated watersheds. Environ Sci Pollut Res Int 21(10):6558–6570

Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil. Environ Sci Technol 27(13):2630–2636

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotech 25(8):356–362

Assunção AGL, Schat H, Aarts MGM (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360

Atafar Z, Mesdaghinia A, Nouri J, Homaee M, Yunesian M, Ahmadimoghaddam M, Mahvi AH (2010) Effect of fertilizer application on soil heavy metal concentration. Environ Monit Assess 160(1–4):83–89

Baldwin PR, Butcher DJ (2007) Phytoremediation of arsenic by two hyperaccumulators in a hydroponic environment. Microchem J 85:297–300

Balestrazzi A, Botti S, Zelasco S, Biondi S, Franchin C, Calligari P (2009) Expression of the $PsMT_{A1}$ gene in white poplar engineered with the MAT system is associated with heavy metal tolerance and protection against 8-hydroxy-2′-deoxyguanosine mediated-DNA damage. Plant Cell Rep 28:1179–1192

Bani A, Pavlova D, Echevarria G, Mullaj A, Reeves RD, Morel JL, Sulçe S (2010) Nickel hyperaccumulation by the species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. Bot Serb 34:3–14

Bañuelos G, Terry N, LeDuc DL, Pilon-Smits EA, Mackey B (2005) Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. Environ Sci Technol 39(6):1771–1777

Barber DA, Lee RB (1974) The effect of microorganisms on the absorption of manganese by plants. New Phytol 73:97–106

Bashan Y, Puente ME, Bashan LE, Hernandez JP (2008) Environmental uses of plant growth-promoting bacteria. In: Barka EA, Clémen C (eds) Plant-microbe interactions. Research Signpost, Kerala, India, pp 69–93

Bauelos GS, Meek DW (1990) Accumulation of selenium in plants grown on selenium-treated soil. J Environ Qual 19:772

Becerra-Castro C, Kidd P, Kuffner M, Prieto-Fernández Á, Hann S, Monterroso C, Sessitsch A, Wenzel W, Puschenreiter M (2013) Bacterially induced weathering of ultramafic rock and its implications for phytoextraction. Appl Eenviron Microbiol 79(17):5094–5103

Becher M, Talke IN, Krall L, Krämer U (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. Plant J 37:251–268

Bennett LS, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EAH (2003) Analysis of transgenic indian mustard plants for phytoremediation of metal-contaminated mine tailings. J Environ Qual 32(2):432–440

Berti WR, Cunningham SD (2000) Phytostabilisation of metals. In: Raskin I (ed) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley-InterScience, New York, NY, pp 71–88

Bissen M, Frimmel FH (2003) Arsenic a review. Part I: occurrence, toxicity, speciation, mobility. Acta Hydrochim Hydrobiol 31(1):9–18

Boyajian GE, Carreira LH (1997) Phytoremediation: a clean transition from laboratory to marketplace? Nat Biotechnol 15:127–128

Branzini A, González RS, Zubillaga M (2012) Absorption and translocation of copper, zinc and chromium by *Sesbania virgata*. J Environ Manag 102:50–54

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702

Brümmer G, Gerth J, Herms U (1986) Heavy metal species, mobility and availability in soils. Z Pflanzenernähr Bodenkd 149:382–398

Burd GI, Dixon DG, Glick BR (1998) A plant growthpromoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation-focusing on accumulator plants that remediate metal contaminated soils. Aust J Ecotoxicol 4:37–51

Chen L, Luo S, Li X, Wan Y, Chen J, Liu C (2014) Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas sp*. Lk9 on soil heavy metals uptake. Soil Biol Biochem 68:300–308

Chen Y, Xu W, Shen H, Yan H, Xu W, He Z, Ma M (2013) Engineering arsenic tolerance and hyperaccumulation in plants for phytoremediation by a PvACR3 transgenic approach. Environ Sci Tech 47(16):9355–9362

Cherian MG, Goyer RA (1978) Metallothioneins and their role in the metabolism and toxicity of metals. Life Sci 23(1):1–9

Chiang PN, Chiu CY, Wang MK, Chen BT (2011) Low-molecular-weight organic acids exuded by millet (*Setaria italica* (L.) Beauv.) roots and their effect on the remediation of cadmium-contaminated soil. Soil Sci 176(1):33–38

Clarkson DT, Luttge U (1989) Mineral nutrition: divalent cations, transport and compartmentalization. Prog Bot 51:93–112

Clarkson TW (1992) Mercury: major issues in environmental health. Environ Health Perspect 100:31–38

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: Understanding and engineering plant metal accumulation. Trend Plant Sci 7:309–315

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Ann Rev Plant Biol 53:159–182

Cosio C, DeSantis L, Frey B, Diallo S, Keller C (2005) Distribution of cadmium in leaves of *Thlaspi caerulescens*. J Expt Bot 56(412):765–775

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ (1991) Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant Soil 130:179–198

Cunningham SD, Berti WR, Huang JW (1995) Phytoremediation of contaminated soils. Trends Biotechnol 13(9):393–397

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110(5):715–719

Daghan H, Arslan M, Uygur V, Koleli N (2013) Transformation of tobacco with *ScMTII* gene-enhanced Cadmium and Zinc accumulation. Clean Soil Air Water 41(5):503–509

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245(1):35–47

Day SD, Wiseman PE, Dickinson SB, Harris JR (2010) Tree root ecology in the urban environment and implications for a sustainable rhizosphere. Arboricult Urban Fores 36(5):193–205

Dominguez-Solis JR, Lopez-Martin MC, Ager FJ, Ynsa MD, Romero LC, Gotor C (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. Plant Biotechnol J 2:469–476

Dräger DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Krämer U (2004) Two genes encoding Arabidopsis halleri MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. Plant J 39:425–439

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human biotoxic effects. Intl J Phys Sci 2(5):112–118

Dushenkov V, Kumar NPBA, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239–1245

Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotech Adv 23:97–114

Edwards CD, Beatty JC, Loiselle JB, Vlassov KA, Lefebvre DD (2013) Aerobic transformation of zinc into metal sulfide by photosynthetic microorganisms. Appl Microb Biotech 97(8):3613–3623

Ernst WHO (1996) Bioavailability of heavy metals and decontamination of soils by plants. Appl Geochem 11(1–2):163–167

Feng RW, Wei CY (2012) Antioxidative mechanisms on selenium accumulation in *Pteris vittata* L, a potential selenium phytoremediation plant. Plant Soil Environ 58(3):105–110

Ferner DJ (2001) Toxicity, heavy metals. eMed J 2(5):1

Ferro AM, Kennedy J, LaRue JC (2013) Phytoremediation of 1, 4-dioxane-containing recovered groundwater. Intl J Phytoremed 15(10):911–923

Förstner U (1995) Non-linear release of metals from aquatic sediments. In: Salomons, W and Stigliani WM, Biogeodynamics of Pollutants in Soils and Sediments Environmental Science, pp 247–307

Foster KJ, Miklavcic SJ (2014) On the competitive uptake and transport of ions through differentiated root tissues. J Theor Biol 340:1–10

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Biores Technol 77(3):229–236

Garbisu C, Alkorta I (2003) Basic concepts on heavy metal soil bioremediation. Eur J Min Proc Environ Protect 3:58–66

Gardner WK, Barber DA, Parbery DG (1983) The acquisition of phosphorus by Lupinus albus L. III. The probabale mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant Soil 70:107–124

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of It's by products. Aust J Energy Environ 6(4):214–231

Ghosh R, Xalxo R, Gope MC, Mishra S, Kumari B, Ghosh M (2011) Estimation of heavy metals in locally available vegetables collected from road side market sites (1-4) of different areas of Ranchi City. PHARMBIT Vol. XXIII & XXIV, No. 1 & 2, Jan–Dec, 2011

Gilbert-Barness E (2010) Teratogenic causes of malformations. Ann Clin Lab Sci 40(2):99–114

Gisbert C, Ros R, de Haro A, Walker DJ, Pilar Bernal M, Serrano R, Avino JN (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303(2):440–445

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Gonzaga MIS, Santos JAG, Ma LQ (2006) Arsenic phytoextraction and hyperaccumulation by fern species. Sci Agric 63:90–101

Gowd SS, Reddy RM, Govil PK (2010) Assessment of heavy metal contamination in soils at Jajmau (Kanpur) and Unnao industrial areas of the Ganga Plain, Uttar Pradesh, India. J Haz Mat 174(1):113–121

Guo J, Xu W, Ma M (2012) The assembly of metals chelation by thiols and vacuolar compartmentalization conferred increased tolerance to and accumulation of Cadmium and Arsenic in transgenic *Arabidopsis thaliana*. J Haz Mat 199:309–313

Guo P, Wang T, Liu Y, Xia Y, Wang G, Shen Z, Chen Y (2014) Phytostabilization potential of evening primrose(*Oenothera glazioviana*) for copper-contaminated sites. Environ Sci Pollut Res 21:631–640

Hammad DM (2011) Cu, Ni and Zn Phytoremediation and translocation by water hyacinth plant at different aquatic environments. Aust J Basic Appl Sci 5(11)

Hani A, Pazira E (2011) Heavy metals assessment and identification of their sources in agricultural soils of Southern Tehran, Iran. Environ Monit Assess 176(1–4):677–691

Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U (2008) Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. Nature 453:391–395

Hegazy AK, Abdel-Ghani NT, El-Chaghaby GA (2011) Phytoremediation of industrial wastewater potentiality by *Typha domingensis*. Intl J Environ Sci Tech 8(3):639–648

Henry JR (2000) In an overview of phytoremediation of Lead and Mercury. NNEMS Report. Washington, DC, pp 3–9

Hsieh JL, Chen CY, Chiu MH, Chein MF, Chang JS, Endo G, Huang CC (2009) Expressing a bacterial mercuric ion binding protein in plant for phytoremediation of heavy metals. J Haz Mat 161(2):920–925

Hu P, Yin YG, Ishikawa S, Suzui N, Kawachi N, Fujimaki S, Igura M, Wu L (2013) Nitrate facilitates cadmium uptake, transport and accumulation in the hyperaccumulator *Sedum plumbizincicola*. Environ Sci Poll Res 20(9):6306–6316

Huang CX, van Steveninck RFM (1989) Effect of moderate salinity on patterns of potassium, sodium and chloride accumulation in cells near the root tip of barley: role of differentiating metaxylem vessels. Physiol Plant 73:525–533

Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134(1):75–84

Jing YX, Yan JL, He HD, Yang DJ, Xiao L, Zhong T, Yuan M, De Cai X, Li SB (2014) Characterization of bacteria in the rhizosphere soils of *Polygonum pubescens* and their potential in promoting growth and Cd, Pb, Zn uptake by *Brassica napus*. Intl J Phytorem 16(4):321–333

Juárez-Santillán LF, Lucho-Constantino CA, Vázquez-Rodríguez GA, Cerón-Ubilla NM, Beltrán-Hernández RI (2010) Manganese accumulation in plants of the mining zone of Hidalgo, Mexico. Bioresource Tech 101(15):5836–5841

Kabata-Pendias A (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton, FL

Kagalkar AN, Jadhav MU, Bapat VA, Govindwar SP (2011) Phytodegradation of the triphenylmethane dye Malachite Green mediated by cell suspension cultures of *Blumea malcolmii* Hook. Bioresource Tech 102(22):10312–10318

Kanu Sheku A, Okonkwo Jonathan O, Dakora Felix D (2013) *Aspalathus linearis* (Rooibos tea) as potential phytoremediation agent: a review on tolerance mechanisms for aluminum uptake. Environ Rev 21(2):85–92

Karenlampi S, Schat H, Vangronsveld J, Verkleij JAC, Lelie D, Mergeay M, Tervahauta AI (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. Environ Pollut 107(2):225–231

Karlson U, Frankenberger WT (1989) Accelerated rates of selenium volatilization from California soils. Soil Sci Soc Am J 53:749–753

Khoudi H, Maatar Y, Brini F, Fourati A, Ammar N, Masmoudi K (2013) Phytoremediation potential of *Arabidopsis thaliana*, expressing ectopically a vacuolar proton pump, for the industrial waste phosphogypsum. Environ Sci Poll Res 20(1):270–280

Kinnersely AM (1993) The role of phytochelates in plant growth and productivity. Plant Growth Regul 12:207–217

Kiyono M, Oka Y, Sone Y, Tanaka M, Nakamura R, Sato MH, Pan-Hou H, Sakabe K, Inoue K (2012) Expression of the bacterial heavy metal transporter MerC fused with a plant SNARE, SYP121, in *Arabidopsis thaliana* increases cadmium accumulation and tolerance. Planta 235:841–850

Knox AS, Gamerdinger AP, Adriano DC, Kolka RK, Kaplan DI (1999) Sources and practices contributing to soil contamination. In: Adriano DC, Bollag JM, Frankenberg WT Jr, Sims RC (eds) Bioremediation of the contaminated soils. Agronomy Series No. 37, ASA, CSSA, SSSA, Madison, Wisconson, USA, pp 53–87

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638

Krämer U, Grime GW, Smith JAC, Hawes CR, Baker AJM (1997) Micro-PIXE as a technique for studying nickel localization in leaves of the hyperaccumulator plant *Alyssum lesbiacum*. Nucl Instr Meth Phy Res B 130(1):346–350

Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator Thlaspispecies. Plant Physiol 122(4): 1343–1354

Krishna MP, Varghese R, Mohamed AA (2012) Depth wise variation of microbial load in the soils of midland region of Kerala: a function of important soil physicochemical characteristics and nutrients. Ind J Edu Inf Manage 1(3):126–129

Kubachka KM, Meija J, LeDuc DL, Terry N, Caruso JA (2007) Selenium volatiles as proxy to the metabolic pathways of selenium in genetically modified *Brassica juncea*. Environ Sci Technol 41(6):1863–1869

Kudo K, Kudo H, Yaeko KF, Kawai S (2013) The release of copper-induced phytosiderophores in barley plants is decreased by cadmium stress. Botany 91(8):568–572

Kukreja S, Goutam U (2013) Phytoremediation: a new hope for the environment. Front Recent Develop Plant Sci 1:149–171

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Tech 29:1232–1238

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212(1):75–84

Langella F, Grawunder A, Stark R, Weist A, Merten D, Haferburg G, Büchel G, Kothe E (2014) Microbially assisted phytoremediation approaches for two multi-element contaminated sites. Environ Sci Poll Res 21:6845–6858

LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP, AbdelSamie M, Terry N (2004) Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation. Plant physiol 135(1):377–383

Lee M, Yang M (2010) Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. *vulgaris*) to remediate uranium contaminated groundwater. J Haz Mat 173(1):589–596

Lee SB (2013) Toxin binding receptors and the mode of action of *Bacillus thuringiensis* subsp. *israelensis* Cry Toxins Ph. D. Thesis, University of California, Riverside

Lenntech (2004) Water treatment and air purification. Lenntech, Rotterdamseweg, The Netherlands

Levine F, Muenke FV (1991) Association with high prenatal lead exposure: similarities to animal models to lead teratogenicity. Pediatrics 87:390–392

Lindblom SD, Fakra SC, Landon J, Schulz P, Tracy B, Pilon-Smits EA (2014) Inoculation of selenium hyperaccumulator *Stanleya pinnata* and related non-accumulator *Stanleya elata* with hyperaccumulator rhizosphere fungi – investigation of effects on Se accumulation and speciation. Physiol Plant 150(1):107–118

Lindqvist O (1991) Mercury in the Swedish environment. Water Air Soil Bull 55(1):23–32

Liu J, Shang W, Zhang X, Zhu Y, Yu K (2014) Mn accumulation and tolerance in Celosia argentea Linn.: a new Mn-hyperaccumulating plant species. J Haz Mat 267:136–141

Liu XM, Wu QT, Banks MK (2005) Effect of simultaneous establishment of *Sedum alfridii* and *Zea mays* on heavy metal accumulation in plants. Int J Phytoremediation 7(1):43–53

Llugany M, Miralles R, Corrales I, Barceló J, Poschenrieder C (2012) *Cynara cardunculus* a potentially useful plant for remediation of soils polluted with cadmium or arsenic. J Geochem Expl 123:122–127

Lorenz A, Rylott EL, Strand SE, Bruce NC (2013) Towards engineering degradation of the explosive pollutant hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine in the rhizosphere. FEMS Microbiol Lett 340(1):49–54

Lu LL, Tian SK, Yang XE, Li TQ, He ZL (2009) Cadmium uptake and xylem loading are active processes in the hyperaccumulator *Sedum alfredii*. J Plant Physiol 166(6):579–587

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579

Ma Y, Rajkumar M, Luo Y, Freitas H (2013) Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. Chemosphere 93(7):1386–1392

Mahdieh M, Yazdani M, Mahdieh S (2013) The high potential of *Pelargonium roseum* plant for phytoremediation of heavy metals. Environ Monit Assess 185:7877–7881

Mahmood T (2010) Phytoextraction of heavy metals – the process and scope for remediation of contaminated soils. Soil Environ 29(2):91–109

Marchiol L, Assolari S, Sacco P, Zerbi G (2004) Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ Pollut 132:21–27

Marques AP, Moreira H, Franco AR, Rangel AO, Castro PM (2013) Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – effects on phytoremediation strategies. Chemosphere 92(1):74–83

Meda AR, Scheuermann EB, Prechsl UE, Erenoglu B, Schaaf G, Hayen H, Weber G, von Wirén N (2007) Iron acquisition by phytosiderophores contributes to cadmium tolerance. Plant Physiol 143(4):1761–1773

Mendoza-Cozatl D, Jobe T, Hauser F, Schroeder J (2011) Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. Curr Opin Plant Biol 14:554–562

Mociardini P, Podini D, Marmiroli N (1998) Exotic gene expression in transgenic plants as a tool for monitoring environmental pollution. Chemosphere 37:2761–2772

Morikawa H, Erkin OC (2003) Basic processes in phytoremediation and some applications to air pollution control. Chemosphere 52:1553–1558

Mueller B, Rock S, Gowswami D, Ensley D (1999) Phytoremediation Decision Tree. – Prepared by – Interstate Technology and Regulatory Cooperation Work Group, pp 1–36

Muthunarayanan V, Santhiya M, Swabna V, Geetha A (2011) Phytodegradation of textile dyes by Water Hyacinth (*Eichhornia Crassipes*) from aqueous dye solutions. Intl J Environ Sci 1(7):1702–1717

Negri MC, Hinchman RR (1996) Plants that remove contaminants from the environment. Lab Med 27(1):36–40

Newman LA, Strand SE, Choe N, Duffy J, Ekuan G, Ruszaj M, Shurtleff BB, Wilmoth J, Heilman P, Gordon MP (1997) Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ Sci Technol 31:1062–1067

Nolan K (2003) Copper toxicity syndrome. J Orthomol Psych 12(4):270–282

Ó Lochlainn S, Bowen HC, Fray RG, Hammond JP, King GJ, White PJ, Graham NS, Broadley MR (2011) Tandem quadruplication of HMA4 in the zinc (Zn) and cadmium (Cd) hyperaccumulator *Noccaea caerulescens*. PLoS One 6:e17814

Park W, Ahn SJ (2014) How do heavy metal ATPases contribute to hyperaccumulation? J Plant Nutr Soil Sci 177(2):121–127

Peers G, Quesnel S, Price NM (2005) Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. Limnol Ocean 50(4):1149–1158

Pérez-Esteban J, Escolástico C, Ruiz-Fernández J, Masaguer A, Moliner A (2013) Bioavailability and extraction of heavy metals from contaminated soil by Atriplex alimus. Environ Exptl Bot 88:53–59

Perronnet K, Schwartz C, Morel JL (2003) Distribution of cadmium and zinc in the hyperaccumulator *Thlaspi caerulescens* grown on multicontaminated soil. Plant Soil 249:19–25

Pivetz BE (2001) Ground water issue: phytoremediation of contaminated soil and ground water at hazardous waste sites. National Risk Management Research Lab ADA OK

Plessl M, Rigola D, Hassinen VH, Tervahauta A, Kärenlampi S, Schat H, Aarts MG, Ernst D (2010) Comparison of two ecotypes of the metal hyperaccumulator *Thlaspi caerulescens* (J & C PRESL) at the transcriptional level. Protoplasma 239:81–93

Prasad MNV, Freitas HMO (2003) Metal hyperaccumulation in plants biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:285–321

Przemeck E, Haase NU (1991) On the bonding of manganese, copper and cadmium to peptides of the xylem sap of plant roots. Water Air Soil Pollut 57(1):569–577

Puschenreiter M, Wittstock F, Friesl-Hanl W, Wenzel WW (2013) Predictability of the Zn and Cd phytoextraction efficiency of a Salix smithiana clone by DGT and conventional bioavailability assays. Plant Soil 369(1–2):531–541

Qiu Z, Tan H, Zhou S, Cao L (2014) Enhanced phytoremediation of toxic metals by inoculating endophytic *Enterobacter* sp. *CBSB1* expressing bifunctional glutathione synthase. J Haz Mat 267:17–20

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotech 8(2):221–226

Rawat K, Fulekar MH, Pathak B (2012) Rhizofiltration: a green technology for remediation of heavy metals. Intl J Inno Biosci 2(4):193–199

Robinson NJ, Tommey AM, Kuske C, Jackson PJ (1993) Plant metallothioneins. Biochem 295:1–10

Römheld V (1991) The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil 130(1–2):127–134

Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic Arabidopsis thaliana plants expressing a modified bacterial *merA* gene. Proc Natl Acad Sci U S A 93:3182–3187

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995a) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biol Technol 13(5):468–474

Salt DE, Prince RC, Pickering IJ, Raskin I (1995b) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109:1427–1433

San Miguel A, Ravanel P, Raveton M (2013) A comparative study on the uptake and translocation of organochlorines by *Phragmites australis*. J Haz Mat 244:60–69

Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–323

Seaward MRD, Richardson DHS (1990) Atmospheric sources of metal pollution and effects on vegetation. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Florida, pp 75–92

Sebastian A, Prasad MNV (2014) Photosynthesis mediated decrease in cadmium translocation protect shoot growth of *Oryza sativa* seedlings up on ammonium phosphate–sulfur fertilization. Environ Sci Poll Res 21(2):986–997

Senden MHMN, Van der Meer AJGM, Verburg TG, Wolterbeek HT (1995) Citric acid in tomato plant roots and its effect on cadmium uptake and distribution. Plant Soil 171(2):333–339

Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042

Shim D, Kim S, Choi YI, Song WY, Park J, Youk ES, Jeong SC, Martinoia E, Noh EW, Lee Y (2013) Transgenic poplar trees expressing yeast cadmium factor 1 exhibit the characteristics necessary for the phytoremediation of mine tailing soil. Chemosphere 90(4):1478–1486

Simmons RW, Chaney RL, Angle JS, Kruatrachue M, Klinphoklap S, Reeves RD, Bellamy P (2014) Towards practical cadmium phytoextraction with *Noccaea caerulescens*. Int J Phytore. doi:10.1080/15226514.2013.876961

Singh NK, Rai UN, Verma DK, Rathore G (2014) Kocuria flava induced growth and chromium accumulation in *Cicer Arietinum* L. Intl J Phytor 16(1):14–28

Soleimani M, Akbar S, Hajabbasi MA (2011) Enhancing phytoremediation efficiency in response to environmental pollution stress. In: Vasanthaiah, HKN and Kambiranda, D Plants and Environment, InTech, Rijeka, Croatia. pp 1–14

Soudek P, Šárka Petrová S, Vaněk T (2012) Phytostabilization or accumulation of heavy metals by using of energy crop *Sorghum* sp. 3rd international conference on biology, environment and chemistry IPCBEE. IACSIT Press, Singapore

Srivastava S (2007) Phytoremediation of heavy metal contaminated soils. J Dept Appl Sci Hum 6:95–97

Sunkar R, Kaplan B, Bouche N, Arazi T, Dolev D, Talke IN, Frans JM, Maathuis FJM, Sanders D, Bouchez D, Fromm H (2000) Expression of a truncated tobacco *NtCBP4* channel in transgenic plants and disruption of the homologous Arabidopsis *CNGC1* gene confer $Pb^{2+}$ tolerance. Plant J 24(4):533–542

Tandy S, Schulin R, Nowack B (2006) The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62(9):1454–1463

Terry N, Banuelos GS (2000) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, FL, p 389

Thayaparan M, Iqbal SS, Chathuranga PKD, Iqbal MCM (2013) Rhizofiltration of Pb by *Azolla pinnata*. Intl J Environ Sci 3(6)

Thomas JC, Davies EC, Malick FK, Endreszl C, Williams CR, Abbas M, Petrella S, Swisher K, Perron M, Edwards R, Ostenkowski P, Urbanczyk N, Wiesend WN, Murray KS (2003) Yeast metallothionein in transgenic tobacco promotes copper uptake from contaminated soils. Biotechnol Prog 19(2):273–280

Trotta A, Falaschi P, Cornara L, Mingati V, Fusconi A, Drava G, Berta G (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. Chemosphere 65:74–81

Tu C, Ma LQ, Bondada B (2002) Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation. J Environ Qual 31:1671–1675

Ukiwe LN, Egereonu UU, Njoku PC, Nwoko CI (2013) Combined chemical and water hyacinth (*Eichhornia crassipes*) treatment of PAHs contaminated soil. Intl J Sci Eng Res 4:1–12

van de Mortel JE, Villanueva LA, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, van Themaat EVL, Koornneef M, Aarts MG (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator Thlaspi caerulescens. Plant Physiol 142(3):1127–1147

Vandenhove H, Van Hees M, Van Winkel S (2001) Feasibility of phytoextraction to clean up low-level uranium-contaminated soil. Int J Phytoremediation 3:301–320

Venkatesan S, Kirithika M, Rajapriya R, Ganesan R, Muthuchelian K (2011) Improvement of economic Phytoremediation with heavy metal tolerant Rhizosphere Bacteria. Intl J Environ Sci 1(7):1864–1873

Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opinion Plant Biol 12:364–372

Veselý T, Tlustoš P, Száková J (2011) The use of water lettuce (*Pistia stratiotes* L.) for rhizofiltration of a highly polluted solution by cadmium and lead. Intl J Phytorem 13(9):859–872

Veselý T, Trakal L, Neuberg M, Száková J, Drábek O, Tejnecký V, Balíková M, Tlustoš P (2012) Removal of Al, Fe and Mn by Pistia stratiotes L. and its stress response. Cent Euro J Biol 7(6):1037–1045

Vishnoi SR, Srivastava PN (2007) Phytoremediation-green for environmental clean. In: Proceedings of Taal2007: the 12th World lake conference 1016:1021

Vivas A, Vörös I, Biro B, Campos E, Barea JM, Azcon R (2003) Symbiotic efficiency of autochthonous arbuscular mycorrhizal fungus (*G. mosseae*) and *Brevibacillus* sp. isolated from cadmium polluted soil under increasing cadmium levels. Environ Pollut 126:179–189

Walsh PR, Duce RA, Finishing JI (1979) Consideration of the enrichment, sources, and flux of arsenic in the troposphere. J Geophys Res 84:1719–1726

Wang F, Zhao L, Shen Y, Meng H, Xiang X, Cheng H, Luo Y (2013) Analysis of heavy metal contents and source tracing in organic fertilizer from livestock manure in North China. Trans Chin Soc Agric Eng 29(19):202–208

Welch RM, Norvell WA, Schaefer SC, Shaff JE, Kochian LV (1993) Induction of iron (III) reduction in pea (*Pisum sativim* L.) roots by Fe and Cu status: does the root-cell plasmalemma Fe(III) – chelate reductase perform a general role in regulating cation uptake? Planta 190:555–561

Wenzel W, Jockwer F (1999) Accumulation of heavy metals in plants grown on mineralised soils of the Austrian Alps. Environ Pollut 104:145–155

Weyens N, Gielen M, Beckers B, Boulet J, Lelie D, Taghavi S, Carleer R, Vangronsveld J (2014) Bacteria associated with yellow lupine grown on a metal-contaminated soil: in vitro screening and in vivo evaluation for their potential to enhance Cd phytoextraction. Plant Biol (Stuttg) 16(5):988–996

Wiseman CL, Zereini F, Püttmann W (2014) Metal translocation patterns in Solanum melongena grown in close proximity to traffic. Environ Sci Poll Res 21(2):1572–1581

Xue L, Liu J, Shi S, Wei Y, Chang E, Gao M, Chen L, Jiang Z (2014) Uptake of heavy metals by native herbaceous plants in an antimony mine (Hunan, China). Clean Soil Air Water 42(1):81–87

Yadav BK, Siebel MA, van Bruggen JJ (2011) Rhizofiltration of a heavy metal (lead) containing wastewater using the wetland plant *Carex pendula*. Clean Soil Air Water 39(5):467–474

Young RA (2005) Toxicity profiles: toxicity summary for cadmium, risk assessment information system, RAIS, University of Tennessee

Zaier H, Ghnaya T, Rejeb KB, Lakhdar SR, Jemal F (2010) Effects of EDTA on phytoextraction of heavy metals (Zn, Mn and Pb) from sludge-amended soil with *Brassica napus*. Biores Technol 101(11):3978–3983

Zhai G, Walters KS, Peate DW, Alvarez PJ, Schnoor JL (2014) Transport of gold nanoparticles through plasmodesmata and precipitation of gold ions in woody poplar. Environ Sci Tech Lett 1(2):146–151

Zhang BZ, Wu ZB, Feng ZH (2011) Study on phytodegradation of Bisphenol A by *Hydrilla verticillata & Myriophyllum verticillatum*. J Huaihai Inst Tech 2:025

Zhang Y, Liu J, Zhou Y, Gong T, Wang J, Ge Y (2013) Enhanced phytoremediation of mixed heavy metal (mercury)-organic pollutants (trichloroethylene) with transgenic alfalfa co-expressing glutathione S-transferase and human P450 2E1. J Haz Mat 260:1100–1107

Zhou ML, Tang YX, Wu YM (2013) Plant hairy roots for remediation of aqueous pollutants. Plant Mol Biol Rep 31(1):1–8

# 11 Phytoremediation Using Leguminous Plants: Managing Cadmium Stress with Applications of Arbuscular Mycorrhiza (AM) Fungi

Rubina Perveen, Shahla Faizan, and Abid Ali Ansari

## 11.1 Introduction

Legumes constitute a major portion of human food throughout the world. In addition, they are good source of oils, fibers, timber, and raw materials for many products. Legumes belong to family *Fabaceae*, are well known for their ability to fix atmospheric nitrogen and to enhance soil nitrogen, consequently leading to the increase in crop growth and yield especially both in conventional or derelict soils. It is known that the interaction between rhizobia and legumes provides nutrients to plants, increases soil fertility, facilitates plant growth, and restores derange ecosystem (Abd-Alla 1999). These characteristics together make the legume an extremely interesting and valuable crop.

Divalent cationic heavy metals may be essential for various metabolic activities of plants and microbes including rhizobia at very lower concentrations (Arora et al. 2009; Mandal and Rabindranath 2012), therefore, regarded as essential metals. At higher concentrations they induce toxicity symptoms in plants. Nonessential heavy metals, however, pose greater survival threat, reducing growth and crop productivity besides contamination of food products (Nellessen and Fletcher 1993; Salt et al. 1995; Akinola and Ekiyoyo 2006). Cadmium is a readily available nonessential heavy metal with high mobility and enrichment ratio in plant tissues. Excessive soil Cd concentration causes undeniable damage to rhizobia, legumes, and their symbiosis. Cadmium and other heavy metals affect the survival and ability of rhizobia to form nitrogen-fixing nodules (Arora et al. 2009; Corticerio et al. 2012). Plants often are screened for their ability to resist the Cd-induced toxicity to manage growth and productivity inducing several internal defense mechanisms. Alternatively, plants do employ different level of avoidance mechanisms to retain Cd in soil, root, efflux excess metal, or defoliate Cd-contaminated lower leaves. Cocultivation of legumes is reported to increase Cd contamination in neighboring crops in Cd-polluted areas (Liu et al. 2012, 2013). Besides, the identification of mechanisms that improve rhizobial tolerance to Cd, its persistence in soil, and ability to improve nodulation efficiency with rhizobia in Cd-contaminated soils is an important issue that requires urgent attention for maintaining soil fertility in metal-polluted lands. Symbiotic relationship of AM fungi with roots is important in many facets for plant growth and productivity. AM fungi colonization with the roots sieve nonessential heavy metal uptake or excess of essential heavy metal uptake while increasing plant access to other minerals (e.g., phosphorus) and moisture, therefore, releasing effects of Cd toxicity on *Rhizbium*–legume symbiosis in heavy metal-contaminated sites (Takas 2012).

### 11.1.1 Heavy Metals, Cadmium, and Biological Functions

The term "heavy metal" encompasses metals and metalloids with the density greater than 5 g/ml. The reason for the choosing certain mono/divalent cations for biological function while other expelling as "nonessential" is yet not well understood. However, the role of "essential" or "beneficial" metal ions as cofactors or in signaling or shaping metabolism is obvious. Therefore, relying on these facts metal ions were categorized as essential, nonessential, or beneficial elements. The role of nonessential metal ions especially in concern with heavy metals is more important as these were regarded to accelerate rate of mortality, reduce potential survival, and induce toxicity symptoms. Since these heavy metals are not vital for plant growth, they are considered as nonessential elements (Velaiappan et al. 2002). On the other hand essential mineral elements find their role in several metabolic reactions, as

R. Perveen, M.Phil. Ph.D. (✉) • S. Faizan
Department of Botany, Environmental Physiology Laboratory, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India
e-mail: rubina.perveen@gmail.com

A.A. Ansari, Ph.D.
Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia

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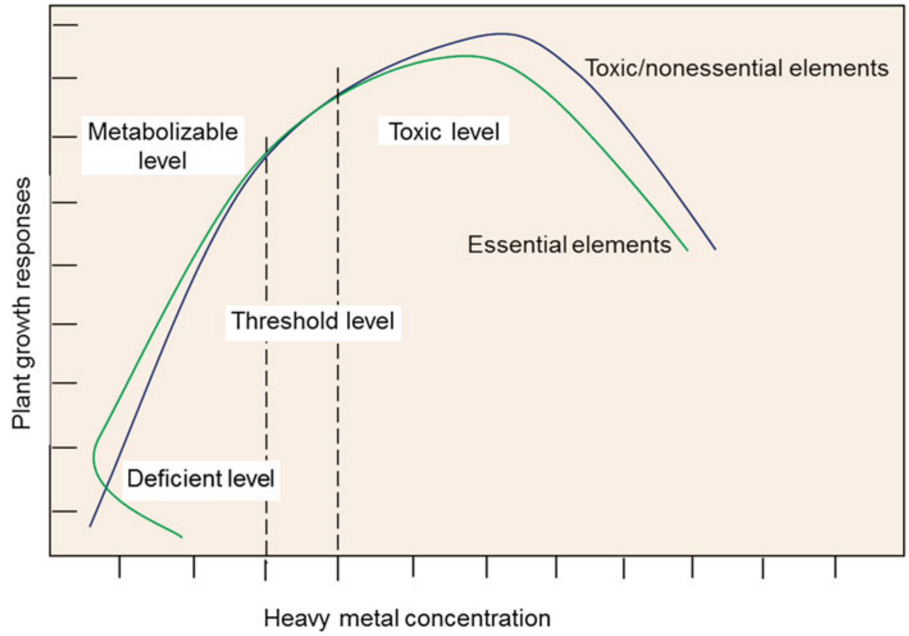


**Fig. 11.1** *Bell-shaped curve* for plant responses to heavy metal uptake

enzyme cofactors of signaling species. Although, they also become toxic beyond a threshold limit (Fig. 11.1).

Recently, another class of heavy metals with their beneficial effect is recognized with no direct role in metabolism, but their presence significantly improves plant health and immunity of plant. These were categorized as beneficial heavy metals. The roles of *Co and †Se are disputed among physiologists to be considered as essential or not. Table 11.1 shows the types of heavy metals/minerals based on the essentiality criteria in plants with their biological roles.

Cadmium is toxic to both plants and human beings (Shukla et al. 2007). Currently, Cd contamination has posed a serious threat to safe food production. It mainly gets accumulated in human kidney and poses a risk of pulmonary emphysema and renal tubular damage (Ryan et al. 1982; Friberg et al. 1986). Extreme cases of chronic Cd toxicity can result in osteomalacia and bone fractures as characterized by disease Itai-Itai, anemia, mainly in women over 40 and induce hormosis (low-dose stimulation and high-dose inhibition) in plant (Calabrese and Baldwin 2004). Toxicity of Cd is a major growth-limiting factor for plants (Mohan and Hosetti 2006). Leaf margins appear with red–brown coloration on the veins and leaves, chlorosis is one of the most important symptoms of Cd toxicity which is due to the consumption with several micronutrients namely, Fe, Ni, Cu (Baccaouch et al. 1998; Siedlecka and Krupa 1999) uptake through the plasma membrane, leading to a deficiency in these essential metal ions in growth metabolism. Cadmium has numerous negative effects on plants cells such as membrane distortion, activity inhibition of several group of enzymes such as those of photosynthetic Calvin cycle (Sandalio et al. 2001), nitrogen metabolism (Boussama et al. 1999), sugar metabolism (Verma and Dubey 2001), and sulfate assimilation (Lee and Leustek 1999), where leaf senescence is accelerated (Siedlecka and Krupa 1999). Cadmium toxicity is also associated with the disturbance in the uptake and distribution of macro- and micronutrients in plants (Sandalio et al. 2001). Cadmium can affect hormonal imbalance and water movement by reduction in the size and number of xylem vessels (Poschenrieder and Barcelo 1999). It antagonizes Zn (Lachman et al. 2004; Kumari et al. 2011) and blocks the Ca-channels (Swandulla and Armstrong 1989) that share the chemical and electronic properties with Cd. In leafy and fruity vegetables Cd is reported at level of 0.6 μg/g tissue fresh mass (Sharma et al. 2009).

Cadmium is a soft, hazardous, heavy metal, and it occurs naturally in the earth's crust. It is placed in II-B group and period fifth of periodic table. It is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g/cm$^3$, melting point 320.9 °C, and boiling point 765 °C. Together with Hg and Pb, Cd is one of the big three heavy metal poisons and is not known for any essential biological function. Cadmium falls under the category of nonessential divalent cations, most abundant and readily available to plant body eliciting toxic responses in aerial parts (Kabata-Pendias 2011).

There are several sources of Cd in soil. Cd reaches into the soil either through anthropogenic processes or via natural (lethogenic and pedogenic factors) ways (Table 11.2). Like other heavy metal ions it could be tolerated at low concentration levels, depending upon species and variety, but at higher concentrations it is toxic, therefore follows bell-shaped relationship of toxicity (Marshner 2012). Soil contaminated with the Cd above the permissible limit leads to a decline in agricultural yield.

**Table 11.1** Classification of metal(oid)s and their role in plant system

| S. No. | Nonessential | Biological significance |
|---|---|---|
| 1. | Pb, Cd, Hg, Al, Cr, As, U, Ti | No biological role. Toxic after a threshold tolerance level |
| | Essential | Biological significance |
| 1. | Fe (iron)<br>$Fe^{2+}$ or $Fe^{3+}$ | In chlorophyll synthesis, component of cytochromes, ferridoxin catalase, peroxidase, nitrogenase, photosynthetic and respiratory chain, leg-hemoglobin |
| 2. | Zn (zinc)<br>$Zn^{2+}$ | In auxin biosynthesis, component of several enzymes or activator (e.g., carbonic anhydrase), maintains ribosomal structure |
| 3. | Mo (molybdenum)<br>$MoO^{4+}$ | In nitrogen fixation and nitrate reductase reduction (nitrate reductase) |
| 4. | B (boron)<br>$BO_3^{3-}$ or $B_4O_7^{2-}$ | Absorption of calcium, role in root nodulation, formation of cell wall |
| 5. | Cu (copper)<br>$Cu^{2+}$ or $Cu^{2}$ | Part of enzymes (e.g., Superoxide dismutase) participates in several oxidation-reduction reactions of electron transport |
| 6. | Mn (manganese)<br>$Mn^{2+}$ | Part of enzymes (e.g., oxygen evolution in photosynthesis, chloroplast integrity) |
| 7. | Mo (molybdenum)<br>$MoO_2^{2+}$ | Part of nitrogenase; nitrogen fixation, in conversion of inorganic phosphate to organic form |
| 8. | Ni (nickel)<br>$Ni^{2+}$ | Nitrogen (urieds) metabolism; its role is disputed among physiologists |
| | Beneficial | Biological significance |
| 9. | *Co (cobalt)<br>$Co^{2+}$ | Part of nitrogenase; nitrogen fixation |
| 10. | †Se (selenium)<br>$Se^{6+}$, $Se^{4+}$ | Improves antioxidant activity |
| 11. | Ca (calcium)<br>$Ca^{2+}$ | In secondary signaling, cell-wall formation, photosynthates allocation, microbial activity stimulation, also work as enzyme activator |
| 12. | Na (sodium)<br>$Na^{2+}$ | Regeneration of phospho-enol pyruvate in CAM and $C_4$ plants. It could substitute K in certain cases |
| 13. | Va (vanadium) | Essential for green algae |
| 14. | Si (silicon) | Cell wall formation, prevents cuticular water loss, improves plant structural defense |

**Table 11.2** Sources of heavy metal pollution

| Types | Sources | References |
|---|---|---|
| Natural sources | Emissions | EMEP/EEA (2009) and EEA (2010) |
| | Transport of continental dust | Crusius et al. (2011) |
| | Withering of metal-enriched rocks | Kimball et al. (2010) |
| Anthropogenic sources | Agrochemicals | Dragovic et al. (2008) |
| | Waste disposal | Fergusson and Kim (1991) |
| | Industries | Adelekan and Abegunde (2011) |
| | Atmospheric deposition | Batisani and Yamal (2010) |
| | Smelting and mining | Cortez et al. (2010) |

Cadmium a nonessential element for a crop is one of the most hazardous heavy metals that exist in the polluted field. The pollution of environment with toxic heavy metals including Cd is spreading fast throughout the world along with industrial revolution (FWPCA 1968). Cadmium ions are taken up readily by plant roots and translocated to the above-ground vegetative parts (Shamsi et al. 2008). The nontoxic level of Cd on soil ranges from 0.04 mM to 0.32 mM. Beyond a certain level of $Cd^{2+}$ in soil, the yield of crops decrease and the quality of field products gets degraded (Hassan et al. 2005). The uptake pattern also depends upon the Cd salt and plant species raised in such soil. For instance; in Chinese cabbage the order of Cd uptake for different Cd compounds is $CdSO_4 > CdCl_2 > CdO > CdCO_3$, whereas in rice it is $CdCl_2 > CdSO_4 > CdO > CdCO_3$ in loamy-sand drab soil with pH 8.2 (Jikai et al. 1982). Cereals and vegetables are most susceptible to increased contamination through raised levels of Cd in the soil, cereal grains ranged from 0.013 to 0.22 mg/kg, grasses ranged from 0.07 to 0.27 mg/kg and protein rich legumes crops varies from 0.08 to 0.28 mg/kg soil (Kabata-Pendias and Pendias 2001). Besides being very toxic to plant metabolism and growth, the enrichment ratio of Cd is more comparing to the other toxic heavy metals.

## 11.2 Effects of Cadmium Toxicity

### 11.2.1 Cadmium Toxicity on Soil Rhizobial Population and Legumes Growth

Higher concentrations of heavy metals severely damages metabolic activities of plants including legumes, e.g., soybean, pea, *Medicago sativa*, and chick pea (Dewdy and Ham 1997; Sandalio et al. 2001; Drazic et al. 2006; Hasan et al. 2008). Nonessential heavy metals are even more toxic to plants at lower concentrations, for instance, Cd (Bahmani et al. 2012). For instance, cadmium competes with mineral uptake causing deficiency of mineral elements (Gadd 2007, 2010), induces oxidative stress, inhibits enzyme activities (Stobart et al. 1985), alters membrane functions (Sandmann and Bflger 1980) and net photosynthesis (Clijsters and Van Assche 1985; Pandey and Tripathi 2011; Chen et al. 2011; Irfan et al. 2013). Soil heavy metal contamination causes significant alteration in the population and activity of soil microbes (Chaudri et al. 1993; Paudyal et al. 2007; Wani et al. 2008a, b; Khan et al. 2009b; Krujatz et al. 2011) to reduce the soil fertility. This indirectly depletes the soil nutrient uptake and plant health (Terry 1981; Karpiscak et al. 2001). Adverse effects of metal-enriched soil have been observed in several legumes also (Wani et al. 2007a, b, 2008a; Wani and Khan 2010). The direct effect of heavy metals has been reported to limit the growth of *Rhizobium* and host legumes (Heckman et al. 1987; Broos et al. 2005). In some legume crops excess heavy metals delay the nodulation processes (Reichman 2007). The decreasing effect of Cd on plant roots inoculated with sensitive and resistant strain reflected the difference of nearly 27 % on nodulation and N level of *S. meliloti* (Sepehri et al. 2006). Contamination with heavy metals had shown inhibition of nitrogen fixation of by the strain of *Rhizobium leguminosarum* in *Trifolium repens* (Hirsch et al. 1993) and in faba bean (Chaudri et al. 2000). The reduction in the activity of nitrogenase in field and pot trials with decreased nodulation, nitrogen metabolism, and plant growth was also reported (Ahmad et al. 2012). Besides, heavy metals at toxic level interfere the induction of root hairs, mineral absorption, normal metabolism, and growth morphology. The interaction of *Rhizobium* in the nodules of chickpea was found to be very sensitive to heavy metals resulting in a decrease in dry mass of chickpea and green gram (Rana and Ahmad 2002). Faizan et al. (2011) reported that the application of Cd enhanced the seedling mortality of six cultivars of chickpea at higher inoculum level. The damaging impact of excessive uptake of Cd on plant growth was marked in various plant species viz. *Glycine max* (Dewdy and Ham 1997), *Pisum sativum* (Sandalio et al. 2001), *Medicago sativa* (Drazic et al. 2006), *Vigna radiata* (Wahid et al. 2007) and *Cicer arietinum* (Hasan et al. 2008). Higher concentrations of Cd decreased the growth of the whole plant (Prasad 1995). Besides morphological challenges, Cd reduces the net photosynthesis, chlorophyll content, activities of carbonic anhydrase and nitrate reductase (Tantrey and Agnihotri 2010), at the cost of stress biochemistry, i.e., lipid peroxidation, production of ROS, membrane disfunctioning, and rise in proline level (Perveen et al. 2011; Vijayaragavan et al. 2011).

### 11.2.2 Cadmium-Induced Cytotoxic Changes

Heavy metals have also been recognized as potent mutagens as they possess genotoxic potential (Winder 1989). Among heavy metals Pb, Hg, and Cd are of special concern as they are found to be genotoxic (Matsumoto and Marin-Morales 2004). Cadmium and Pb are found normally in soil in at least trace quantities. However, due to anthropogenic activities their concentration has been increased in the soil. The most pronounced effect of heavy metals on plant development is growth inhibition, which is inseparably connected with cell division. Cadmium along with other heavy metals induced various degrees of mutations in *Pisum sativum* with strong genotoxic effects Von Rosen (1954). Heavy metal exposure shows various anomalies in plants such as chromosome lesion or division anomalies (Ramel 1973) and poor stem and root elongation (Fargasova 1994). Dimitrova (1993) reported that high concentration of Pb, Zn, Cd, and Cu suppressed the growth of vegetative organs. Mutagenic potential of heavy metals (Pb, Cd, and Hg) in causing changes in the banding patterns of $M_2$ seed storage protein in *Vicia faba* plants was reported by George (2000). Cadmium toxicity induces abnormalities such as stickiness, precocious movements, secondary associations, and laggards in addition to other abnormalities such as unequal separation, scattering, nonsynchronous division, and micronuclei and binucleate cells. in soybean (Kumar and Rai 2007). Formation of micronuclei was the most prominent type of aberration in *Vicia faba* as a result of Cd toxicity (Baeshin and Qari 2003).

### 11.2.3 Generation of Reactive Radicals and Oxidative Stress

Cadmium induces production of toxic metabolites and reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($^{\cdot}OH$), singlet oxygen ($O_2^1$), and hydroperoxyl radical ($H_2O^{\cdot}$). This results in growth inhibition and photosynthesis (Shamsi et al. 2008). To survive against Cd stress, the plants evolved defense mechanisms of mitigating these radicals and repairing the ROS-induced damages (Overmyer et al. 2003). These are specific but complex mechanisms involving morphological,

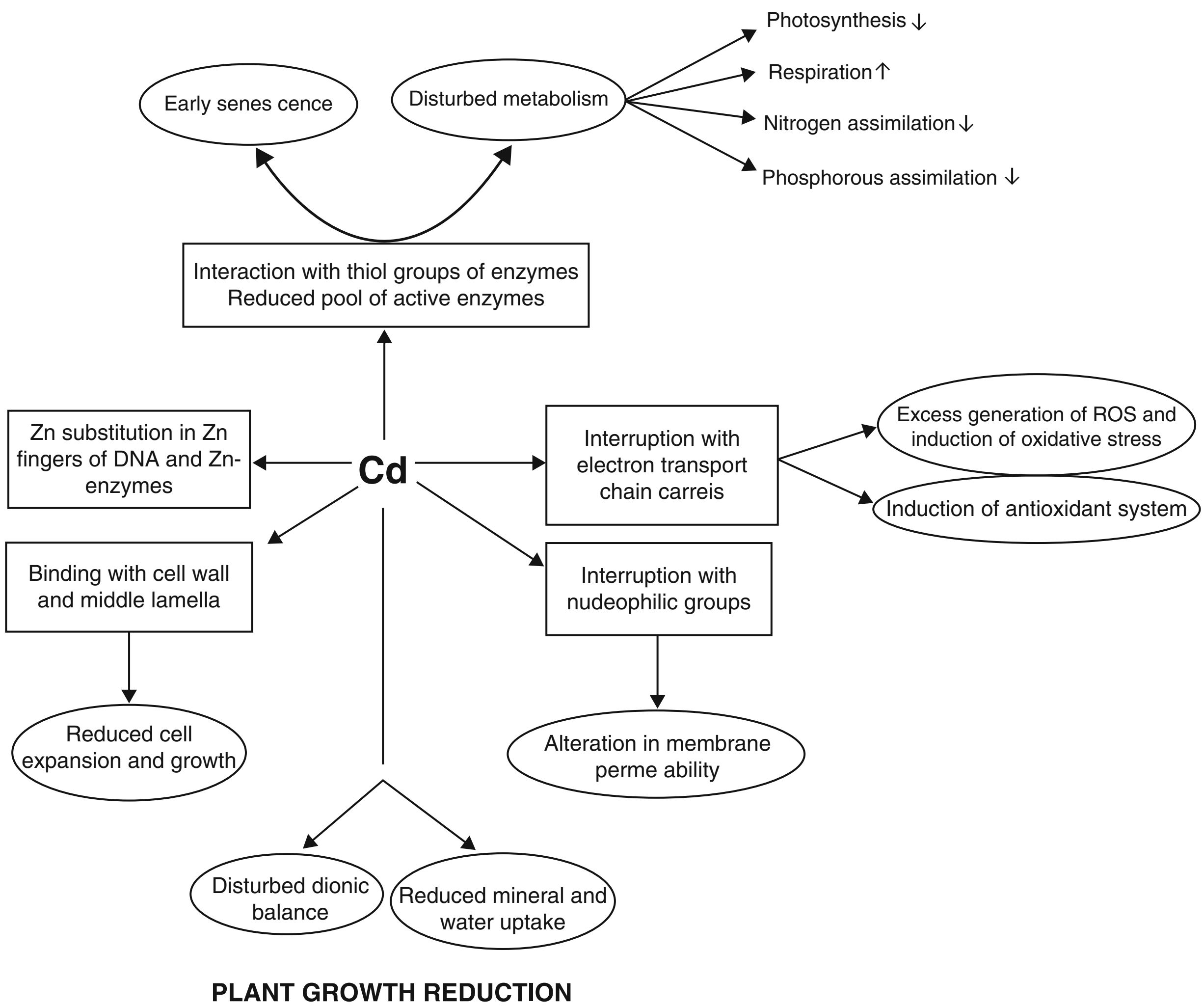


**Fig. 11.2** Cadmium-induced toxicity processes at cellular level

physiological, and biochemical adaptation. In particular, the ROS-combating antioxidant system consists of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), and nonenzymatic system consists of glutathione (GSH) and ascorbic acid (Gill and Tuteja 2010). Plants' resistance to environmental stresses is dependent on the nature and the amount of antioxidants. Reactive oxygen radicals at supraoptimal level starts stress signaling at the cost of growth signaling claiming induction of defense mechanisms. Declined growth, chlorophyll content, photosynthesis, and stomatal conductance ($g_s$), along with enhanced malondialdehyde (MDA) content, SOD, and POD activities, were found in soybean plants exposed to Cd stress (Shamsi et al. 2008). In addition Cd caused significant inhibition in the activity of POD, CAT in assistance with nitrate reductase (NR) and nitrite reductase (NiR) activities in *Arachis hypogea* seedlings are under Cd stress compromising the plant growth (Damodharam et al. 2009) (Fig. 11.2).

### 11.2.4 Morpho-anatomical Changes Under Heavy Metal Stress

Several workers have observed the changes in the morphological and molecular level of many crops by addition of Cd and other heavy metals. Ahmad et al. (2005) observed the effect of different concentrations of Cd and Pb on morphological and anatomical features of *Trigonella foenum graecum* at pre-flowering, flowering, and postflowering stages. Size of stomata and stomatal pore and the density of stomata on both epidermises were significantly reduced under metal stress at all the developmental stages. Trichome length on both the epidermises increased while their density decreased under metal stress. Under Cd stress, proportion of pith and vasculature decreased but cortex increased in the stem. While on the other hand, lead stress, proportion of pith, and vasculature increased but cortex decreased in the stem. In the root, proportion of vasculature and pith increased and cortex decreased in response to both Cd and Pb stresses. Dimensions of vessel element and xylem fiber in the wood of stem and root decreased under the Cd and Pb stresses. Plants grown under Cd and Pb stresses reflected more pronounced decrease in the density of stem and root vasculature with the age advancement (Ahmad et al. 2005)

## 11.3 Strategies to Recover Heavy Metal Toxicity

A distinct group of plants have been shown to tolerate high metal concentrations. These hyper-accumulating plants are capable of sequestering metals in their tissues at remarkable high concentrations that would be toxic to most organisms, being important for phytoremediation of polluted soils. The majority of research carried out so far has focused on physiological mechanism of metal uptake, transport, and sequestrations, but little is known about the genetic basis of hyper-accumulation. There are known cases of major genetic polymorphism in which some members of species are capable of hyper-accumulation and others are not. This is in contrast to the related phenomenon of metal tolerance. Plant species those possess any metal tolerance are polymorphic, and evolving tolerance only in local populations on metalliferous soils (Pollard et al. 2002).

### 11.3.1 Plant Strategies to Augment Heavy Metal Stress

Alteration in root morphology, direction of growth, and activation of pumps to efflux metal ions are the important strategies to avoid metal uptake. Secondarily, plant growth promoting microorganisms (PGPMs) potentially could sieve at the interface of root and soil further diluting the metal uptake through root system. Arbuscular mycorrhizial fungi, phosphate solubilizing bacteria (PSB), root noodling Rhizobia, etc., are good examples of rhizo-filteration of toxic level of heavy metals (Ganesan 2012). Plants growing in a community could dilute the toxicity effect of these metals to escape the survival threat of several sensitive species (Rather 2013). Pulses, on the other hand have been found to redirect the Cd accumulation in the neighboring crops (Liu et al. 2012, 2013). Inoculation of AM fungi with Rhizobial strain further detoxifies the metal in soil and discourages its uptake. However, it is not possible for a plant growing in heavy metal-polluted area to completely avoid the toxic level of essential or nonessential heavy metal. Therefore, plants growing in such area are naturally selected inducing defense mechanisms to counter their toxicity. The activation of defense is a costly trade-off and channelizes the growth metabolisms, hence, plants often compromises growth, get threatened or eliminated from the community (Rather 2013). In agriculture programs often crops have been screened against locally prevailing different stress factors. Alternatively, plants species rich in sulfur compounds or other chelating agents could reach to such a limit that they could hyper-accumulate these heavy metals (Na and Salt 2011). These crops potentially could be used or optimized in phytoremediation programs for heavy metal detoxification of polluted sites and phyto-mining, which is considered as a safe and natural alternative of other strategies of metal decontamination of such sites. Often agricultural crops are discouraged for such programs. Although some degree of hyper-accumulation occurs in all members of the species that can hyper-accumulate, their evidence of quantitative genetic variation in the ability to hyper-accumulate, both between and within population (Pollard et al. 2002). The genetic basis of Cd tolerance and hyper-accumulation investigated in *Arabidopsis halleri* has demonstrated that Cd tolerance may be governed by more than one major gene (Bert et al. 2003). The mechanism of Cd tolerance and hyper-accumulation in *Thalaspi caerulescens* hairy roots has been shown to be due to the ability of this plant species to withstand the effects of plasma membrane depolarization (Boominathan and Doran 2003).

### 11.3.2 Role of AM Fungi in Prevention of Cadmium Toxicity in Legumes

The importance of legumes has been attributed in determination of N economy of various ecosystems (Makoi et al. 2009) including arable lands by forming root nodules (Ma et al. 2006). Nonessential heavy metals and excess of essential heavy metals have strong negative correlation with plant growth (Vyas and Puranik 1993; Shetty et al. 1994). Cadmium, being nonessential metal inhibits nodule formation and nitrogen fixation in legumes (Hernandez et al. 1995), decreases nutrient uptake (Obata and Umebayashi 1997), photosynthetic activity (Kumar and Kumar 1999), and finally biomass production (Leita et al. 1993). The deleterious effect of heavy metals taken up by soil environment could be discouraged by use of PGPR (Khan et al. 2009a, c; Kumar 2012) or mycorrhizae (Heggo et al. 1990; Saraswat and Rai 2011) called as rhizo-remediation (Kuiper et al. 2004). Soil microbial pool detoxifies heavy metals such as Cd, Hg, and Pb (Aiking et al. 1985). Plant root–mycorrhizal symbiosis is one of the important associations among plant–microbe interaction. It is so important that over 95 % of the plant families are known to have some sort of mycorrhizal association under normal conditions. The use of mycorrhizae could potentially minimize the fertilization and water. It aids nutritional supplementation to the host plant at the cost of carbohydrate. The nutritional availability where on the one hand strengthens plant immunity against abiotic and biotic stress, fungus protects plants from certain root pathogens, metal toxicity, and water-stress. Role of mineral nutrition in minimizing Cd accumulation by plants in agricultural fields is discussed by Sarwar et al. (2010). Among different flowering plants which associate AM fungi with them, legumes are of special mention. Legumes

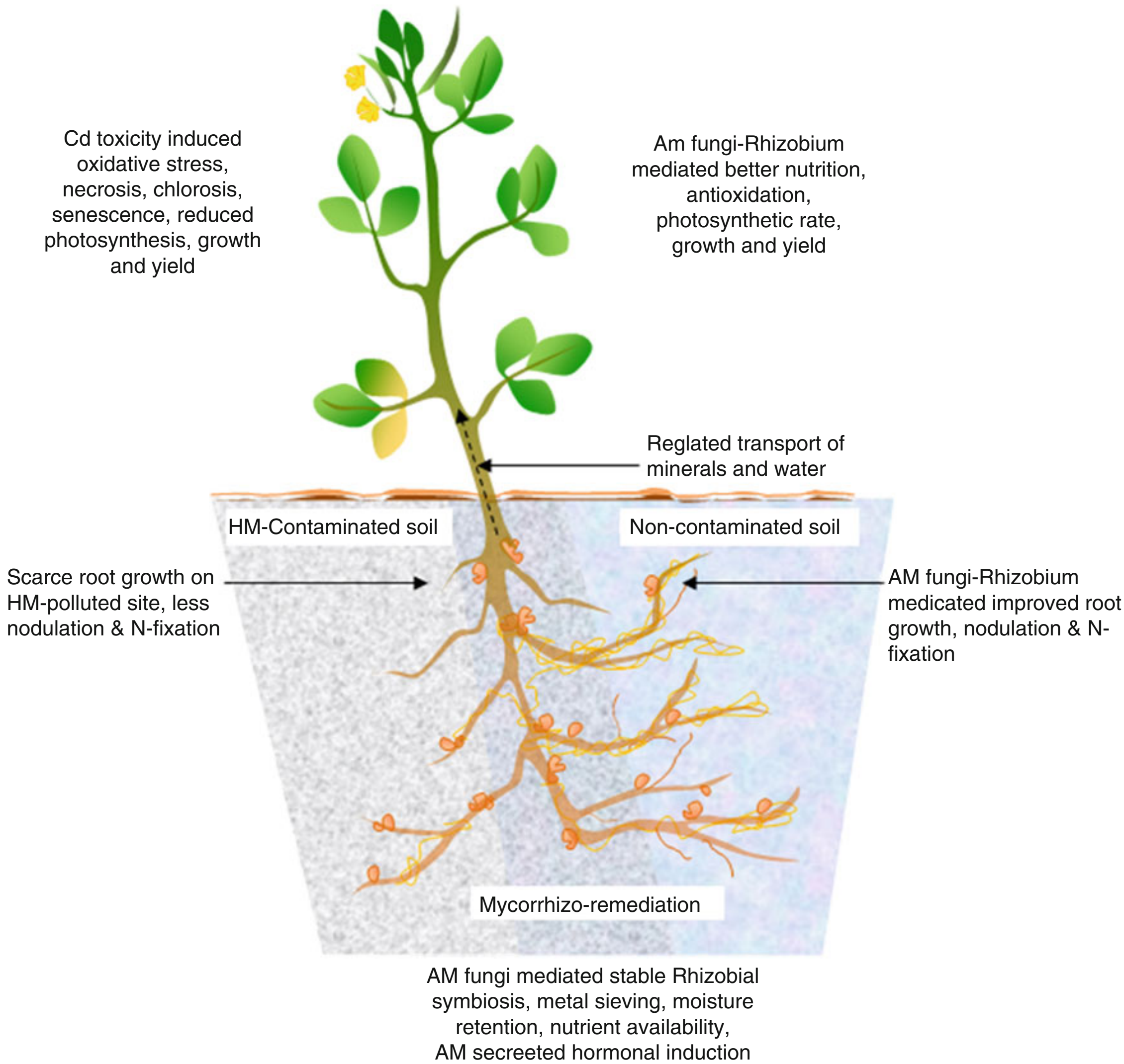


**Fig. 11.3** Role of AM fungi–*Rhizobium*-mediated alleviation of Cd toxicity in legumes

substantially contribute to the soil N pool and productivity in terrestrial ecosystems (Cleveland et al. 1999). It is also observed that AM fungus, when associating themselves in the tripartite relationship with *Rhizobium*-legume roots, strengthens the nodulation frequency and nitrogen fixation efficiency of host plant. Since heavy metals persist in soil for a long time they are very resistant to chemical degradation or physical removal or immobilization (Kroopnick 1994). The site-specific management, excavation, and disposal is cumbersome and uneconomic (Parker 1994; Elliott et al. 1989). Bioremediation in this context is better substitute (Leyval et al. 2002) involves microbes, level type of contamination, and climatic conduciveness (Brar et al. 2006). Researches have shown that AMF inoculation significantly improved the tolerance of legumes to heavy metal toxicity in different growing conditions (Chen et al. 2007). Mycorrhizae and Rhizobia colonization besides protection of plant growth also secretes phytohormones viz. cytokinins and gibberellins, which induce cell division, stem elongation, seed germination, and other functions of host plants. Positive benefits of composite inoculation of AM fungi and *Rhizobium* with legumes grown in metal-contaminated soil are also reviewed by Muleta and Woyessa (2012). The diagrammatic representation of role of legume mycorrhization in preventing *Rhizobium* symbiosis and plant growth under heavy metal stress is shown in Fig. 11.3. Various provisions and mechanisms of tripartite association in legume improvement are tabulated in Table 11.1.

Microbes themselves deploy several strategies to check metal uptake viz. cell wall biosorption, incorporation into enzymes and pigments (Gadd 2009, 2010), removal by efflux pumps and metal binding proteins and peptides (Silver 1996). Low molecular weight organic acids are secreted by plant roots such as oxalate and citrate to mobilize metals in soil. Synergy with AM fungi improves this mobilization to detoxify these meals as fungi are aided with plethora of diverse phytochleatins (PCs), metallothionines (MTs), and organic acids (Joner et al. 2000a, b) and easily change their strains as per requirement as compared to complex eukaryotes. Site-specific optimization of AM fungi-mediated mycorrhiza-remediation is effective tool for restoring the eco-economics of soil and plant (Takas 2012) particularly in legumes (Muleta and Woyessa 2012). However, contradictory reports regarding increase or decrease of metal concentration of mycorrhized plant are available (Gildon and Tinker 1983; Heggo et al. 1990; Tonin et al. 2001; Karimi et al. 2011). Enhanced uptake of heavy metals is the part of phytoremediation and reclamation of contaminated soil, whereas, heavy metal sieving via fungal mat is desired in the crop plants to check metal accumulation (Rivera-Becerril et al. 2002; Jamal et al. 2002; Audet and Charest 2007) in the plant tissue for safe food. Muleta (2010) discussed the dependency of legumes on mycorrhizal associations. This association has shown to supply high P for nodulation and N-fixation as P is known as critical element for nodule formation (Barea and Azcon-Aguilar 1983). The effect of dual inoculation of AMF and bacteria remarkably improve the heavy metal tolerance of plant (Vivas et al. 2003a, b; Muleta 2010). The mechanisms of heavy metal tolerance by mycorrhizal legumes were discussed by Malcova et al. (2003), Cardoso and Kuyper (2006), and Garg and Aggarwal (2011).

### 11.3.3 Benefits of Tripartite Relation for Soil Health

Evidence of existence of symbiosis of plant roots with AM fungus has been found in fossils dating back 460 million years. Mycorrhizal symbiosis predates the evolution of nodulation by approximately 400 million years. The sharing of two symbiotic pathways was shown to be present in cereals and essential for mycorrhizal signaling (Hayat et al. 2012). AM fungus ensures the absorption of N, P, K, and S uptake besides the absorption of mineral elements, e.g., Cu, Fe, Ni, Co, and Zn. The fungal hyphae keeps check on the root uptake of excess level of Zn, Cd, and Mn from soil. This protects the plant roots and enhances the plant's ability to re-vegetate and stabilize the soils of reclaimed mines that may be high in heavy metals. Increased stability of *Rhizobium*–legume symbiosis ensures the fixation of optimum level of nitrogen from air. The glycoprotein glomalin excreted by fungal hyphae act as glue leading soil particles to stick together, or aggregate. Soil aggregates are resistant to breakdown by water and enhance the soil's physical characteristics, such as soil erosion by water and air. The fungal hyphae also physically entangle nonaggregated soil particles together, facilitating other bacterial and fungal compounds to form these particles into aggregates besides enriching rhizosphere with the activity of other beneficial microbes such as PGPRs (Kumar 2012). These aggregates are important for the soil food web (Table 11.3).

**Table 11.3** Mechanism and ways of heavy metals (including Cd) rhizo-remediation and legume growth improvement in tripartite association

| | |
|---|---|
| **A.** | **Rhizobium associated metal rhizo-remediation** |
| 1. | Strengthening plant immunity to combat metal stress providing N-feed |
| 2. | Heavy metal dilution incorporating them in *Rhizobium* metabolism |
| 3. | Bio-sorption of heavy metals in cell wall, other structural components, e.g., pigments, or as enzyme cofactors |
| 4. | Plant growth induction through the secretion of phytohormones |
| **B.** | **Mycorrhiza-mediated metal detoxification rhizo-remediation** |
| 1. | Sieving of heavy meals to reach into roots of legumes |
| 2. | Detoxification of heavy metals through the secretion of organic acids, metallo-thionines, etc. |
| 3. | Nutritional supplementation to strengthen plants' internal resistance viz. phosphates, mineral elements, and soil organic compounds |
| 4. | Enrichment of rhizosphere with PGPRs |
| 5. | Plant growth improvement through secretion of phytohormones |
| 6. | Protection from root disease-causing pathogens |
| 7. | Protection from water stress |
| 8. | Incorporation of heavy metals in fungal metabolism (metal sink) |
| 9. | Protection of *Rhizobium*–legume symbiosis |
| **C.** | **Plant strategies and defense mechanisms to overcome metal stress** |
| 1. | Root growth direction and morphology |
| 2. | Root metal retention and other |
| 3. | Transportation to metabolically inactive parts |
| 4. | Cellular detoxification and sequestration to vacuoles |
| 5. | Secretion of phyto-chelatins (PCs) and metalothionines (MTs) |
| 6. | Antioxidant enzymes (CAT, POX, SOD, GR, etc.) and molecules (glutathione, ascorbate etc.) |
| 7. | Reactive oxygen species quenching metabolites and osmo-protectants (proline, polyamines, betains, and sugar alcohols) |
| 8. | Root excretion of organic acids |
| 9. | Efflux of heavy metals activating metal exporters |

## 11.4 Conclusion

The necessity of increased establishment and capacity of nitrogen fixing nodules in legumes becomes more important in stressed habitats, for instance, heavy metal-contaminated sites. Therefore, the research advancement regarding the isolation, characterization, and selection of metal-resistant rhizobial strains and legume varieties is accelerating rapidly. Besides, research work is also going on to screen and optimize the legume varieties with resistant *Rhizobium* strain to restore the soil fertility in heavy metal stressed habitats particularly emphasizing the protection of nitrogen fixing activity (Coba de la Peña and Pueyo 2012). The role of glutathione in the resistant *Rhizobium* strain was confirmed against Cd stress for effective symbiosis and bioremediation of metals (Sofia et al. 2012; Mandal and Bhattaccharyya 2012). The site-specific optimization of mycorrhization of plants has also emerged as a promising strategy for metal detoxification which might improve the legume production in such disturbed cropping locations (Takas 2012). Molecular studies of signaling in this perspective will further disclose the mechanism of interaction of AM fungi with Rhizobium enable restoration of nitrogen fixation, would enlighten our current understanding.

## References


Abd-Alla MH (1999) Nodulation and nitrogen fixation of Lupinus species with Bradyrhizobium (lupin) strains in iron-deficient soil. Biol Fert Soils 28:407–415

Adelekan BA, Abegunde KD (2011) Heavy metals contamination of soil and ground water at automobile mechanic villages in Ibadan Nigeria. Int J Phys Sci 6:1045–1058

Ahmad E, Zaidi A, Khan MS, Oves M (2012) Heavy metal toxicity to symbiotic nitrogen-fixing microorganism and host legumes. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 29–44. doi:10.1007/978-3-7091-0730-0_9

Ahmad SH, Reshi Z, Ahmad J, Iqbal MZ (2005) Morpho-anatomical responses of *Trigonella foenum graecum* Linn. to induced cadmium and lead stress. J Plant Biol 48:64–84

Aiking HH, Goves H, Riet JV (1985) Detoxification of mercury, cadmium and lead in *Klebsiella aerogenes* NCTC418 growing in continuous culture. Appl Environ Microbiol 50:1262–1267

Akinola MO, Ekiyoyo TA (2006) Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odonla area of Ikorodu, Lagos state. J Environ Biol 27:597–599

Arora NK, Khare E, Singh S, Maheshwari DK (2009) Effect of Al and heavy metals on enzymes of nitrogen metabolism of fast and slow growing rhizobia under explanta conditions. World J Microbiol Biotechnol. doi:10.1007/s11274-009-0237-6

Audet P, Charest C (2007) Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation. Meta-analytical and conceptual perspectives. Environ Polut 147:609–614

Baccaouch S, Chaoui A, Ferjani EE (1998) Nickel induced oxidative damage and antioxidant responses in *Zea mays* shoot. Plant Physiol Biochem 36:689–694

Baeshin NA, Qari SH (2003) Combined genotoxic and cytotoxic effects of cadmium chloride and carbofuran in root meristems of *Vicia faba*. Saudi J Bio Sci 10:107–119

Bahmani R, Bihamta MR, Habibi D, Forozesh P, Ahmadvand S (2012) Effect of cadmium chloride on growth parameters of different bean genotypes (*Phaseolus vulgaris* L.). ARPN J Agric Biol Sci 7:35–40

Barea JM, Azcon-Aguilar C (1983) Mycorrhizas and their significance in nodulating nitrogen-fixing plants. Adv Agron 36:1–54

Batisani N, Yamal B (2010) Rainfall variability and trends in semi-arid Botswana: implications for climate change adaptation policy. Appl Geogr 30:483–489

Bert V, Meerts P, Saumitou-Laprade P, Salis P, Gruber W, Verbruggen N (2003) Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. Plant Soil 249:9–18

Boominathan R, Doran PM (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator. *Thlaspi caerulescens*. Biotechnol Bioeng 83:158–167

Boussama N, Ouariti O, Suzuki A, Ghorbel MH (1999) Cd-stress on nitrogen assimilation. J Plant Physiol 155:310–317

Brar SK, Verma V, Surampalli RY, Misra K, Tayagi RD, Meunier N, Blais JF (2006) Bioremediation of hazardous wastes—a review. Pract Period Hazard Toxic Radioactive Waste Manag 10:59–72

Broos K, Beyens H, Smolders E (2005) Survival of rhizobia in soil is sensitive to elevated zinc in the absence of the host plant. Soil Biol Biochem 37:573–579

Calabrese EJ, Baldwin LA (2004) Chemotherapeutics and hormesis. Crit Rev Toxicol 33:215–304

Cardoso IM, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agric Ecosyst Environ 116:72–84

Chaudri AM, Allain CM, Barbosa-jefferson VL, Nicholson FA, Chambers BJ, McGrath SP (2000) A study of the impacts of Zn and Cu on two rhizobial species in soils of a long term field experiment. Plant Soil 22:167–179

Chaudri AM, McGrath SP, Giller KE, Reitz E, Suerbeck DR (1993) Enumeration of indigenous *Rhizobium leguminosarum biovar. trifolii* in soils previously treated with metal contaminated sewage sludge. Soil Biol Biochem 25:301–309

Chen BD, Thu YG, Duan J, Xiao XY, Smith SE (2007) Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environ Pollut 147:374–380

Chen JW, Zhang Q, Li XS, Cao KF (2011) Steady and dynamic photosynthetic responses of seedlings from contrasting successional groups under low-light growth conditions. Physiol Plant 141:84–95

Cleveland CC, Townsend AR, Schimel DS et al (1999) Global patterns of terrestrial biological nitrogen ($N_2$) fixation in natural ecosystems. Global Biogeochem Cycles 13:623–645

Clijsters H, Van Assche F (1985) Inhibition of photosynthesis by heavy metals. Photosynth Res 7:31–40

Coba de la Peña T, Pueyo JJ (2012) Legumes in the reclamation of marginal soils, from cultivar and inoculant selection to transgenic approaches. Agro Sust Develop 35:65–91

Cortez H, Pingarron J, Munoz JA, Ballester A, Blazquez ML, Gonzalez F, Garcia C, Coto O (2010) Bioremediation of soils contaminated with metalliferous mining wastes. In: Plaza G (ed) Trends bioremediation phytoremediation. Research Signpost, Trivandrum, pp 283–299

Corticerio S, Pereira S, Lima A, Figueira E (2012) The influence of glutathione on the tolerance of *Rhizobium leguminoserum* to cadmium. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer-Verlag, Wien, pp 89–100

Crusius J, Schroth AW, Gasso S, Moy CM, Levy RC, Gatica M (2011) Glacial flour dust storms in the Gulf of Alaska: hydrologic and materiological controls and their importance as a source of bioavailable iron. Geophys Res Lett 38:5

Damodharam T, Dinakar N, Nagajyothi PC, Suresh S, Suresh C (2009) Cadmium induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in *Arachis hypogea* L. J Environ Biol 30:289–294

Dewdy RH, Ham GE (1997) Soybean growth and elemental content as influenced by soil amendments of sewage sludge and heavy metals: seedling studies. Agron J 69:300–303

Dimitrova I (1993) Studies of grass plants under conditions of industrial pollution with heavy metals and sulfur dioxide. Author's summary of Ph.D. Dissertation, "P. Hilendarski." Plovdiv University

Dragovic S, Uscumlic M, Radojevic V, Cicmil M (2008) Water quality for vegetable irrigation from the aspect of safety. Ekoloski Pokret Novog Sada, Novi Sad, special edition, II International ECO-Conference "SAFE FOOD," pp 75–81

Drazic G, Mihailović N, Lojić M (2006) Cadmium accumulation in *Medicago sativa* seedlings treated with salicylic acid. Biol Plant 50:239–244

EEA (2010) European Union emission inventory report 1990-2008 under the UNECE convention on long-range trans-boundary air pollution (LRTAP). Copenhagen, Technical report number 7

Elliott HA, Linn JH, Sheilds GA (1989) Role of Fe in extractive decontamination of Pb polluted soils. Hazards Mater Hazards Waste 6:223–229

EMEP/EEA (2009) The EMEP/EEA air pollutant emission inventory guidebook.http://www.eea.europa.eu/publications/emep-eea-emission-inventory-guidebook-2009. Accessed 31 Oct 2014

Faizan S, Kausar S, Perveen R (2011) Varietal differences for cadmium-induced seedling mortality, foliar toxicity symptoms, plant growth, proline and nitrate reductase activity in chickpea (*Cicer arietinum* L.). Biol Med 3:196–206

Fargasova A (1994) Toxicity determinations of plant growth hormones on aquatic alga *Scenedesmus quadricauda*. Bull Environ Contam Toxicol 52:706–711

Fergusson JE, Kim ND (1991) Trace elements in street and house dusts: sources and speciation. Sci Total Environ 100:125–150

Friberg L, Elinder C-G, Kjellstrom T, Nordberg GF (eds) (1986) Cadmium and health: a toxicological and epidemiological appraisal, vol. 1. CRC Press, Boca Raton, FL

FWPCA (1968) Water quality criteria. Report of the National Technical Advisory Committee to the Secretary of the Interior. Federal Water Pollution Control Administration, Corvallis, OR, pp 32–34

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bio-weathering and bioremediation. Mycol Res 111:3–49

Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiol 156:609–643

Ganesan V (2012) Rhizoremediation: a pragmatic approach for remediation of heavy metal-contaminated soil. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer-Verlag, Wien, pp 147–161

Garg N, Aggarwal N (2011) Effects of interaction between cadmium and lead on growth, nitrogen fixation, phytochelatin, and glutathione production in mycorrhizal *Cajanus cajan* (L.) Millsp. J Plant Growth Regul 30:286–300

George NM (2000) Evaluation on mutagenic effects of the three heavy metals on *Vicia faba* plants. Cytologia 65:75–82

Gildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular arbuscular mycorrhizas. New Phytol 95:247–261

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Hasan SA, Hayat S, Ali B, Ahmad A (2008) 28-homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidant. Environ Poll 151:60–66

Hassan MJ, Shao GS, Zhang GP (2005) Influence of cadmium toxicity on growth and antioxidant enzyme activity in rice cultivars with different grain cadmium accumulation. J Plant Nutr 28:1259–1270

Hayat S, Irfan M, Wani AS, Alyemeni MN, Ahmad A (2012) Salicylic acids: local, systemic or inter-systemic regulators? Plant Signal Behav 7:1–10

Heckman JR, Angle JS, Chaney RL (1987) Residual effects of sewage sludge on soybean II. Accumulation of soil and symbiotically fixed nitrogen. J Environ Qual 16:117–124

Heggo A, Angle JS, Chaney RL (1990) Effects of vesicular arbuscular mycorrhizal fungi on heavy metal uptake by soybean. Soil Biol Biochem 22:865–869

Hernandez LE, Garate A, Carpena-Ruiz R (1995) The effect of cadmium on symbiotic nitrogen fixation of pea (*Pisum sativum*) plant grown in perlite and vermiculite. J Plant Nutr 18:287–303

Hirsch PR, Jones MJ, McGrath SP, Giller KE (1993) Heavy metals from past applications of sewage sludge decrease the genetic diversity of *Rhizobium leguminosarum biovar. trifolii* populations. Soil Biol Biochem 25:1485–1490

Irfan M, Hayat S, Ahmad A, Alyemeni MN (2013) Soil cadmium enrichment: allocation and plant physiological manifestations. Saudi J Biol Sci 20:1–10

Jamal A, Ayub N, Usman Mand Khan AG (2002) Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by bean and lentil. Int J Phytoremed 4:205–221

Jikai R, Qinglang C, Lingzhi C, Rongzhuang H, Yiqun Y, Fanzhi K, Yougui M (1982) The soil contaminated by cadmium and crop. Chinese J Plant Ecol 6:131–141

Joner EJ, Briones R, Leyval C (2000a) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 222:227–234

Joner EJ, Ravnskov S, Jakobsen I (2000b) Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radiolabeled inorganic and organic phosphate. Biotechnol Lett 22:1705–1708

Kabata-Pendias A (2011) Trace elements in soils and plants, 3rd edn. CRC Press LLC, Boca Raton

Kabata-Pendias A, Pendias H (2001) Trace element in soils and plant. CRC Press, London

Karimi A, Habib K, Mohzan S, Mirhassan RS (2011) Arbuscular mycorrhizal fungi and heavy metal contaminated soils. Afr J Microbiol Res 5:1571–1576

Karpiscak MM, Whiteakeriola JF, Foster KE (2001) Nutrient and heavy meal uptake and storage in constructed wetland systems in Arizona. Water Sci Technol 44:455–462

Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M (2009a) Functional diversity among plant growth-promoting rhizobacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, pp 105–132

Khan MS, Zaidi A, Wani PA, Oves M (2009b) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19

Khan S, Farooq R, Shahbaz S, Khan MA, Sadique M (2009c) Health risk assessment of heavy metals for population via consumption of vegetables. World Appl Sci J 6:1602–1606

Kimball BA, Runkel RL, Walton-Day K (2010) An approach to quantify sources, seasonal change, and biogeochemical processes affecting metalloading: facilitating decisions for remediation of mine drainage. Appl Geochem 25:728–740

Kroopnick PM (1994) Vapor abatement cost analysis methodology for calculating life cycle costs for hydrocarbon vapor extracted during soil venting. In: Wise DL, Trantolo DJ (eds) Remediation of hazardous wastes. Marcel Dekker, New York, pp 779–790

Krujatz F, Haarstrick A, Nortemann B, Greis T (2011) Assessing the toxic effects of nickel, cadmium and EDTA on growth of the plant growth-promoting rhizobacterium *Pseudomonas brassicacearum*. Water Air Soil Pollut. doi:10.1007/s11270-011-09440

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant microbe interaction. Mol Plant Microbe Interact 17:6–15

Kumar A (2012) Role of plant growth promoting rhizobacteria in the management of cadmium contaminated soil. In: Zaidi A, Wani PA,

Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 163–198
Kumar CL, Kumar SS (1999) Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. Plant Physiol Biochem 37:297–303
Kumar G, Rai P (2007) Genotoxic potential of mercury and cadmium in soybean. Turk J Biol 31:13–15
Kumari M, Sinhal VK, Srivastava A, Singh VP (2011) Zinc alleviates cadmium induced toxicity in *Vigna radiata* (L.) Wilczek. J Phytol 3:43–46
Lachman J, Dudjak J, Miholová D, Kolihová D, Pivec V (2004) Effect of cadmium stress on the uptake and distribution of microelements copper and zinc in plant parts of barley (*Hordeum sativum* L.). Sci Agric Biochem 35:81–86
Lee S, Leustek T (1999) The effect of cadmium on sulfate assimilation enzymes in *Brassica juncea*. Plant Sci 141:201–207
Leita L, De Nobili M, Mondini C, Baca-Garcia MT (1993) Response of leguminaseae to cadmium exposure. J Plant Nutr 16:2001–2012
Leyval C, Joner EJ, del Val C, Haselwandter K (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. Birkhauser, Switzerland, pp 175–186
Liu L, Zhang Q, Hu L, Tang J, Xu L, Yang X, Yong JWH, Chen X (2012) Legumes can increase cadmium contamination in neighboring crops. PLoS One 7(8):e42944
Liu Y, Zhuang P, Li Z, Zou B, Wang G, Li N, Qiu J (2013) Cadmium accumulation in maize monoculture and intercropping with six legume species. Acta Agric Scand 63:376–382
Ma Y, Dickinson NM, Wong MH (2006) Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous tress on Pb/Zn mine tailings. Soil Biol Biochem 38:1403–1412
Makoi JHJR, Chimphango SBM, Dakora FD (2009) Effect of legume plant density and mixed culture on symbiotic N2 fixation in five cowpea genotypes in South Africa. Symbiosis 48:57–67
Malcova R, Vosatka M, Gryndler M (2003) Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L. Appl Soil Ecol 23:255–267
Mandal SM, Bhattaccharyya R (2012) *Rhizobium*-Legumes symbiosis: a model system for the recovery of metal-contaminated agricultural land. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 115–128
Mandal SS, Rabindranath R (2012) *Rhizobium*-Legume symbiosis: a model system for the recovery of metal-contaminated agricultural land. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 115–128
Marshner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, London, UK
Matsumoto ST, Marin-Morales MA (2004) Mutagenic potential evaluation of the water of a river that receives tannery effluent using the *Allium cepa* test system. Cytologia 69:399–408
Mohan BS, Hosetti BB (2006) Phytotoxicity of cadmium on the physiological dynamics of *Salvinia natans* L. grown in macrophyte ponds. J Environ Biol 27:701–704
Muleta D (2010) Legume response to arbuscular mycorrhizal fungi inoculation in sustainable agriculture. In: Khan MS, Zaidi A, Mussarrat J (eds) Microbes for legume improvement. Springer, Wien, pp 293–323
Muleta D, Woyessa D (2012) Importance of arbuscular mycorrhizal fungi in legume production under heavy metal contaminated sites. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 219–241
Na G, Salt DE (2011) The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. Environ Exp Bot 72:18–25
Nellessen JE, Fletcher JS (1993) Assessment of published literature pertaining to the uptake/accumulation, translocation, adhesion and biotransformation of organic chemicals by vascular plants. Environ Toxicol Chem 12:2045–2052
Obata H, Umebayashi M (1997) Effects of cadmium on mineral nutrient concentrations in plants differing in tolerance for cadmium. J Plant Nutr 20:97–105
Overmyer K, Brosché M, Kangasjarvi J (2003) Reactive oxygen species and hormonal control of cell death. Trends Plant Sci 8:335–342
Pandey P, Tripathi AK (2011) Effect of heavy metals on morphological and biochemical characteristics of *Albizia procera* (Roxb.) Benyh. seedlings. Int J Environ Sci 5:1009–1018
Parker R (1994) Environmental restoration technologies. EMIAA Yearbook, pp 169–171
Paudyal SP, Aryal RP, Chauhan SVS, Maheshwari DK (2007) Effect of heavy metals on growth of *Rhizobium* strains and symbiotic efficiency of two species of tropical legumes. Sci World 5:5
Perveen R, Faizan S, Tiyagi SA, Kausar S (2011) Performance of Cd stress condition on growth and productivity parameters of *Trigonella foenum-graecum* L. World J Agric Sci 7:607–612
Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. Critical Rev Plant Sci 21:539–566
Poschenrieder C, Barcelo J (1999) Water relations in heavy metal stressed plants. In: Prasad MNV, Hagemeyer J (eds) Heavy metal stress in plants: from molecules to ecosystems. Springer, Heidelberg, pp 207–230
Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. Environ Exp Bot 35:525–545
Ramel C (1973) The effect of metal compounds on chromosome segregation. Mutat Res Lett 21:45–46
Rana A, Ahmad M (2002) Heavy metal toxicity in legume microsymbiont system. J Plant Nutr 25:369–386
Rather GM (2013) Studies of response of single and multi-plant species systems to heavy metal contamination and its significance for community characteristics. Ph.D. Thesis. Aligarh Muslim University, Aligarh.
Reichman SMA (2007) The potential use of the legume-*Rhizobium* symbiosis for the remediation of arsenic contaminated sites. Soil Biol 39:2587–2593
Rivera-Becerril F, Catherine C, Katarzyna T, Caussanel J-P, Belimov AA, Gianinazzi S, Strasser RJ, Gizninazzi-Pearson V (2002) Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycrorrhiza in three *Pisum sativum* L. genotypes. J Exp Bot 53:1177–1185
Ryan JA, Pahren HR, Lucas JB (1982) Controlling cadmium in the human food chain: a review and rationale based on health effects. Environ Res 28:251–302
Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet L, Raskin L (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnol 13:468–474
Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC, del Rio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot 52:2115–2126
Sandmann G, Bflger P (1980) Copper mediated lipid peroxidation processes in photosynthetic membranes. Plant Physiol 66:797–800
Saraswat S, Rai JPN (2011) Prospective application of *Leucaena leucocephala* for phytoextraction of Cd and Zn and nitrogen fixation in metal polluted soils. Int J Phytoremed 13:271–288
Sarwar N, Saifullah MSS, Zia MH, Naeem A, Bibi S, Farid G (2010) Role of mineral nutrition in minimizing cadmium accumulation by plants. J Sci Food Agric 90:925–937
Sepehri M, Rastin NS, Rahmani HA, Alikhani H (2006) Effects of soil pollution by cadmium on nodulation and nitrogen fixation ability if native strains of *Sinorhizobium meliloti*. J Sci Technol Agric Nat Res 10:153–163
Shamsi IH, Wei K, Zhang GP, Jilani GH, Hasan MJ (2008) Interactive effects of cadmium and aluminium on growth and antioxidative enzymes in soybean. Biol Plant 52:165–169

Sharma RK, Agrawal M, Marshall FM (2009) Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food Chem Toxicol 47:583–591
Shetty KG, Hetrick BA, Schwab AP (1994) Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ Poll 86:181–188
Shukla V, Dhankhar M, Prakash J, Sastry KV (2007) Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. J Environ Biol 28:395–397
Siedlecka A, Krupa Z (1999) Cd/Fe interaction in higher plants—its consequences for the photosynthethic apparatus. Photosynthetica 36:321–331
Silver S (1996) Bacterial resistances to toxic metal ions—a review. Gene 179:9–19
Sofia C, Sofia P, Ana L, Etelvina F (2012) The influence of glutathione on the tolerance of *Rhizobium leguminosarum* to cadmium. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 89–100
Stobart AK, Griffiths WT, Ameen-Bukhari I, Sherwood RP (1985) The effect of $Cd^{2+}$ on the biosynthesis of chlorophyll in leaves of barley. Physiol Plant 63:293–298
Swandulla D, Armstrong CM (1989) Calcium channel block by cadmium in chicken sensory neurons. Proc Natl Acad Sci U S A 86:1736–1740
Takas T (2012) Site-specific optimization of arbuscular mycorrhizal fungi mediated phytoremediation. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 179–202
Tantrey MS, Agnihotri RK (2010) Chlorophyll and proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatments. Res J Agri Sci 1:119–122
Terry N (1981) An analysis of the growth responses of *Beta vulgaris* L. to phytotoxic trace elements. II. Chromium. Final report to the Kearney foundation of soil science. July 1975–June 1980
Tonin C, Vandennkoornhuyse P, Joner EJ, Straczek J, Leyval C (2001) Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 10:161–168
Velaiappan A, Melchias G, Kasinathan P (2002) Effect of heavy metal toxicity on the nodulation pattern of legume cultivars. J Ecotoxicol Environ Monitor 12:17–20
Verma S, Dubey RS (2001) Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biol Plant 44:117–123
Vijayaragavan M, Prabhahar C, Sureshkumar J, Natarajan A, Vijayarengan P, Sharavanan S (2011) Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (*Vigna unguiculata* L.) plants. Int Multidisc Res J 1:01–06
Vivas A, Marulanda A, Ruiz-Lozano JM, Barea JM, Azcon R (2003a) Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG induced drought stress. Mycorrhiza 13:249–256
Vivas A, Voros I, Biro B, Campos E, Barea JM, Azcon R (2003b) Symbiotic efficiency of autochthonous arbuscular mycorrhizal fungus (*G. mosseae*) and *Brevibacillus brevis* isolated from cadmium polluted soil under increasing cadmium levels. Environ Pollut 126:179–189
Von Rosen G (1954) Mutation induced by the action of metal ions in *Pisum sativum*. Hereditas 43:644–650
Vyas J, Puranik RM (1993) Inhibition of nitrate reductase activity by mercury in bean leaf segments. Ind J Plant Physiol 36:57–60
Wahid A, Ghani A, Ali I, Ashraf MY (2007) Effect of cadmium on carbon and nitrogen assimilation in shoots of mungbean [*Vigna radiata* (L.) Wilczek] seedlings. J Agron Crop Sci 193:357–365
Wani PA, Khan MS (2010) Bacillus species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. Food Chem Toxicol 48:3262–3267
Wani PA, Khan MS, Zaidi A (2007a) Cadmium, chromium and copper in green gram plants. Agron Sustain Dev 27:145–153
Wani PA, Khan MS, Zaidi A (2007b) Impact of heavy metal toxicity on plant growth, symbiosis, seed yield and nitrogen and metal uptake in chickpea. Aus J Exp Agric 47:712–720
Wani PA, Khan MS, Zaidi A (2008a) Effect of metal-tolerant plant growth-promoting *Rhizobium* on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol 55:33–42
Wani PA, Khan MS, Zaidi A (2008b) Chromium reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium amended soil. Biotechnol Lett 30:159–163
Winder C (1989) Reproductive and chromosomal effect of occupational exposure to lead on the male. Reprod Toxicol 3:221–223

# 12 Phytoremediation of Copper-Contaminated Soil

Anna Karczewska, Andrzej Mocek, Piotr Goliński, and Mirosław Mleczek

## 12.1 Current Problem of Worldwide Contamination of Soils with Copper

Copper occurs in the environment at trace levels; its average concentrations in the earth crust and world average content in topsoils, reported by various authors, remain in the ranges 24–68 mg/kg and 14–30 mg/kg, respectively. The origin of copper present in soil is usually both natural and anthropogenic. The main natural sources of soil Cu are parent rocks, or more precisely the products of their weathering, supplied by the influx with water and airborne deposition. Typical concentrations of Cu in granitic igneous rocks are lower than those in basic or ultrabasic rocks, such as basalt or peridotite. Limestones are usually relatively poor in Cu. Fine-textured sedimentary rocks, such as clays and shales, thanks to their sorption properties, contain higher concentrations of Cu than sandstones or loose sandy rocks. Accordingly, light-textured soils are naturally poorer in Cu than clays or clayey loams (Adriano 1986; Kabata-Pendias and Pendias 2001; FOREGS 2005; Alloway 2013).

According to FOREGS (2005), based on the data from 840 sampling sites, the concentrations of Cu in European topsoils vary in a broad range (0.8–256 mg/kg) and are strongly related to regional and local geology, with additional impacts from anthropogenic pollution. The median total Cu content in topsoil is 13.0 mg/kg. Soils with low Cu content (below 8.0 mg/kg) are typical for southern Fennoscandia, the Baltic states, and the Quaternary plain of northern Central Europe, with a well-marked southern limit of the glacial drift. Separate zones with low soil Cu also occur in northeastern Italy and central Portugal. Soils enriched in Cu, containing over 20 mg/kg Cu, are typical for the Mediterranean zone, as well as for southwest England and Ireland.

An important anthropogenic source of local soil enrichment in Cu is deposition of airborne dust released from metal ore mining smelting, processing, and waste disposal. Urban and industrial areas are affected by a wide variety of activities, such as traffic, housing, and metal industry that may contribute to increased Cu concentrations in soils. Particular sources of such enrichment are often difficult to unambiguously identify. Considerably enhanced concentrations of Cu in soils of agricultural lands, distant from industrial and urban sites, may be caused by common agricultural practices: the use of pesticides, in particular fungicides containing Cu as an active ingredient, and the use of contaminated sewage sludge, solid wastes, or fertilizers as soil amendments (Kabata-Pendias and Pendias 2001; Alloway 2013).

The source and nature of soil enrichment in Cu is of crucial importance for its fate in the environment, its bioavailability, environmental risk, and hence for the decisions on need for soil remediation and its most appropriate methods.

Significant soil pollution with copper occurs both in Europe and in the world, only on a local scale. This applies mainly to heavily industrialized and urban areas, with historical or currently operating copper ore mines, smelters, and metallurgical plants.

In the areas of historical mining and ore processing, beginning from Roman and medieval times, metallic Cu was produced in crude, highly polluting technologies, and certain amounts of Cu were released to the environment from various facilities, usually scattered over the land. Throughout the centuries, the gangue rock material was disposed on mine spoils, and processing wastes and slags were dumped on heaps, in tailings impoundments, and in landfills. As an effect of land redevelopment, this waste material used to be spread

A. Karczewska, Ph.D.
Institute of Soil Science and Environmental Protection, Wrocław University of Environmental and Life Sciences, Grunwaldzka 53, 50-357 Wrocław, Poland

A. Mocek
Department of Soil Science and Land Protection, Poznań University of Life Sciences, Szydłowska 50, 60-656 Poznań, Poland

P. Goliński • M. Mleczek, Ph.D. (✉)
Department of Chemistry, Poznań University of Life Sciences, Wojska Polskiego 75, 60-625 Poznań, Poland
e-mail: mirekmm@up.poznan.pl

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over the soil surface, for its leveling or road hardening, which led to Cu dispersion in the environment. Ore smelting processes released huge amounts of Cu-rich dust that was subsequently deposited on the areas adjacent to smelters (Thornton 1996; Li and Thornton 2001; Karczewska and Bogda 2006; Wilson and Pyatt 2007a, b; Chopin and Alloway 2007; Abreu et al. 2008). Small-sized aerosols were transported over a long distance, sometimes thousands of kilometers (Hong et al. 1996). For historical mining areas, it is often quite difficult to distinguish between natural soil enrichment in Cu and the effects of human activities, related to various stages of ore processing. The assessment of associated environmental risk will also be difficult because the potential mobility and bioavailability of soil Cu depend on its origin and speciation. Typically, the solubility of Cu in metallurgical dust is greater than that in the unprocessed ores.

### 12.1.1 Present Mining and Ore Processing as a Source of Soil Pollution

Copper ore mining areas are intrinsically enriched in Cu. In those areas where Cu occurs in shallow ore deposits, exploited by open-pit mining, soil enrichment in Cu may be caused both by natural rock mineralization, and by depositions of dust released from ore mining, transportation, crushing, and further processing, as well as from the dispersion of mining wastes in the environment (Salomons 1995; Thornton 1996; Bech et al. 1997; Lottermoser 2003; Ikenaka et al. 2010). Cu-rich dust can also be blown away from waste dumps and tailing impoundments (Kabała et al. 2008). Copper originating from these sources occurs usually in the same form as in the ore itself, i.e. as sulfide, oxide, carbonate, or silicate minerals. In the areas of underground Cu mining, associated with deep ore deposits, the enrichment of soils in Cu is usually purely anthropogenic.

Almost 90 % of global copper production is based on sulfide ores. Oxide and carbonate ores are of local importance (USGS 2013; InfoMine 2014). Copper ores are usually polymetallic, and contain considerable amounts of other elements such as Ni, Co, Sn, W, Mo, Zn, Pb, Sb, Bi, As, Se, Te, and Au, Ag, and Hg. Table 12.1 provides information about the largest copper mines in the world. Undoubtedly, in the surroundings of these mines, the enrichment of soils in Cu occurs on the largest scale. However, no aggregated data on the ranges of Cu content in rocks and soils, or its spatial distribution patterns, in those areas are available.

**Table 12.1** The largest copper mines in the world, by yearly production in 2013 (InfoMine 2014)

| No. | Mine | Country | Deposit type | Mine type | Yearly Cu production, 2013 | Yearly Cu capacity, 2012 |
|---|---|---|---|---|---|---|
| | | | | | $10^3$ metric tons | |
| 1. | Escondida | Chile | Porphyry | Surface | 1,194 | 1,150 |
| 2. | El Teniente | Chile | Porphyry | Underground | 450 | 433 |
| 3. | Collahuasi | Chile | Porphyry | Surface | 445 | 520 |
| 4. | Antamina | Peru | Skarn | Surface | 427 | 450 |
| 5. | Los Pelambres | Chile | Porphyry | Surface | 420 | 470 |
| 6. | Los Bronces | Chile | Porphyry | Surface | 416 | 416 |
| 7. | Grasberg Complex | Indonesia | Porphyry | Surface and underground | 415 | 750 |
| 8. | Radomiro Tomic (Codelco Norte) | Chile | Porphyry | Surface and underground | 380 | 840 |
| 9. | Chuquicamata (Codelco Norte) | Chile | Porphyry | Surface/underground | 339 | |
| 10. | Morenci | USA | Porphyry | Surface | 301 | 420 |
| 11. | Cerro Verde | Peru | Porphyry | Surface | 300 | 320 |
| 12. | Taimyr (Norilsk—Polar Division) | Russia | Mafic intrusive (basal) | Underground | 297 | 430 |
| 13. | Kansanshi | Zambia | Structurally-controlled vein-hosted | Surface | 271 | 285 |
| 14. | Tenke Fungurume | DR Congo | Sediment hosted | Surface | 264 | No data |
| 15. | Andina | Chile | Porphyry | Surface | 237 | 300 |
| 16. | Mopani | Zambia | Sediment hosted | Underground and surface | 212 | No data |
| 17. | Bingham Canyon | USA | Porphyry | Surface | 211 | 280 |
| 18. | Rudna (KGHM) | Poland | Sediment hosted | Underground | 209 | 215 |
| 19. | Polkowice-Sieroszowice (KGHM) | Poland | Sediment hosted | Underground | 205 | No data |
| 20. | Candelaria | Chile | Hydrothermal (iron-oxide-Cu-Au) | Surface and underground | 191 | No data |

The data on mine capacities refer to the year 2012, according to ICSG 2013

### 12.1.2 Soil Contamination in the Surroundings of Copper Smelters

Copper metallurgy was for many centuries, and particularly in the twentieth century, a large source of emissions containing Cu and other metals, such as Pb, Cd, sometimes Ni and highly toxic, semimetallic As. Since most of the copper deposits mined presently are sulfide ones, their processing releases considerable amounts of $SO_2$, which, if not entrapped, may lead to strong soil acidification, thereby causing highly increased solubility of Cu. High concentrations of copper and other metals, together with the effects of soil acidification and sometimes enhanced salinity, were responsible for the formation of barren lands, highly vulnerable to water and wind erosion.

Such effects have been reported from several areas in the world. Industrial barren lands are frequently associated with historical smelting sites. The best known examples from North America are nickel and copper smelters in Sudbury (Ontario, Canada) that started operation in the eighteenth to nineteenth centuries (McCall et al. 1995), and smelters in the United States: MT and Anaconda-Deer Lodge Valley in southwestern Montana (Burt et al. 2003), the Asarco smelter in Tacoma (Washington) (Crecelius et al. 1974) and Copper Hill and Duck Town in Tennessee (Quinn 1991), the largest US emitters of metals and sulfur dioxide in the 1970s and 1980s. In Australia, one of the important producers of copper, there were hot disputes about the environmental impacts of the Port Kembla copper smelter in New South Wales (Martley et al. 2004). The largest European center of Cu mining and processing is that situated in SW Poland (Fore-Sudetic Monocline, i.e. the area of Legnica and Głogów), where Permian sedimentary Cu deposits are mined and processed by the company KGHM Polska Miedź S.A. (KGHM 2013). Dust and sulfur dioxide discharged into the atmosphere from two smelters in the last decades of the twentieth century caused severe environmental pollution within a large area of a 1,000 ha. There are several other copper metallurgy centers in Europe that also exerted a considerable impact on soils in their surroundings, such as Falun in central Sweden (Ek et al. 2001), where mining and ore processing activities lasted for over 1,000 years, nickel and copper smelter Harjavalta in Finland (Derome 2000; Nieminen et al. 2002; Nikonov et al. 2001), several smaller centers in the UK, such as Devon Great Consols Mine in the Tamar valley in Devon (Lombi et al. 2004), and near Avonmouth (Spurgeon and Hopkin 1996). In the south of Europe, Rio Tinto in Spain is one of the greatest ore smelting centers, where over the centuries copper has been produced from polymetallic ores. The main environmental concern in Rio Tinto is contamination of water, sediments, and soils with arsenic rather than with copper (Sáinz et al. 2004).

The countries of South America, Asia, and Africa, which currently host the world's largest copper industry plants, have been affected by their activities to various extents. Most of the most modern smelters are equipped with highly efficient installations of air protection, and therefore their possible environmental impacts should be substantially reduced. On the other hand, however, due to their high costs, such installations will be placed in service only when their operation is enforced by efficacious legal regulations. The list of the ten most polluting industrial plants in the world, published by Blacksmith Institute (2008a, b) includes, among others, the Zambia Consolidated Copper Mines in Kabwe, shut down in 1994, the Chinese Shenyang Smelting center (running from 1936 to 2003), Russian Copper Company smelters in Norilsk and in Karabash (the Chelyabinsk region of Russia's Ural Mountains), as well as Romanian plants Copsa Mica. The center of mining and metallurgy Severonikiel in Monchegorsk (the Kola Peninsula) is considered one of the most polluted areas in Russia (Barcan 2002; Kozlov 2005; Kozlov and Zvereva 2007). Several papers confirm considerable soil pollution caused by the Chinese smelters at Guixi, Huangshi, and Shuikoushan (Hu et al. 2006; Yan et al. 2007; Wei et al. 2009), as well as by others, for example in Peru (e.g. the region of Ilo), South Korea, Indonesia, and in other countries. The Blacksmith Institute's database contains more than 350 sites polluted by ore mining and smelting, that potentially put more than 6.7 million people at risk. Most of the modern facilities, built or modernized in 1990s or later, including the majority of smelters in Chile, currently the world's largest copper producer, cause a much smaller impact on the environment. However, it would not be possible to completely cut out all the hazardous effects of mining and metallurgy on the environment (Ginocchio 2000). The world production of metallic copper has reached recently (in 2011) 17 million tons per year (USGS 2013), half of which is produced in Asia, 30 % in China and 9 % in Japan. Not earlier than 20 years ago, Cu production in Asia remained below three million tons. Such rapid development of the Cu smelting industry, particularly in China, as well as in India and South Korea, is quite a new phenomenon, and information on the impact exerted on the soil environment by new facilities is for the time being difficult to acquire. Table 12.2 shows the current list of the largest copper smelters in the world.

Massive depositions of airborne particulate matter, together with gaseous sulfur dioxide, have strongly affected the environment in the immediate vicinities of large copper smelters, leading to the development of industrial barren lands, almost completely deprived of plant cover. A brief description of 36 such objects, published by Kozlov and Zvereva (2007), shows that most of those areas developed in the 1970s, and 25 of them evolved in the surroundings of either Cu or Cu-Ni smelters, mainly in Russia

**Table 12.2** The largest copper smelters in the world (2012), according to ICSG 2013, extended

| No. | Smelter | Country | Smelting process | Year of foundation | Yearly Cu capacity $10^3$ metric tons |
|---|---|---|---|---|---|
| 1. | Guixi (Jiangxi Copper Corp.) | China | Outukumpu Flash | 1977 | 900 |
| 2. | Birla Copper | India | Outukumpu Flash, Ausmelt, Mitsubishi Cont. | 1998 | 500 |
| 3. | Codelco Norte | Chile | Outukumpu/Teniente Conv. | 1952 | 450 |
| 4. | Hamburg | Germany | Outukumpu, Contimelt, Electric | 1948 | 450 |
| 5. | Beshhi/Ehime (Toyo) | Japan | Outukumpu Flash | 1905 | 450 |
| 6. | Saganoseki/Ooita | Japan | Outukumpu Flash | 1916 | 450 |
| 7. | El Teniente | Chile | Reverberatory/Teniente Conv. | 1905 | 400 |
| 8. | Jinchuan | China | Reverberatory/Kaldo Conv. | 1960 | 400 |
| 9. | Xiangguang Copper | China | Outukumpu Flash | 2007 | 400 |
| 10. | Norilsk (Nikelevy, Medny) | Russia | Reverb., Electric, Vanyukov | 1942 | 400 |
| 11. | Sterlite (Tuticorin) | India | Isasmelt Process | 1996 | 400 |
| 12. | Ilo | Peru | Isasmelt Process | 1983 | 360 |
| 13. | Onahama/Fukushima | Japan | Reverberatory | 1965 | 354 |
| 14. | Altonorte (La Negra) | Chile | Normanda Continuous | 1993 | 350 |
| 15. | Jinlong (Tongdu) | China | Flash Smelter | 1997 | 350 |
| 16. | Yunnan | China | Isasmelt Process | 1958 | 350 |
| 17. | Naoshima/Kagawa | Japan | Mitsubishi Cont. | (1917) 1974 | 342 |
| 18. | Pirdop | Bulgaria | Outukumpu Flash | 1958 | 330 |
| 19. | Onsan II | S. Korea | Mitsubishi Cont. | 1979 | 320 |
| 20. | Huelva | Spain | Outukumpu Flash | 1970 | 320 |
| 21. | Garfield | USA | Konnecott/Outukumpu | 1906 | 320 |
| 22. | Głogów I and II[a] | Poland | Blast Furnace/Outukumpu Flash Electrorefining | 1971 | 460 |

[a]The complex of two smelters [(KGHM 2013), www.kghm.pl]

(Kola Peninsula and the Southern Ural region), the United States, and Canada, as well as in Poland, Chile, and Slovakia. In many of the listed areas, plant cover has already been partially restored due to organized reclamation, or by slow processes of spontaneous succession, after the closure of installations or substantial reduction of their emissions.

The spatial extent of serious environmental impacts caused by copper smelters depends on a set of various factors, of which the most important are local topography and height of chimneys, as well as the kinds and quantities of pollutants released to the atmosphere, and in particular the size of metal-laden particles. The largest smelters, operating for many years, have caused soil contamination within distances of several kilometers, and sometimes even more. The amounts of contaminants deposited on the land surface, and hence the concentrations of Cu in soils, generally decrease along with increasing distance from the emission source, according to typical logarithmic dependence, related to a normal particle size distribution of dust (Zwoździak and Zwoździak 1982; Hutchinson and Whitby 1977). Environmental impacts caused by the emissions from three major Sudbury copper smelters, i.e. Copper Cliff, Coniston, and Falconbridge, were found at tens of kilometers. Copper (and nickel) concentrations in soils at the distance of 13.4 km away from the Copper Cliff smelter still exceeded 1,000 mg/kg (Table 12.3).

The extent of impacts exerted by the Russian smelter Severonickel was assessed by Barcan and Kovnatsky (1998) as 20 km, and Cu content in topsoils at the distance of 10–15 km from the smelter remained in the range of 51–384 mg/kg. The emissions from the Norwegian Sulitjelma smelter caused apparent soil enrichment in airborne Cu over 25 km away from the plant (Løbersli and Steinnes 1988), which should be assigned to the high frequency of strong winds in the coastal zone of the sea. Numerous reports indicate a significant impact of copper smelters on the soil environment at a distance up to 10 km, as it was proved for example for the smelter Legnica in Poland (Roszyk and Szerszeń 1988; Szerszeń et al. 1993; Karczewska 1996) and the copper smelter in Port Kembla, Australia (Martley et al. 2004). The range of this impact is sometimes much smaller, as in the case of the smelters at Tharsis, Ríotinto, and Huelva, SW Spain, where it was assessed as 2–3 km (Chopin and Alloway 2007).

Some of the world's largest emitters of Cu have already been closed, and most of those still operating have radically reduced the amounts of emitted dusts and gases. An example illustrating reduction of emissions from all divisions of KGHM, presently the largest copper producer in Europe, is presented in Table 12.4.

Copper, similarly to Pb and As, usually accumulates in topsoil, where it is strongly bound, and therefore the problem of

**Table 12.3** Examples of Cu concentrations in topsoil at various distances from copper smelters

| Smelter | Country | Distance (km) | Depth (cm) | Cu (mg/kg) | Reference |
|---|---|---|---|---|---|
| Sudbury: Coniston Smelter | Canada | 1.5 | 0/5 | 2,007/1,864 | Hutchinson and Whitby (1977) |
| | | 7.4 | | 1,425/1,621 | |
| | | 19.3 | | 730/597 | |
| | | 49.8 | | 31/27 | |
| Severonickel | Russia | 3–10 | Forest litter and mineral soil to the depth of max. 15 | 246–4,622 | Barcan and Kovnatsky (1998) |
| | | 10–15 | | 51–384 | |
| | | Over 20 | | 13–34 | |
| Harjavalta | Finland | 0.5 | Humus layer/mineral soil 0–5 | 2,304/259 | Derome and Lindroos (1998) |
| | | 3 | | 1,079/29.1 | |
| | | 4 | | 525/4.3 | |
| | | 8 | | 125/1.3 | |
| Sulitjelma | Norway | 1 | 3–5 | 2,500 | Løbersli and Steinnes (1988) |
| | | 27 | | 10×BL | |
| | | 37 | | 10 | |
| Las Ventanas I Chagres | Chile | 2.6–8 | 0–20 | 113–384 | De Gregori et al. (2000) |
| | | 13.5–26 | | 62–89 | |
| Legnica | Poland | 1 | 0–20 | 750–986 | Karczewska (1996) |
| | | 2 | | 250–280 | |
| | | 3 | | 100–248 | |
| | | 4 | | 75–101 | |
| Głogów | Poland | 0.5 | 0–20 | Up to 1,710 | Kabała and Singh (2001), Karczewska (1996) |
| | | 1 | 0–18 | 369 | |
| | | 3 | | 426 | |
| | | 6 | 0–27 | 115 | |

*BL* background level

**Table 12.4** The amounts of gaseous and particulate pollutants released into the atmosphere from mining and smelting facilities of KGHM S.A. during the period 1980–2010 (KGHM 2013)

| Pollutant | 1980 | 1990 | 2000 | 2010 |
|---|---|---|---|---|
| Sulfur dioxide (t/year) | 154,245 | 48,719 | 6,202 | 4,518 |
| Copper (t/year) | 2,968 | 204 | 23 | 10.6 |
| Lead (t/year) | 3,119 | 124 | 14 | 4.9 |

soil contamination in smelter-affected areas will still exist for a long time, even though the emissions have been drastically reduced. The concentrations of the three above-mentioned elements in soils surrounding the Legnica and Głogów copper smelters remain in fact unchanged for many recent years (Karczewska et al. 2010; Szerszeń et al. 1991, 2004).

### 12.1.3 Cu Contamination of Urban Soils

Soil contamination in the urban environment and associated human health implications caused by heavy metals has been recently a matter of emerging studies (Wong et al. 2006). High spatial variability of soil properties and frequent occurrence of local contamination are the characteristic features of soils in urban and industrialized centers (Pasieczna et al. 2003; Wong et al. 2006; Kabała et al. 2009). Pollution of urban soils may be caused by emissions from traffic (fossil fuel combustion, wear and tear of vehicular parts, leakage of motor oils), industry-specific activities, disposal of municipal waste (incineration and landfilling), and the corrosion of constructions. Moreover, soils in the urban environment are usually highly disturbed, and various exogenous materials, often polluted, of unknown origin, may be used for land leveling and landscaping. Organic waste materials commonly used for soil improvement and fertilization in urban parks, lawns, and allotments (Alloway 2004; Kabała et al. 2009) are often rich in metals. High Cu concentrations in soils of the old city centers may also be derived from historical, ancient activities (Alexandrovskaya and Alexandrovskiy 2000).

Studies carried out by a number of authors in various cities show that the mean Cu concentrations in surface levels of urban soils are usually much higher than the values of the local geochemical background (Ajmone-Marsan and Biasioli 2010). Locally, urban soils contain extremely high levels of copper, recorded from the industrial parts of the towns, and also from the parks, recreational areas, and allotments. Table 12.5 summarizes the data on Cu concentrations in soils reported from various cities. A review study published by

**Table 12.5** Cu concentrations in urban soils

| City | N | Depth (cm) | Usage | Total Cu (mg/kg) Range | Mean | Median (or geometric mean) | Reference |
|---|---|---|---|---|---|---|---|
| Aviles | 40 | 0–15 | Various | 19–1,040 | x | (63) | Ordóñez et al. (2003) |
| Aveiro (Portugal) | 26 | 0–10 | Parks | 8–61 | 18 | 16 | Madrid et al. (2006) |
| Bangkok | 30 | 0–5 | Various | 5–283 | 42 | 27 | Wilcke et al. (1998) |
| Beijing | 771 | 0–20 | Various | 2–282 | 24 | (21) | Zheng et al. (2008) |
| Berlin | 2,182 | 0–20 | Various—city | ?–12,300 | 80 | 31 | Birke and Rauch (2000) |
| Gibraltar | 120 | 0–15 | Various | <1–12,500 | x | 40 | Mesilio et al. (2003) |
| Glasgow | 27 | 0–10 | Parks | 24–113 | 73 | 71 | Madrid et al. (2006) |
| | 80 | 0–5, 5–10 | Parks and allotments | 58–484 | 140 | x | Hursthouse et al. (2004) |
| Hong Kong | 236 | 0–10 | Parks and lawns | 1–277 | 16 | 10 | Lee et al. (2006) |
| Ibadan | 106 | 0–15 | Various | 7–248 | 47 | 32 | Odewande and Abimbola (2008) |
| Ljubljana | 25 | 0–10 | Parks | 21–78 | 33 | 31 | Madrid et al. (2006) |
| Mexico City | 135 | | Traffic | 15–398 | x | 54 | Morton-Bermea et al. (2009) |
| Moscow | 3 | 0–10 | Various | 99–125 | 111 | 108 | Alexandrovskaya and Alexandrovskiy (2000) |
| Montreal | 8 | 0–15 | Polluted soils | 32–640 | x | >100 | Sauvé et al. (1996) |
| Newcastle upon Tyne | 163 | 0–5 | Various | 20–12,107 | 233 | 77 | Rimmer et al. (2006) |
| Poznań | 350 | 0–20 | Various | 3–120 | 16 | 11 | Grzebisz et al. (2002) |
| | 29 | 0–20 | Traffic | 12–149 | 86 | 82 | Diatta et al. (2003) |
| Richmond upon Thames | 214 | 0–15 | Various | 38–1,130 | x | (30) | Kelly et al. (1996) |
| Seville | 32 | 0–10 | Parks | 30–72 | 48 | 47 | Madrid et al. (2006) |
| | 62 | 0–10, 10–20 | Parks | 14–698 | 73 | x | Hursthouse et al. (2004) |
| Stockholm | 42 | 0–5 | Various | 7–1,315 | 71 | x | Linde et al. (2001) |
| Torino | 25 | 0–10 | Parks | 44–123 | 87 | 83 | Madrid et al. (2006) |
| | 70 | 0–10 | Urban | 34–283 | 90 | 76 | Biasioli et al. (2006) |
| | 40 | 0–10, 10–20 | Parks | 20–293 | 111 | x | Hursthouse et al. (2004) |
| Uppsala | 25 | 0–10 | Parks | 8–90 | 36 | 31 | Madrid et al. (2006) |
| Warsaw | 36 | 0–20 | Various | 7–65 | 25 | x | Pichtel et al. (1997) |
| Wolverhampton | 178 | 0–15 | Residential | 10–841 | x | (62) | Kelly et al. (1996) |
| | 25 | 0–15 | Industrial | 18–2,750 | x | (139) | |
| | 58 | 0–15 | Recreational | 12–387 | x | (55) | |
| Wrocław | 120 | 0–20 | Allotments | 13–595 | 63 | 39 | Kabała et al. (2009) |
| | 12 | 0–5 | Lawns | 8–52 | 22 | 16 | Dradrach et al. (2012) |

Ajmone-Marsan and Biasioli (2010) revealed that soils with Cu content over 120 mg/kg (Italian soil quality standard for residential and recreational areas) were present in 47 cities of the 153 covered by the analysis. According to Italian legal regulations, such soils should be considered for remediation. The highest concentrations of Cu in urban soils were found in industrial cities in the UK (Newcastle upon Tyne and Wolverhampton) and in large, industrialized European cities (Berlin, Glasgow, and Turin). Extremely high levels of Cu, vastly exceeding the value of 1,000 mg/kg, were recorded locally from urban soils in Gibraltar (12,500 mg/kg), Newcastle upon Tyne (12,107 mg/kg) and Wolverhampton (2,750 mg/kg), Berlin (12,300 mg/kg) and Stockholm (1,315 mg/kg) (Table 12.5), as well as in Osnabrück (1,570 mg/kg) and Jakobstad (2,612 mg/kg) (Ajmone-Marsan and Biasioli 2010).

All cases of such strong soil contamination with copper should be carefully examined from the standpoint of environmental hazards and human health risks. In some cases, soil remediation should be recommended. Particular concern should be given to highly Cu-enriched soils of allotment gardens in Berlin (Cu up to 1,280 mg/kg) (Birke and Rauch 2000), Wroclaw (up to 595 mg/kg) (Kabała et al. 2009) and Glasgow (up to 484 mg/kg) (Hursthouse et al. 2004).

### 12.1.4 Fertilizers and Pesticides as Sources of Cu in Agricultural Soils

Cu-based pesticides and some fertilizers may contribute to significant increase of Cu concentrations in agricultural soils, particularly if applied repeatedly for many years.

**Table 12.6** Cu concentrations in vineyard soils

| Region | Country | Depth (cm) | Cu (mg/kg) | Reference |
|---|---|---|---|---|
| Victoria | Australia | 0–1 | 9–249 | Pietrzak and McPhail (2004) |
| South Australia, Murray Valley, Grampians | Australia | 0–5 | 19–162 | Wightwick et al. (2013) |
| Zelina and Plesivica | Croatia | 0–10 | 30–700 | Romić et al. (2004) |
| Reuilsur Marne, near Reims | France | 0–3 | 264–519 | Besnard et al. (2001) |
| | | 15 | Mean: 149 | |
| | | 60 | Mean: 18 | |
| Herault, southern France | France | 0–15 | 14–251 | Brun et al. (2001, 2003) |
| Burgundy | France | 0–10 | 15–430 | Jacobson et al. (2005) |
| Rio Grande do Sul State | Brazil | 0–5 | 37–3,216 | Mirlean et al. (2007) |
| Rio Grande | Brazil | 0–5 | 433–517 | Mirlean et al. (2009) |
| Bohemian and Moravian region | Czech Republic | 0–20 | 20–168 | Komárek et al. (2008b) |
| Serra Gaúcha of Rio Grande do Sul | Brazil | 0–20 | 51–665 | Casali et al. (2008) |
| Various regions | Slovenia | 0–20 | 87–120 | Rusjan et al. (2007) |
| 3 various regions | Slovenia | 0–20 | 23–265 | Pavlovic (2011) |
| Galicia (NW Spain) | Spain | 0–20 | 25–272 | Fernández-Calviño et al. (2008a) |
| Galicia (NW Spain) | Spain | 0–20 | 61–434 | Fernández-Calviño et al. (2008b) |
| Galicia (NW Spain) | Spain | 0–10 | 125–603 | Nóvoa-Muñoz et al. (2007) |
| Dalmatian coast | Croatia | 0–20 | 105–553 | Milko et al. (2007) |
| Vojvodina | Serbia | 0–15 | >60–112 | Ninkov et al. (2012) |

Various Cu-based fungicides, such as copper sulfate, Bordeaux mixture ($CuSO_4 + Ca(OH)_2$), Cu-oxychloride, and many other preparations based on copper oxide and copper salts have been used since the end of the nineteenth century to control fungal diseases in fields, orchards, vineyards, and various plantations. Their regular application and subsequent wash-off from the treated plants have resulted in extensive Cu accumulation in vineyard soils (Komarek et al. 2010). The average yearly dosage of Cu applied with fungicides in French vineyards is as high as 5 kg/ha. There are numerous reports that confirm considerable Cu enrichment of vineyard soils and apparent increase of Cu concentrations in soils along with increasing vineyard age (Mirlean et al. 2009; Komárek et al. 2010; Pavlovic 2011). The highest Cu concentrations occur usually in the surface soil layers (Pietrzak and McPhail 2004; Wightwick et al. 2006; Mirlean et al. 2007), where they may be as high as in the range 500–1,000 mg/kg. The maximum value of 3,216 mg/kg was reported from a subtropical region in Brazil (Mirlean et al. 2007). Compared with those values, Cu content in vineyard soils in the Czech Republic, Slovenia, and Serbia, in the range 20–265 mg/kg (Table 12.6), should be assessed as relatively low. Comprehensive review papers on contamination of vineyard soil have been recently published by Komárek et al. (2010) and Mackie et al. (2012).

### 12.1.5 Other Sites with Cu-Contaminated Soils

A particular example of sites considerably contaminated with copper, chromium, and arsenic is wood treatment facilities in which wood preservatives (Cu sulfate or chromated Cu arsenate (CCA)) were used for many years to protect wood against insect and fungal attacks (Mench and Bes 2009). Many of the large facilities that presently apply CCA, or used to apply it in the past, caused local soil pollution with Cu (Zagury et al. 2003; Mench and Bes 2009; Kumpiene et al. 2006; Morrell and Hoffman 2004; Bes and Mench 2008). Cu concentrations in those soils may significantly exceed 1,000 mg/kg (Buchireddy et al. 2009). Some of such sites have turned into derelict areas, either barren or with poor vegetation (Mench and Bes 2009). Enhanced Cu concentrations were also reported from the underneath of CCA-treated wood structures, such as wooden bridges (Townsend et al. 2003).

All the kinds of metallurgical plants, such as secondary smelters or rolling mills, should also be mentioned as significant sources of copper release into the environment (Lepp et al. 1997).

## 12.2 Methods of Remediation of Soils Polluted with Copper

### 12.2.1 The Aim of Soil Remediation

According to the definition published in the proposal of the EU Soil Directive (2006), *soil remediation shall consist of actions on the soil aimed at the removal, control, containment or reduction of contaminants so that the contaminated site, taking account of its current use and approved future use, no longer poses any significant risk to human health or the environment.*

The decision on the necessity of reclamation and remediation methods should be consistent with the current and approved future use of contaminated land. Accordingly, soil remediation does not necessarily mean the need for cleanup. Several other strategies, alternative to decontamination, may be applied for soil restoration to obtain the required level of functionality.

Legal regulations concerning remediation are in various countries quite different, and essentially independent of each other (Carlon 2007). The policy and practice of managing the contaminated sites vary significantly across Europe, depending on different national approaches and various legal requirements. Most European countries, including Poland, have established national soil screening values (soil quality standards) aimed at regulating various issues related to land contamination—from setting long-term quality objectives, via triggering further investigations, to enforcing remedial actions. According to present Polish and some other countries' regulations, excessive amounts of pollutants should be removed from soils wherever the standard values are exceeded. There is, however, a strong tendency in EU environmental policy to move away from the arbitrary set standard values and to replace them with more general guidelines focusing on human health risk assessment and ecological risk assessment. Consequently, all the decisions concerning remediation need, its strategy, the most appropriate methods, and time requirements should be based on specific, site-related conditions, including the categories of land use and assessed environmental risk and human health risk.

Two opposing strategies may be applied to soils contaminated with heavy metals, including copper, with the aim of soil remediation:

- Immobilization—based on reduction of environmental risk by limiting metal solubility, bioavailability, and leaching.
- Decontamination, i.e. removal of excessive amounts of pollutant (Cu) from soil, or alternatively, removal of contaminated soil material by excavation and landfilling.

In Poland, as in several other countries, the strategy of *immobilization* combined with soil protection against erosion was considered for many years the best way to manage and revitalize land contaminated with heavy metals. The effect of copper immobilization in the soil solid phase may be in fact easily obtained by improvement of those soil properties that govern Cu sorption and release from the solid phase, in particular by modification of soil pH and cation exchange capacity. A fundamental soil treatment that reduces Cu mobility in acidified soils is liming (Kabata-Pendias et al. 1993; Adriano 1986; Bade et al. 2012). Application of high doses of lime proved to efficiently immobilize toxic metals, in particular Cu, in soils affected by emissions from the copper smelters at Legnica and Głogów, even though the Cu concentrations in the surface soil layers considerably exceeded the value of 150 mg/kg, established as the Polish soil quality standard for agricultural lands and forests. Coal fly ash also turned out to be a good soil amendment for metal immobilization (Ciccu et al. 2003; Kumpiene et al. 2007). It should be mentioned, however, that in strongly alkaline conditions, at pH above 8, copper may get remobilized from the solid phase by the formation of easily soluble complexes with ammonium, hydroxyl ions, or organic substances (Karczewska 2002; Ciccu et al. 2003; Cuske et al. 2013).

Light-textured sandy or gravely soils, poor in organic matter, require considerable improvement of sorption properties to effectively reduce Cu mobility. For this purpose, a range of materials with very good sorption capacity may be used as soil amendments (Kumpiene et al. 2008). They induce different sorption processes, such as ion exchange, specific adsorption to mineral surfaces, surface precipitation, as well as formation of complexes with stable organic matter. Precipitation as salts and coprecipitation can also contribute to reducing Cu mobility. Organic matter rich additives, such as peat, ground lignite, composts, sawdust, and woodchips, as well as green manure, were efficient in Cu immobilization (Ruttens et al. 2006; van Herwijen et al. 2007; Soler-Rovira et al. 2010; Mench et al. 2010). In recent years, extensive studies have also been carried out to examine short- and long-lasting effects of soil amendment with biochar, which appeared to be particularly efficient in immobilizing heavy metals in soil.

At this point, however, it should be emphasized that not all commonly available organic-rich materials may be safely applied to Cu contaminated soils. The well-proved high affinity of Cu to organic matter may have two-sided consequences, as the solubility of organically complexed Cu depends on the molecular size of the ligand. Various kinds of organic matter may, therefore, variously influence Cu mobility. Well-humified biosolids, such as mature composts, will be effective in Cu immobilization. In contrast, fresh biosolids, with a low degree of humification, rich in low molecular weight organic acids, may contribute to Cu mobilization rather than to its retention (Ruttens et al. 2006; Schwab et al. 2007; Kumpiene et al. 2008). Hence, such organic amendments as manure, fresh sewage sludge, or immature composts, rich in soluble organic fractions, should not be applied to Cu contaminated soils. Conclusions drawn from various experiments carried out to examine the impact of amendments rich in dissolved organic matter on Cu solubility in soil are, in fact, fairly inconsistent. An initial effect of Cu mobilization may be diminished or totally reduced via secondary sorption of soluble chelates on clay or a high molecular weight, insoluble organic fraction.

Various mineral amendments, such as clay-rich materials—bentonite, montmorillonite (Lottenbach et al. 1999; Zhang et al. 2011), as well as natural or synthetic zeolites (Gworek 1993; Shi et al. 2009)—proved to effectively support Cu immobilization. Sandy soils may also be supplied and ploughed with clay-rich soil material (Greinert 1995). Efficacious chemical sorption, i.e. precipitation in the forms of insoluble Cu salts or formation of ternary cation–anion complexes on the surface of Fe and Al oxy-hydroxides, may be brought about by soil amendment with phosphate-containing materials, such as ground phosphate rocks or hydroxyapatite (Ruby et al. 1994; Ma et al. 1995; Cotter-Howells and Caporn 1996; Berti and Cunningham 2000; Kumpiene et al. 2008). Application of iron and aluminum rich waste materials, such as iron grit, iron oxides, or red mud (a waste product of bauxite processing), usually efficient in immobilizing other heavy metals, has often failed to reduce Cu solubility in contaminated soils (Gray et al. 2006; Kumpiene et al. 2008).

Soil improvement based on an immobilization strategy was for a long time the only method of reclamation applied to metal contaminated soils, particularly those in the vicinities of copper smelters in Poland and in other countries. Its unquestionable beneficial effects on soil properties were obtained by easily available, inexpensive techniques that did not require any specialized equipment. Moreover, various waste materials were thereby utilized as soil amendments. These factors should be considered as very important advantages of the immobilization strategy. This strategy was applied as a standard in various copper smelter affected areas all over the world, and soil liming was usually considered the crucial, primary step in soil reclamation (Vangronsveld et al. 1996; Winterhalder 1999, 2000; Derome 2000). Neutralization of soil pH enabled the retrieval of soil biological activity and successful restoration of plant cover in barren areas around the smelters in Sudbury, Tacoma, Harjavalta, and Falun.

Despite all its unquestionable outcomes, the method of metal immobilization in soils is often considered as temporary and therefore insufficient. In fact, it reduces, but does not eliminate, the potential risk of metal remobilization after possible changes of soil conditions. Therefore, the pollutants immobilized in soils are often referred to as a kind of time bomb. Moreover, insoluble metals adsorbed on the soil solid phase may still pose harmful effects on soil biology, for example on earthworms, and metal bearing particles may be carried away due to water or wind erosion, leading to secondary environmental pollution.

Particular methods of metal immobilization in soils are the technologies of solidification and vitrification that mechanically or physically fix the contaminants in the soil solid phase. These methods are of particular importance when hazardous substances occur in soils at shallow depth, within relatively small areas. Soil solidification involves physical encapsulation of contaminants in the soil matrix by injection of solidifying agents, such as cement-based mixtures, fly ash, bitumen, or liquid monomers that polymerize. Vitrification is a process requiring thermal energy, based on melting the soil together with contaminants at a high temperature generated by electric or microwave energy (Mulligan et al. 2001; Abramovitch et al. 2003; Chen et al. 2005; Kumpiene et al. 2008; Voglar and Leštan 2010). Both these kinds of treatments can be used either in situ or ex situ, after soil excavation. The methods are, however, highly energy-consuming and expensive, and therefore they have rarely been applied to Cu contaminated soils.

### 12.2.2 Soil Decontamination (Cleanup)

One of the simplest solutions for effective removal of contaminants from highly polluted areas may be exchange of the surface soil layer, i.e. the excavation and disposal (landfilling) of polluted topsoil, followed by the coverage of remaining subsoil with extraneous uncontaminated soil or other potentially productive material. Such a measure will be technically feasible and economically justified in those cases where soil contamination is confined to the topsoil within a relatively small area, for example in the immediate vicinities of dust emitters from Cu smelting processes. The exchange of topsoil was accomplished, for instance, in the internal areas of the Legnica and Głogów copper smelters in the late 1990s, shortly after installing highly efficient devices capable of separating dust and sulfur dioxide from the industrial waste gas.

The basic principle of soil cleanup involves, however, the removal of pollutant from the soil and recovery of decontaminated soil. Various technical methods have been developed for cleaning up soils contaminated with heavy metals. All of them are technologically much more advanced than the methods of metal immobilization; they are at the same time much more expensive. Their crucial disadvantage, however, is that they usually cause a temporary increase of metal solubility, thus leading to deterioration or complete destruction of living organisms, loss of biological activity, and worsening of soil physical and chemical properties. The only advantage of this type of treatment seems to be a relatively quick and definitive removal of pollutants. In cases where Cu is the main soil contaminant, in the absence of other much more toxic and hazardous substances, this type of action does not seem to be justified.

Cleanup treatments aimed at removing heavy metals from soils can be performed in two ways:

- In situ—at the site where contamination took place.
- Ex situ (off-site)—in special installations, where soil must be transferred after excavation.

From the technological standpoint, soil decontamination processes comprise two separate stages: (1) desorption of contaminant from the solid phase, associated with radical increase of its solubility, and (2) removal of soil solution, or soil extract, that contains solubilized metals, which requires an effective mechanism to separate the liquid and solid phases. The available technical methods that may be used for decontamination of Cu-polluted soils are:

- Soil washing (in situ) or extraction (ex situ)
- Electrochemical methods—in situ
- Combined extraction and electrochemical method—ex situ.

Several other technologies of soil decontamination, based on biological processes, such as bioleaching and phytoextraction, may also be applied to metal polluted soils. These methods will be described further on, in a separate subsection.

In the method of soil washing in situ (soil flushing), water with or without additives infiltrates into soil and solubilizes contaminants. Water application may be via surface flooding, a system of sprinklers, or vertical and horizontal drains. Various reagents may be added to flushing water to support Cu solubilization: diluted mineral acids (HCl or $HNO_3$), organic acids that act additionally as complexing agents (citric acid, acetic acid) or chelating agents such as EDTA, NTA, or glycine (Fischer et al. 1992; Lim et al. 2004; Dermont et al. 2008; Leštan et al. 2008; Zou et al. 2009; Arwidsson et al. 2010). The efficiency of extraction depends highly on soil permeability.

The same effect of metal washing from soils can also be achieved ex situ, in reactors or as heap leaching, where the soil, crushed and sieved prior to treatment, is shaken or leached with an excessive amount of water containing a solubilizing agent. This method, which uses a high water-to-soil ratio, is more effective than soil washing in situ, but—because of the costs of soil excavation and transport—is also much more expensive. Soils that contain over 10–20 % clay or that are rich in organic matter cannot be effectively decontaminated in this way, because of difficulties with clay and humic fraction recovery from the slurry. Flotation as a separation method may be helpful in such cases (Dermont et al. 2010), but ex situ extraction methods are usually applied to sandy and sandy-gravelly soils, poor in clay fractions.

Electrochemical methods (Page and Page 2002; Virkutyte et al. 2002) involve a mechanism of electrolysis for the removal of metals from soils. Ions and small charged particles are transported between the electrodes embedded in waterlogged soil that should be acidified prior to treatment, so that metallic elements are transformed into the form of free cations. Electrochemical methods can be used in situ; then the electrodes are imbedded relatively close to each other, at a distance of 1–6 m (Virkutyte et al. 2002). Better results may be obtained by electrokinetic processes carried out ex situ (Chung 2009). In practice, however, the removal of metallic pollutants, including Cu, from soils by electrochemical methods poses a lot of problems. It is difficult to remove metal fractions strongly associated with the soil solid phase and those that occur in solution in complexed forms rather than as free cations (Karczewska 2002; Virkutyte et al. 2002; Suèr et al. 2003). This is a specific feature of copper. The treatment can be supported by using various additives (Zhou et al. 2004), but these methods still remain at the stage of laboratory or pilot scale experiments. Electrochemical methods are very expensive, and in environmental terms quite risky, as they usually require soil acidification and application of a strong electric field, which adversely affects soil biota. Their advantage is that, unlike the methods of soil washing, they can be used for treatment of clay rich soils with low permeability.

Among various soil reclamation methods, the one that uses conditioned zeolite, proposed by Gworek (1993), should also be mentioned. The method combines two concepts of Cu immobilization and soil decontamination. Granulated zeolite with a high capacity to absorb Cu, fixed to briquetted carriers, is placed in soil and immobilizes the soluble Cu fraction. After a certain time, the briquettes can be easily removed from the soil (possibly by using a potato digger) and then regenerated. Pot experiments proved good suitability of this method for Cu removal from soils collected from the surroundings of copper smelters, but the results of field experiments were less encouraging. The method did not find practical application due to its high costs.

All the technical methods of soil decontamination presented above raise many objections. The common disadvantage of all described techniques is their high cost. Natural scientists, including soil scientists, lay emphasis on the environmental aspects of their application. The idea itself to clean up soils seem to be questionable, as the efficiency of decontamination treatments is directly related to the step of contaminant solubilization. Chemical reagents used for this purpose, as well as various kinds of physical operations, act as detrimental factors on soil biota and must cause irreversible destruction of biological activity. Therefore, the product obtained from decontamination is no longer soil, but a lifeless material, with altered or completely destroyed structure. Biologically active soil may be produced from this material, but this will require long-term biological reclamation.

### 12.2.3 Bioleaching

In recent years, much attention has been paid to bioleaching, a biological method of soil decontamination. This method, based on the same principles as "*biomining*," uses the abilities of certain microorganisms to dissolve metal-containing minerals. In the process of biomining, several metals of value can be extracted from poor quality ores, unsuitable for conventional processing. The technologies of biomining involve using consortia of acidophilic bacteria, such as *Acidithiobacillus ferrooxidans*, and Archaea to cause oxidative dissolution of sulfide minerals. As the major ore reserves of copper in the world contain sulfide minerals (such as chalcopyrite, chalcocite, or bornite) prone to biooxidation and bioleaching, biomining has found technical application for Cu recovery from poor ores. The method was patented and first applied in the 1960s by Kennecott Copper Corporation to extract copper from mine waste rock dumps at the Bingham Canyon mine in Utah and at the Chino mine in New Mexico. Since then, biomining has been successfully introduced in several other mines in the world, including Chile and China (Watling 2006; Rawlings and Johnson 2007; Brierley 2008; Johnson 2010). Technical solutions for biomining involve two techniques: irrigated systems and stirred tanks.

Bioleaching can be used to leach copper from contaminated soils—both in situ and ex situ, on the heaps or in bioreactors (Johnson 2001, 2010). The technology involves different groups of bacteria, usually of the genera *Acidithiobacillus*, *Acetobacter*, *Acidiphilum*, *Arthrobacter,* and *Pseudomonas* (Mulligan and Cloutier 2003; Deng et al. 2012). Several *Thiobacillus* spp. bacteria (renamed *Acidithiobacillus*), most often used in this process, require soil acidification to pH 4 and the optimal temperature, in the range of 15–55 °C, depending on the strain. Bioleaching of metals from bottom sediments and other deposits under anoxic conditions is easier than from the soil exposed to oxidizing conditions. Intensive research has recently been carried out on optimization of soil bioleaching with respect to soil properties, including initial pH, substrate concentration, pulp density, hydraulic retention time, etc., as well as different groups of bacteria and various strains to be applied (Naresh Kumar and Nagendran 2007, 2009).

Another group of biotechnologies developed for remediation of soils contaminated with metals, such as copper, is based on metal leaching from soil by organic acids, such as citric and gluconic acids, produced by fungi and actinomycetes, mostly of the genus *Aspergillus*, e.g. *Aspergillus niger*, as well as other genera, including *Penicillium* and *Fusarium* (Burgstaller and Schinner 1993; Valix et al. 2001; Deng et al. 2012). Organic acids of biological origin act both as acidifying and chelating agents. Soil should be additionally supplied with a carbon substrate, which is essential to accelerate the leaching of metals (Mulligan et al. 2001).

The methods of bioleaching are, definitely, closer to the technical approach of soil cleanup than to natural processes that normally occur in a terrestrial environment. Bioleaching has not been so far commonly applied for in situ remediation of soils, but if it were, it probably would not gain social acceptance, as in fact it cannot ensure a gentle, environment-friendly process of soil cleaning. Undoubtedly, the methods to be potentially applied for reclamation of contaminated soils, that might be commonly accepted by both decision-makers and the general public, are those referred to as "green technologies." These methods are intrinsically based on plants, and generally termed "phytoremediation."

### 12.2.4 Position of Phytoremediation Among Other Remediation Methods

The term "phytoremediation," derived from the Greek *phyton* = plant, and Latin *remedy* = healing, applies to a group of technologies that use either naturally occurring or genetically engineered plants to improve the properties of polluted soils or to remove contaminants from soils. The main aim of such measures is restoration of contaminated sites to conditions suitable for public or private applications. In the 1980s and 1990s, these environment-friendly methods of land reclamation gained great popularity, social acceptance, and a lot of attention from scientists, particularly in the U.S. and Western Europe. In the vast literature concerning phytoremediation, several techniques are distinguished, of which two may be considered for application to Cu-contaminated soils: phytostabilization and phytoextraction (Cunningham and Ow 1996; Chaney et al. 1997; Ensley 2000; Meagher 2000; Terry and Banuelos 2000; Grudziński et al. 2000).

Phytostabilization may be basically considered as an extension of in situ immobilization technology. It is a technique aimed to diminish the risk caused by the presence of contaminants in soil by the use of amendments that reduce contaminant solubility, and by introduction of appropriate plants (Terry and Banuelos 2000; Berti and Cunningham 2000; Chaney et al. 1997; Raskin and Ensley 2000). Phytostabilization focuses on long-term stabilization and containment of pollutants in soil by their sequestration in the plant rhizosphere and chemical fixation with amendments. The main objective of phytostabilization is not to remove contaminants, but to stabilize them and reduce the risk to human health and to the biota.

Plants used for phytostabilization should be tolerant to high metal levels and other limiting factors (such as soil pH, salinity, etc.) and indicate low ability of metal uptake from soil and translocation to the shoots. Fast growing, perennial plants, forming a tight cover over the soil, with a dense or deep rooting system and high transpiration rates (such as grasses, sedges, reeds, and various tree species) are particularly suitable for this purpose. Phytostabilization methods are widely used for biological reclamation of mining sites, including mine spoils and tailing impoundments, smelter-affected soils, different landfills, and repository sites, particularly when dumped material contains medium or high concentrations of metals. In order to obtain effective plant growth, it is important to chemically immobilize metals by chemical amendments, adjust pH to a plant-tolerable level, and provide nutrients necessary for plant growth. The plants will stabilize the surface, thereby preventing wind and water erosion, reduce water percolation down the soil profile, and prevent animals and humans from making direct contact with pollutants. Plants may also help to stabilize contaminants in soil by precipitating toxic elements in the rhizosphere or by sorption on root surfaces. Root exudates, rhizospheric bacteria, and mycorrhiza assist in altering the chemical forms of metals and reducing their solubility (Cotter-Howells and Caporn 1996; Maier 2004; Mench et al. 2006).

Phytoextraction is a concept of soil decontamination that involves the use of plants to remove (extract) pollutants, especially heavy metals and metalloids, from soil by root uptake and subsequent transport to aerial parts of plants (Raskin et al. 1997; Terry and Banuelos 2000; Lasat 2002). Unlike those recommended for phytostabilization, the plants suitable for phytoextraction should intensively take up metals and translocate them to the aboveground parts to be harvested. The idea of phytoextraction, although impressive and socially acceptable, raises a number of questions and concerns. The main problem is the real efficiency of this method and its capacity to bring the concentrations of pollutants in soil to a desired level, required by legal regulations, within a reasonable, relatively short period of time. Another important issue to be addressed is production of highly contaminated biomass that should be utilized (Sas-Nowosielska et al. 2008). Therefore, suitable and environmentally safe technologies of biomass processing should be developed together with optimizing phytoextraction technology. Energy biomass production and utilization of metal-rich ash in smelting processes seem to be proper directions in this respect.

Practical solutions for phytoextraction, reported in the literature, and tested in the field, involve the following methods.

- The use of accumulating or hyperaccumulating plants with natural capability of intensive metal uptake from soil;
- The use of fast-growing, high-biomass plants;
- Supported (assisted, induced) phytoextraction—an approach based on enhanced metal uptake by plants induced for instance by application of metal-solubilizing agents (usually chelators) to the soil.

Possible applications of these methods for remediation of copper-contaminated soils will be presented below in more detail.

## 12.3 Phytostabilization and Phytoextraction: Their Use for Reduction of Risk Caused by Copper Presence in Soils

### 12.3.1 Phytostabilization

Phytostabilization is a method that combines the effects of immobilization of pollutants in soil with mechanical protection against erosion and direct contact with humans and animals. Plants reduce water percolation into the soil, and—in the case when certain amounts of metals have been incidentally released from the solid phase—take them up, preventing water pollution. An amplified effect of metals' immobilization in soil is usually caused by their accumulation by the roots, in particular fine roots, and by precipitation in the form of insoluble compounds with root exudates and rhizosphere-produced substances (Mench et al. 2006; Kumpiene et al. 2008). The presence of plants positively influences site biodiversity. It is recommended that plant species and genotypes used for phytostabilization be of local, native origin. Phytostabilization turned out to be particularly efficient in managing sites heavily contaminated with metals, including copper. It proved successful in the vicinity of the largest metal smelters, as well as in Cu mining sites—in rehabilitation of mining dumps and tailings impoundments.

Soil amendments applied to support phytostabilization are quite often based on waste material, mainly for economic reasons. Soil liming or amendment with other alkalizing materials is used as a principle for remediation of acidic soils. The other, commonly used amendments, already mentioned above, are: weathering clay-rich rocks, gravel sludge, various organic additives such as compost, stabilized sewage sludge, paper mill wastes, woodchips, and sawdust, waste materials rich in Fe, Al, Mn, and Ti oxides, and a range of P-containing materials (such as hydroxyapatite, phosphate rocks, or phosphoric acid) (Mench et al. 2010). Recently, soil amendment with biocarbon has also been extensively tested (Karami et al. 2011). Mineral fertilization may be additionally beneficial for improvement of plant growth. Intensive studies are presently being carried out focusing on biological support of plant growth by the use of mycorrhizae and plant growth-promoting bacteria (PGPR) (Wang et al. 2007; Grandlic et al. 2008; Verdugo et al. 2010).

Among the first sites where the technique of phytostabilization was successfully used in a large-scale field application were tailings impoundments of the Canadian mines Copper Cliff (with an area of over 2,200 ha) and Elliot Lake (Peters 1995; Mench et al. 2006). The surface of strongly acidic tailings waste, rich in sulfides, was partly covered with a mulch of straw and biosolids, then limed with ground limestone or dolomite, amended with mineral fertilizers (740 kg/ha), and subsequently sown with a mixture of grasses and legumes. In a short time, a tight turf covered the surface, and the area was immediately colonized by woody species including birch, aspen, and willows. Native coniferous species, as well as some other trees, such as black locust or European larch, were planted in groups to form a seed source for further colonization (Winterhalder 1996). Satisfactory growth of plants and succession of species from the local flora proved successful phytostabilization. Cu content in the aerial parts of plants remained at a level typical for uncontaminated areas. The phytostabilization method has proved to be inexpensive, simple, and effective.

The main rules of revegetation for copper tailings impoundments, particularly those located in arid and semiarid ecosystems, were developed in the years 2005–2010, under the U.S. EPA superfund basic research program (Maier 2004; Mendez and Maier 2008). Presently, numerous different scale experiments aimed at optimizing phytostabilization of tailings impoundments are still being carried out, in both operating and historical copper mining sites (Mench et al.

2006, 2010; Santibáñez et al. 2008; Karami et al. 2011; Spiak and Gediga 2009; Verdugo et al. 2010). Each of such objects usually requires an individual approach, because of different characteristics of gangue rock and different climatic conditions that determine proper selection of plant species and may strongly affect their growth. The availability of inexpensive soil amendments for phytoremediation is usually determined by specific local conditions. The tailings produced by copper ore processing are often highly acidic, particularly when they originate from porphyry Cu deposits. In some other cases, however, the tailings may contain large amounts of calcium and dolomite, such as those obtained from sedimentary deposits mined by KGHM in Poland (Karczewska and Milko 2010).

Successful experiments on phytostabilization of tailings impoundments in the so-called Old Copper Mining Region in SW Poland, which remained barren for over 30 years, involved application of quite unusual amendments. Tailings, rich in calcium carbonate (40–50 %) and silicate clay, were amended with suitable waste materials available in the vicinity of the object, i.e. sandy overburden from a nearby quarry (aimed at loosening the structure of deposits) and strongly acidic phosphogypsum disposed on a nearby industrial dump, supplemented by initial fertilization with nitrogen, and then sown with appropriate mixtures of grass and legumes (Spiak and Gediga 2009). Similarly, phytostabilization of mine waste dumps and barren lands that have developed in various copper mining areas requires individual treatment, adapted to local, site-specific conditions (Vangronsveld et al. 1996; Alvarenga et al. 2008).

Phytostabilization is a basic, commonly applied in practice, and highly effective method of remediation and revitalization of large, often barren areas contaminated by emissions from copper smelters. Remediation of those soils, usually strongly acidified, involves first of all neutralization by liming or application of alkaline fly ash, combined with soil amendment with various materials, usually industrial by-products or wastes, intended to increase copper sorption and precipitation (such as red mud or iron-rich wastes) and to enrich soil in organic matter (mature compost, composted sewage sludge, etc.). Then, grass or mixtures of grass with legumes are sown to quickly create dense plant cover on the soil surface. Eventually, trees or shrubs may be planted, in accordance with site specifics and its functions. The methods of phytostabilization were applied as pioneering measures for reclamation of highly contaminated areas surrounding copper smelters in Sudbury, Canada (Winterhalder 1996) and several others in the United States, for example in the area of Anaconda, Montana (Redente and Richards 1997). Similar phytostabilization measures were also effective in revegetation of barren lands in the neighborhoods of the Polish copper smelters at Legnica and Głogów (in the 1980s and 1990s), the Harjavalta smelter in Finland (Kiikkilä 2003), smelters in Chile, for instance in the Puchuncavi valley (Goecke et al. 2011), as well as in Peru and Russia. An interesting kind of treatment involved in phytostabilization, tested on the forest soils in the vicinity of the Harjavalta smelter, was soil mulching with a mixture of compost and woodchips in order to convert copper into less toxic forms prior to planting the tree seedlings into pockets filled with mulch (Kiikkilä et al. 2001). The long-term effects of this treatment, however, are so far not known.

Lack of comprehensive information about the long-term effects of phytostabilization is often pointed out as one of the major shortcomings of this technique (Mench et al. 2006, 2010; Kumpiene et al. 2008). Soils subject to phytostabilization remain a kind of "time bomb" (Adriano 1986). The species in which Cu occurs in the reclaimed soil may undergo considerable changes in various geochemical processes, such as mineral weathering. Moreover, the changes in Cu speciation and potential mobility may also result from biochemical processes associated with improved soil biological activity. Oxidation of sulfides present in the tailings can lead to severe acidification, often on a scale that is difficult to predict on the basis of models (Mench et al. 2010). Most research on the remediation of soils contaminated with copper focuses on the fate of this particular element. At the same time, however, biogeochemical transformations may lead to changes in the availability or toxicity of other toxic components or nutrients. Easily soluble fractions of the organic matter released into soil by the decomposition of organic amendments, plant residues, or forest litter act as chelating compounds, causing secondary mobilization of Cu and other toxic components. The latter kind of risk applies particularly to copper, an element with known affinity to organic matter (Nowack et al. 2006; Karczewska and Milko 2010). In addition, well-developed plant root systems may act as a sort of drainage facilitating the processes of metal leaching from soil.

Another problem that may arise in apparently well-phytostabilized sites is the lack of micro-organisms and soil flora and fauna, normally crucial for transformation of organic matter and the supply of available nutrients. Several-year lasting laboratory experiments often confirmed the need for regular soil fertilization. Satisfactory results obtained in medium-term laboratory or field experiments do not guarantee that the same effects will occur in practice, under the impact of various unpredictable factors, such as weather conditions. The guidelines for the practical application of phytostabilization suggest that for ensuring better long-term results, multi-species mixtures of plants should be applied rather than monocultures.

The sites subject to phytostabilization should always be examined in long-term monitoring, focusing on possible changes of pollutant speciation, soil biological activity, and the dynamics of plant succession and plant quality, depending on the specific climatic and environmental conditions

(Mench et al. 2010). Regularly repeated assessment of environmental risk and the risk to human health should be carried out along with any changes in soil properties and evolving composition of phytocenosis.

### 12.3.2 Phytoextraction

#### 12.3.2.1 Plant Species That Accumulate or Hyperaccumulate Cu

Since the early 1990s, scientists around the world have been intensively working on application of selected hyperaccumulating species for phytoextraction of metals and semimetallic elements from contaminated soils (Brown et al. 1994; Brooks 1998; Chaney et al. 2005; Grudziński et al. 2000; Rascio and Navari-Izzo 2011). The phenomenon of hyperaccumulation may be explained as an effect of active uptake of metals from the soil. The term "hyperaccumulating species" has been precisely defined by plant physiologists, although the definition is based on arbitrarily set threshold values rather than on clearly specified physiological features. Plant species may be classified as hyperaccumulators if there has been at least one documented case of exceeded threshold concentrations of metal in the aerial parts of plants growing in their natural habitats. The minimum metal content (related to dry mass) for hyperaccumulation are: 1 % in the case of Ni, Zn, and Mn; 0.1 % in the case of Cu, Pb, Se, As, and Co; and 0.01 % for Cd (Brooks 1998). Over 400 plant species that take up metals in such large quantities have been identified (Blaylock and Huang 2000). Most of them are metallophytes, occurring endemically, known primarily from the areas enriched in heavy metals of either lithogenic, or sometimes anthropogenic origin. The species that hyperaccumulate nickel are primarily associated with serpentine soils. Some hyperaccumulators, such as *Alyssum* spp. (Ni), *Thlaspi caerulescens* (Zn and Cd), and *Pteris vittata* (As), are extensively tested for their suitability for cleaning up metal-polluted soils.

High concentrations of copper are highly toxic to plants and therefore most plants have developed an efficacious defense mechanism against toxicity, based mainly on the formation of complexes with citric acid, phytochelatins PC2 and PC3, and metallothioneins (Peer et al. 2005). Consequently, the majority of Cu-tolerant plant species demonstrate a strategy typical for excluders, while the mechanism of intensive accumulation of Cu by plants is very rare (Peer et al. 2005). The phenomenon of Cu hyperaccumulation was reported from southeastern Zaire (Katanga region, presently DR Congo). The group of plants identified as Cu (and Co) hyperaccumulators has nearly 40 species, including *Haumaniastrum katangense* (Lamiaceae) (Brooks 1998). The search for copper hyperaccumulating species undertaken elsewhere in the world, outside Katanga and Copperbelt, has generally been unsuccessful. Moreover, several recent studies have led to critical reexamination of the Cu/Co hyperaccumulator list (Faucon et al. 2007, 2009). The plants representative of species for which hyperaccumulation ability was confirmed in Katanga usually took up considerably smaller amounts of Cu when they grew outside that region (Paton and Brooks 1996). Moreover, some species classified as hyperaccumulators on the basis of Katangan samples will probably be deleted from the list, as their ability to take up extremely high amounts of Cu was not confirmed under controlled conditions, and the high concentrations in plant samples collected from the field were probably due to leaf surface contamination. Plant material thoroughly cleaned and deprived of mechanically adsorbed particles contained much lower concentrations of Cu than those reported in the literature. Only 9 % of the tested plants met the requirements for hyperaccumulation, i.e. Cu concentration above 1,000 mg/kg d.m. (Faucon et al. 2007, 2009). In China, several studies are currently being carried out focusing on locally occurring, Cu-tolerant species, considered to be hyperaccumulators, such as *Commelina communis* and *Elsholtzia splendens* (Wang et al. 2004, 2005). The latter, however, proved to be a tolerant excluder rather than accumulator, similarly to *Elsholtzia argyi*, *Silene vulgaris,* and *Mimulus guttatus* (Song et al. 2004; Peer et al. 2005).

Ineffective trials to identify Cu hyperaccumulators outside of Africa, and perhaps China, as well as failed attempts to introduce Katangan hyperaccumulating species to other habitat conditions, or to make them intensively take up Cu beyond their natural environments, put into question the concept of using hyperaccumulation for decontamination of Cu-polluted soils.

#### 12.3.2.2 High-Biomass Plants

Another idea of phytoextraction, alternative to hyperaccumulating plants, assumes that extremely high concentrations in plant shoots are not necessarily a prerequisite for efficient removal of metals from polluted soils. Some authors posit that a comparable effect of aggregate metal uptake from soil may be achieved with plants that contain medium concentrations of metals, but yield high biomass, such as willow, poplar, hemp, Virginia mallow, miscanthus, and many others (Terry and Banuelos 2000; Raskin and Ensley 2000). It can, however, be demonstrated that, in the case of most heavy metals beside Cd and Zn, the rates of their removal from heavily or moderately contaminated soils cannot guarantee successful decontamination within a reasonable timespan (Karczewska et al. 2009a, 2011). This concern applies particularly to copper, toward which most of the plants execute a strategy of avoidance (excluding). Copper concentrations in plant shoots usually remain below 20 mg/kg d.m. (Fernandes and Henriques 1991; Kabata-Pendias and Pendias 2001; Reichman 2002; Yruela 2005). However, considerably higher Cu concentrations in nonhyperaccumulating plants have also frequently been reported. The plants proposed for the high-biomass option of phytoextraction must be tolerant to high content of copper in soil and produce high biomass yields. The most frequently considered groups of plants that meet these requirements are certain species of trees and shrubs, as well as fast-growing annual crops that produce high biomass and may be further utilized, for example as biofuels (Terry and Banuelos 2000; Raskin and Ensley 2000). Among tree species, certain varieties and clones of poplar (*Populus* spp.) (Buczkowski et al. 2002; Komárek et al. 2007, 2008a) and willow (*Salix* spp.), particularly osier (*Salix viminalis*) (Pulford and Watson 2003; Dickinson and Pulford 2005; Klang-Westin and Perttu 2002; Jensen et al. 2009; Mocek et al. 2001), proved to be highly resistant to enhanced soil concentrations of heavy metals, including Cu. Annual biomass yield for these species, obtained from 3 to 4 year old plantations, usually exceeds 25–40 t d.m./ha. Different varieties and clones of willow and poplar demonstrate high diversity of resistance to soil metals and various abilities to translocate metals to the shoots. Particularly high capability of Cu accumulation was confirmed for *Salix nigra* (Kuzovkina et al. 2004). Among high-biomass agricultural crops, those particularly resistant to high metal concentrations in soils are: miscanthus (*Miscanthus giganteus*), cultivated as an energy plant; hemp (*Cannabis sativa*) and Virginia mallow (*Sida hermaphrodita*), which represent fiber plants; as well as maize (*Zea mays*) and sunflower (*Helianthus annus*). Extensive research has also been carried out on other crops, including cereals, with moderately high biomass, such as wheat (*Avena sativa*), barley (Hordeum vulgare), Indian mustard (*Brassica juncea*), and pea (*Pisum sativa*) (Salt et al. 1995; Ebbs and Kochian 1998; Wenger et al. 2002).

The deep rooting system of trees and most other high biomass plants allows take-up of metals, such as Cu, not only from the topsoil but also from deeper layers of contaminated soils. Unlike in the case of hyperaccumulators, for most of the trees and agricultural crops, the principal rules of their agrotechnics, as well as the conditions required for cultivation, are well known. An important advantage of the high-biomass option of phytoextraction is also the possibility of biomass utilization for energy purposes. A fundamental disadvantage is a low, definitely unsatisfactory aggregate uptake of metals from soils. It may be easily proved that the potential capacity of Cu phytoextraction by high biomass plants is much lower compared to that which theoretically might be obtained with the use of hyperaccumulators. Soil fertilization, applied to improve crop yields, can to a certain extent increase the amounts of plant-accumulated metals (Wu et al. 2004; Mleczek et al. 2013), but several studies have proved that due to the effect of metal "dilution" in larger biomass, the efficiency of phytoextraction, expressed as aggregate metal uptake from soil, remained at a basically unchanged level (Pulford and Watson 2003).

Relatively low efficiency of Cu phytoextraction from contaminated soils was confirmed in an experiment carried out with five clones of willow grown on soils contaminated by emissions from copper smelters, containing high concentrations of Cu in the range 300–1,060 mg/kg Cu, with a mean value of 650 mg/kg Cu (Mocek et al. 2001; Mocek 2012). A field experiment was established on soils developed from silty loam with slightly acidic or neutral pH ($pH_{KCl}$ 6.50–6.95), fairly rich in organic matter (with a mean organic C content in topsoil at the level of 16.8 g/kg). Plant material collected from different experimental plots contained unusually high copper concentrations. The mean value of Cu concentrations in dry mass of leaves was as high as 429 mg/kg, while the twigs contained considerably less Cu: 27.5 mg/kg d.m. on average. It was believed that such high concentrations of copper in willow leaves, together with a high yearly biomass yield (approximately 15–20 t d.m./ha shoots and 20 t d.m./ha leaves), would result in great amounts of Cu being withdrawn from soil with harvested willow shoots. The calculations showed that the mean aggregate yearly uptake of Cu with leaves was not higher than 8.5 kg/ha and that with twigs was assessed as 0.6 kg/ha. This means that the yearly removal of Cu with harvested willow shoots was at the level of about 9 kg Cu/ha, whereas the top layer (20 cm) of soil humus horizon in the experimental area contained over 2,000 kg/ha of accumulated Cu. Assuming that the leaves will be every year thoroughly collected and removed from the plantation (in fact, they fall onto the soil surface, forming thereby a secondary source of Cu in the topsoil), and provided that the uptake of Cu from soil remains constant over time (although it would probably decrease after using up the best available Cu forms), the period of time required to bring soil concentrations to an acceptable level turns out to be about 220 years. These results illustrate clearly that the method of Cu phytoextraction by willow cannot be applied for successful decontamination of soils considerably polluted by copper smelters.

It should be noted, however, that cultivation of high-biomass, Cu-tolerant plants on contaminated soils has unquestionable advantages, similar to those typical for phytostabilization. The plants that accumulate relatively high concentrations of Cu in their shoots, such as *Salix* spp., in fact do not meet phytostabilization requirements, as they extract considerable amounts of Cu from soils. Nevertheless, their cultivation has several positive aspects. The uptake of Cu from soil goes on continuously, in a "gentle" way, without any drastic changes in soil properties, and thus without considerable impacts on biological equilibrium and the stability of the ecosystem. As a matter of fact, growing plants take up only the most easily soluble forms of Cu, which otherwise could pose a risk to the ecosystem or might get leached from the soil. Although the efficiency of phytoextraction, understood as a decrease in soil Cu concentration, remains exceptionally low, the plants provide a sort of ecological safety, as they ensure continuing removal of the most dangerous, mobile Cu forms. Therefore, the cultivation of traditional crops for energy biomass should be considered as a good method for the management of Cu-contaminated sites. It cannot result, however, in any significant reduction of soil Cu concentrations.

#### 12.3.2.3 Enhanced Phytoextraction

Considerations on the potential use of phytoextraction as a method to clean up polluted soils lead to the concept of joining two approaches, i.e. high biomass plants should intensively accumulate heavy metals. This concept was a basis for developing the idea of enhanced phytoextraction. All the comprehensive knowledge on metal speciation and dynamics in soils, as well as on the mechanisms of metal phytoaccumulation and phytotoxicity, should be involved in the studies on considerably increasing the rate of metal uptake by nonhyperaccumulating plants. The effect of magnified metal accumulation by plants can be achieved using one of three basic mechanisms, or combinations of them:

- Genetically based accumulation, i.e. development of transgenic high biomass plants with the capability of hyperaccumulation and hypertolerance which may be achieved by cloning all the relevant genes and expressing them in high biomass yielding crops, or—less likely—by genetic modification of hyperaccumulating species to adapt them to new habitats and increase their biomass;
- Chemically assisted phytoextraction (induced hyperaccumulation, enhanced phytoextraction), based on increasing metal solubility in soil by application of chelating agents, which further stimulates the plants to intensively take up soluble metals from the soil;
- Biologically assisted phytoextraction, based on increasing soil solubility of metals caused by natural compounds of microbial origin released to soil by several kinds of bacteria.

#### 12.3.2.4 Genetic Modification

The first successful attempts to produce transgenic plants able to take up extraordinarily high amounts of metals from soil were undertaken by Meagher (2000), who developed transgenic plants expressing modified bacterial mercuric reductase (MerA), and organic-mercurial lyase (MerB) that allowed plants to phytovolatilize Hg from Hg-rich soil. Further trials did not result, however, in development of plants capable of accumulating high amounts of Hg in their shoots (Chaney et al. 2007). Nevertheless, the experience gained from development of genetically engineered Hg volatilizing plants, as an example of successful gene transfer in the field of phytoremediation-oriented studies, prompted further research focusing on phytoextraction of other metals, including Cu (Krämer and Chardonnens 2001; Papoyan and Kochian 2004; Cherian and Oliveira 2005; Eapen and D'Souza 2005; Kotrba et al. 2009). Considerably advanced studies are underway aimed at identifying and cloning the groups of genes responsible for metal tolerance, root-shoot transfer, and cellular accumulation. Examination of genes expressed in *Thlaspi caerulescens* and *Arabidopsis thaliana* provided valuable knowledge on the mechanisms of hyperaccumulation. Cloning the genes responsible for overproduction of the enzyme glutathione synthetase and its expression in Indian mustard (*Brassica juncea*) resulted in a significant increase of metal tolerance and accumulation of Cd, Zn, Cr, Cu, and Pb by this species (Zhu et al. 1999; Bennett et al. 2003). Despite unquestionable achievements, no reports have been published so far on development of a complete transgenic hyperaccumulator that is close to field testing or practical application. Additionally, several questions must be raised concerning biological consequences that might result from potential introduction of engineered hyperaccumulators into the environment.

#### 12.3.2.5 Chemically Assisted Phytoextraction

First, very promising results on so-called "induced hyperaccumulation" were published nearly 20 years ago. The technology was based on stimulation of intensive uptake of metals from soil after their solubilization caused by chelating agents. Extensive research work in this field, initiated by Huang and Cunningham (1996), was carried out in numerous research centers (Blaylock et al. 1997; Huang et al. 1997; Chen and Cutright 2001; Römkens et al. 2002; Sas-Nowosielska et al. 2008; Karczewska et al. 2011), mainly in pot experiments, and also in field trials on a semitechnical scale (Blaylock 2000). In their first experiments, Huang, Cunningham, and Blaylock examined Pb phytoextraction by Indian mustard and maize, induced by application of a synthetic chelating agent, EDTA (ethylene diamine tetraacetic acid). Not only did EDTA cause Pb mobilization into soil solution, but it also enabled leakage of Pb in the form of Pb-EDTA through the root membranes, followed by its passive transport to plant shoots powered by transpiration. Many other complexing agents, including low molecular organic acids and amino acids, as well as various plant species, were examined afterwards (Chen et al. 2003; Meers et al. 2005). The most frequently tested plants were high biomass crop plants with relatively high tolerance to heavy metals, in particular maize, sunflower, Indian mustard, rape, and turnip rape. The technology involves initial plant cultivation at optimal possible soil conditions to enable their satisfactory growth and obtain a sufficiently large biomass. This prerequisite might require immobilization of metals by soil liming and use of appropriate amendments. Application of chelating agents onto the soil surface takes place when the plants are large enough. Then, metals are rapidly solubilized and taken up by plants. Their unusually high concentrations in the shoots are highly toxic, and cause death of the plants shortly after the phytoextraction effect has been achieved.

The very first papers that reported the results of research on chemically assisted phytoextraction did not touch the issue of adverse environmental effects that may possibly be caused by introduction of chelating agents into the soil. Therefore, the method gained common acceptance and was recommended as an inexpensive and environmentally friendly way to clean up contaminated soils (Salt et al. 1995; Garbisu and Alkorta 2001; Chaney et al. 1997; Terry and Banuelos 2000; Chen and Cutright 2001; Mejare and Bulow 2001). The results of subsequent studies indicated, however, that it could not be applied in practice due to its several unforeseen drawbacks and unavoidable side-effects. EDTA and other synthetic aminopolycarboxylic acids are not metal-specific, and therefore their effects on solubility of the target metal depends strongly on interactions with other heavy metals such as Pb, Cu, or Zn as well as other cationic elements present in the soil at high concentrations, such as Ca or Fe (Grčman et al. 2003; Wu et al. 1999; Chandra Sekhar et al. 2005). No wonder that the first enthusiastic opinions on induced phytoextraction were followed by a number of definitely critical papers that pointed out its limited soil-, metal-, chelant-, and plant-related efficiency, but in the first place the unavoidable side-effects, which involve a long-term increase in the concentration of heavy metals in soil solution and the inevitable hazard of metal leaching into groundwater (Römkens et al. 2002; Madrid et al. 2003; Karczewska et al. 2009b, 2011). The assessment made by several authors indicated that the amounts of metals taken up by plants are far smaller than those to be leached into water. In most cases, the plant uptake was not higher than a few percent of the pool of metals solubilized due to chelation (Grčman et al. 2001; Wenzel et al. 2003; Evangelou et al. 2007; Karczewska et al. 2011).

All the drawbacks of enhanced extraction prompted further studies aimed at reducing its side-effects, particularly the long-term increase in the solubility of metals. Biodegradable chelating agents, such as EDDS (ethylenediamine-disuccinic acid), a natural amino-polycarboxylic acid, produced by many microorganisms (Nishikiori et al. 1984; Goodfellow et al. 1997), were tested in place of EDTA. Several studies (Karczewska et al. 2009b; Kos and Leštan 2003; Luo et al. 2005, 2007; Meers et al. 2005; Tandy et al. 2006; Quartacci et al. 2007) confirmed selective affinity of EDDS to copper. This fact, as well as EDDS biodegradability, opened new perspectives for reconsidering the method of induced phytoextraction for possible remediation of Cu-contaminated soils. It was found, however, that Cu uptake by plants (maize, Indian mustard) from sandy and loamy soils was satisfactory only with application of large EDDS doses (1 mM/kg or more). As a consequence, a major part of complexed Cu was susceptible to leaching, while only a small portion of Cu was accumulated by plants. Various attempts to reduce the effect of Cu leaching, made by several authors, were presented and discussed in detail in a review paper by Evangelou et al. (2007). Examined solutions included splitting the chelant dosage, its application in the form of slow-release granules, application of metal-absorbing permeable barriers, and several other quite sophisticated solutions, but they did not bring about a solution to the problem of metal leaching. Most of the authors of recently published review papers on chemically assisted phytoextraction expressed the opinion that it would not be reasonable to continue this approach (Evangelou et al. 2007). The main disadvantages of the method are very low efficiency of Cu phytoextraction and side-effects, particularly the inevitable hazard to groundwater arising when the chelating agents are applied at rates high enough to radically increase Cu uptake by plants. Many papers that appear nowadays, however, obstinately continue to emphasize mainly the positive aspects of this method (Ciura et al. 2005; Sun et al. 2011; Smolińska and Król 2012; Ali et al. 2013).

#### 12.3.2.6 Biologically Supported Phytoextraction

Although numerous previous attempts to apply phytoextraction for effective removal of copper from soils did not bring satisfactory results, research focusing on improvement of phytoextraction efficiency is still being continued. Phytoextraction seems to be the only reasonable solution to the problem of soil pollution in vineyards, where in fact no other methods can be applied (Pietrzak and Uren 2011; Mackie et al. 2012).

Interesting research works have been recently carried out on a relatively new approach oriented at microbially assisted phytoextraction. Various groups of microorganisms may contribute to mobilization of metals, such as Cu, from soil, via complexation by either their metabolites or siderophores, or as a result of methylation (Gadd 2004). Siderophore producing bacteria are used in practice to increase Fe and Cu bioavailability in poor, sandy soils. A similar effect can be expected in Cu contaminated soils (Wenzel 2009; Rajkumar et al. 2010; Kidd et al. 2009; Gadd 2004). Factually, Andreazza et al. (2010) demonstrated in laboratory experiments that Cu-resistant bacteria *Pseudomonas putida* A1, A2 *S. maltophilia* and *Acinetobacter calcoaceticus* A6 increased efficiency of Cu phytoextraction from contaminated vineyard soil. Several other experiments confirmed the new perspectives for microbially-assisted phytoextraction (Lebeau et al. 2008; Weyens et al. 2009). At the moment, these methods clearly need further, long-term studies that will allow for comprehensive assessment of their short- and long-term effects. Another direction of research that may have application in the field of phytoextraction is examining the effects of PGPR on the yields of Cu accumulating plants, which can result in increasing amounts of copper to be taken up from soils by those plants (Kidd et al. 2009; Ma et al. 2009).

## 12.4 Prospects for Practical Applications of Hyperaccumulators

The potential use for hyperaccumulators, estimated at about 450 plant species (Miransari 2011), especially in phytoremediation of areas highly polluted with trace elements, is one of the most important chances to shorten the generally long duration of this process. Many plant hyperaccumulators are known to be able to accumulate some trace elements in significantly greater amounts than other plants (Brooks et al. 1977; Poschenrieder et al. 2006), even when considering enhanced trace element phytoextraction (Bhargava et al. 2012). In relation to the tested element present in soil and its concentration, accumulation of hyperaccumulators is different. Hyperaccumulators of Cd are able to take up >100 mg/kg d.w. (Baker et al. 1994; Reeves and Baker 2000; Wei et al. 2005), while hyperaccumulators of As, Co, Cu, and Ni (the most numerous group of known hyperaccumulators) or Pb are able to take up >1,000 mg/kg d.w. (Reeves and Baker 2000; Ma et al. 2001; Srivastava et al. 2006; Tappero et al. 2007). To date the highest accumulation has been described for Mn and Zn, where concentration of these elements was above 10,000 mg/kg d.w. (Reeves and Baker 2000; Yang et al. 2008). Hyperaccumulation in plant organs is not limited only to the above-mentioned elements, because we know plants able to accumulate significant amounts of, for example, Al (Jansen et al. 2002), B (Babaoğlu et al. 2004), Cr (Zhang et al. 2007) or Fe (Rodríguez et al. 2005).

The use of hyperaccumulators or other plants with specific strategies to survive and the effective element uptake in Cu-polluted areas have been presented in numerous studies (Boojar and Goodarzi 2007; Jiang et al. 2004; Lamb et al. 2012). Cu is an element of great importance in today's industry, but this element is present in many soils in significant amounts (especially in industrial soil). It is particularly important to remove Cu from the environment or to retrieve this element in the pure form. It stands to reason that the use of biological methods or biological methods in combination with engineering methods is necessary in practical applications (Koppolu and Clements 2003; Reijnders 2003). Hyperaccumulators have some significant traits distinguishing them from nonhyperaccumulators such as rapid accumulation of trace elements, a significant ability to detoxify elements in plant organs (especially in leaves), easy adaptation to new environmental conditions and fast translocation of elements to aerial parts of plants (Martens and Boyd 2002; Ghaderian and Ghotbi Ravandi 2010; Rascio and Navari-Izzo 2011). These traits are caused by different regulation and expression of genes; therefore we are not able to observe the toxic influence of high concentrations of trace elements on plant morphology. Additionally, the significant advantage of hyperaccumulators is connected with the possession of genes encoding transmembrane transporters (ZIP, YSL, or MTP). Some hypotheses have been proposed on the causes of hyperaccumulation in plants (Miransari 2011; Boyd 2012), but in practice the most important is the last observed result.

For now we know some hyperaccumulators of Cu, tested in different environmental and artificial conditions, and some plant species with significant potential for Cu accumulation from soil. The most interesting plants include *Lecanora polytropa* (Purvis et al. 2008), *Polycarpaea longiflora*, *Hyptis capitata,* and *Nicotiana tabacum* (Nedelkoska and Doran 2000), *Elsholtzia haichowensis* (Lou et al. 2004), *Crassula helmsii* (Küpper et al. 2009), *Helianthus annuus* L. and *Kalanchoe serrata* L. (Wilson-Corral et al. 2011) as well as the moss *Scopelophila cataractae* (Nakajima et al. 2011), which have very many unique genetic and biochemical properties. The use of different hyperaccumulating plant species in decontamination of Cu-polluted areas is considerably limited by the generally too low biomass of these plants (Miransari 2011), but in our opinion when we are able to identify hyperaccumulating genes and make use of them in relation to high biomass-producing plants, this situation will change. Phytoextraction is a cost-effective method to decontaminate Cu-polluted areas; therefore an increase of biomass with simultaneous high efficiency for the accumulation of one or more elements can be the starting point for the practical application of not only hyperaccumulators, but also nonhyperaccumulators. Additionally, high concentrations of trace elements in modified plants with "hyperaccumulation" genes can be interesting and a valuable substrate in phytomining (Brooks and Robinson 1998). Within the last 20 years, the concept of combining two traits—high biomass and high trace element (also Cu) accumulation capacity—in one plant has been the subject of many studies (Kumar et al. 1995; Volk et al. 2006; Šyc et al. 2012). Brooks et al. (1998a) described phytomining as a useful 'green' alternative method and proposed a model for a possible economic phytomining system. This method has a great potential for a wide range of trace elements (especially Cu, Ni, and Zn) and it is quite simple to perform in some stages. In the case of soil polluted with copper the first stage is the choice and cultivation of a specific hyperaccumulator or another plant with a highly efficient accumulation rate of this element. When plants grow, they have to be harvested and the material may be (a) burnt for energy, (b) smelt bio-ore for metal recovery. After these two procedures, the capital return is facilitated. According to Brooks et al. (1998a), these stages can be repeated with or without fertilizers and/or chelates to increase biomass and elemental uptake by plants, respectively, if the soil metal concentration is high enough for other economic crops. If not, the last question is whether the ore body is exhausted. If it is, the phytomining process is completed; when it is not, the addition of new (fresh) soil is necessary to replace the destroyed topsoil, which has to be removed for the next crop. Generally, as regards economic aspects of applied biological methods, the use of each plant (hyperaccumulators/nonhyperaccumulators/modified plants) with high phytoextraction abilities, high resistance to new environmental conditions and high biomass production in phytomining could be the future of environment decontamination. The use of hyperaccumulators in practice is usually difficult due to the limited size of plants and problem of their harvesting. As regards this conclusion and the time of phytomining, this method is usually used less frequently than biomining (Gałuszka 2005).

Nevertheless, the use of selected plant taxa in phytomining facilitates decontamination of mine tailings, sewage sludge, and soils. The first concept of practical hyperaccumulator use was presented by Chaney in 1983 and Baker and Brooks in 1989 (Anderson et al. 1999). At the beginning of the twenty-first century we have identified 24 Cu hyperaccumulators (for natural and induced hyperaccumulation) and only some of them have been analyzed as potential plants for use in phytomining. At this moment we know about 34 Cu hyperaccumulators (Bhargava et al. 2012) and the main attention is focused on combined phytoremediation and phytomining as regards the costs. The use of plants with high biomass is the cause of higher profits in phytomining (biomass burning), but only when phytoextraction of metals is high enough. On the other hand, the higher biomass is the cause of higher material transport costs. For this reason a significant aspect is connected with the economic analysis prior to decontamination of areas polluted with Cu or other metals. Brooks and Robinson (1998b) presented the elemental contents that would be required in used plants with fertilized biomasses for the phytomining process to become profitable. It is relatively obvious that a decrease of biomass or trace element(s) phytoextraction efficiency reduces the potential use of many plants (including selected hyperaccumulators). It should be stressed here that phytomining, while being an interesting method as regards recruitment energy and expensive in metals industry, is fast and relatively cheap in comparison to other methods.

## 12.5 Estimation of Real Time Necessary in Phytoextraction Practice

The presence of copper in soils worldwide varies as regards anthropogenic activity and the concentration of this metal in some mineral forms such as chalcopyrite ($CuFeS_2$), chalcocite ($Cu_2S$), bornite ($Cu_5FeS_4$), azurite ($Cu_3(CO_3)_2(OH)_2$), and also malachite ($Cu_2CO_3(OH)_2$). Regardless of the type of polluted soil, concentrations of copper and other trace elements, cation exchange capacity, differences in water availability, pH, Eh, organic substances, or plant taxa used in phytoextraction, a significant aspect, although ignored in many scientific works, is connected with the determination of tentative phytoextraction duration. An example is provided in a study by Van Nevel et al. (2007), where a simple, but highly useful and reliable method for necessary phytoextraction time estimation was presented. Those authors pointed to real drawbacks of phytoextraction, hindering the practical use of this method, or that cause prolonged duration of this process. As regards the usually small root system, phytoextraction is limited only to the decontamination of topsoil, with the efficiency of this process depending not only on the used plant species/varieties, but to a greater degree on the concentration and form of metals available for plants. Another problem is connected with the small biomass increase of effective accumulation of trace elements by plants. Based on the above-mentioned limitations, it needs to be stressed that the attitude of Van Nevel et al. (2007) and Ernst (2005) about the more appropriate use of phytostabilization than phytoextraction for decontamination of soils polluted by significant amounts of trace elements, especially in deeper layers of the soil profile, is correct (Prasad 2003). Thanks to the theoretical calculations, Van Nevel et al. (2007) and also Sheoran et al. (2011) presented two significant values: "A," defined as the amount of metal to be removed per hectare (mg/ha), and "*t*," described as phytoextraction time (yr). Literature sources provided some data concerning the calculation of phytoextraction time, presented also by Van Nevel et al. (2007).

Generally, the use of hyperaccumulating plant species is connected with phytoextraction time ranging from 2 to 60 years, whereas nonhyperaccumulating plants require 25–2,800 years. These differences both between two plant groups and between plants inside each group result from differences in plants' potential for trace element accumulation, biomass production and also amounts of available metals in soil. Taking into consideration the diverse plant needs, their survivability and resistance and also adaptability to new environmental conditions, each analysis of metal accumulation by tested plant species/varieties should be completed by determination of the time required to reduce metal concentrations to levels indicated by environmental law regulations.

Efficiency of phytoextraction (the selection of specific plant taxa) and biomass are usually considered as major factors for this process duration; however, modification of plant growth conditions also plays a significant role. Based on the model by Van Nevel et al. (2007) and according to our own calculations, it may be stated that modification of plant biomass or element accumulation rates is only sufficient for the reduction of phytoextraction time—but how significantly? The phytoextraction process time can be shortened by addition to soil of selected microorganisms, fungi (Göhre and Paszkowski 2006) or chelators (Eapen and D'Souza 2005). Generally, a greater effect is observed for accumulation rate than plant biomass modification. For example, decreasing Cu concentration in polluted soil (about 82 mg/kg d.w.) to a normal geochemical level by the most effective *Salix* taxa as one of many energy plants (Labrecque et al. 1997; Stolarski et al. 2008) requires about 1,200–2,400 years. Modification of biomass in these *Salix* taxa makes it possible to shorten this time to 530–790 years. On the other hand, genetic modification of plants raises considerable controversies related to improvement of accumulation and biomass stimulation facilitating the same phytoextraction efficiency within 21–35 years (the author's studies). Based on the short time for particular plants as short rotation coppice (SRC), this time may be considered acceptable. The same situation is observed for other elements, such as Zn. The particular phytoextraction time for the most interesting *Salix* taxa growing in soil with about 22 mg/kg d.w. is 92 (no modification), 34 (biomass modification), and as little as 2 years (genetic modification). Taking into consideration the previous information about phytomining technology, these plants could be a significant source of metals for industry.

## 12.6 Conclusions

The extensive research on phytoextraction carried out in recent decades has not brought any breakthrough or innovative developments that might be applied for Cu-contaminated soils. Unlike zinc and cadmium, copper usually demonstrates poor phytoavailability and therefore its uptake by most plant species is very low. The trials to make phytoaccumulation of Cu more intensive were in fact unsuccessful. The methods of chemically aided phytoextraction should be considered ineffective and hazardous to the environment; hence, a further development of this approach does not seem to be reasonable. Microbiologically supported phytoextraction still remains in its infancy and requires comprehensive examination. The results obtained so far, however, do not create grounds to expect a multiplication, or at least a radical improvement, of phytoextraction rates. Genetic engineering, although encouraging and scientifically sound, has not yielded any promising Cu-related outcomes. Moreover, there are several concerns about the idea of aided phytoextraction itself. Presuming that the plants capable of accumulating large Cu concentrations in their aerial parts have been successfully engineered or stimulated, the sites where they will grow should be well protected to prevent uncontrolled input of copper into the food chain, for example via animals, and its secondary dispersion in the environment. Theoretical considerations proved that in the vicinities of copper smelters the risk to human health caused by possible Cu ingestion may be feasible (Seeley et al. 2013). This seems to be an additional contribution to the thesis that from among several phytoremediation methods that might be applied for Cu-contaminated soil, phytostabilization will be much better justified and—paradoxically—environmentally safer than various options of phytoextraction.

The above chapter can be concluded as follows:

- Concentrations of Cu in European topsoils vary in a broad range.
- Excessive levels of copper concentration in soil (dangerous for humans, animals, and plants) need to be reduced.
- Soil cleanup of pollutant presence is technologically advanced but also expensive.
- Bioleaching involves different groups of bacteria.
- Phytoremediation represents the technologies that use either naturally occurring or genetically engineered plants to remove contaminants from soils.
- Phytostabilization is a kind of chemical fixation of trace elements by sequestration of natural products by plants.
- Hyperaccumulation of copper is an effect of active uptake of metals from the soil—due to defense mechanism against toxicity—based mainly on the formation of complexes with chelating molecules.
- Remediation of polluted soil is time consuming and in hyperaccumulating plants takes 2–60 years while in non-hyperaccumulating plants it takes 25–2,800 years.

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## References

Abramovitch RA, Lu CQ, Hicks E, Sinard J (2003) In situ remediation of soils contaminated with toxic metal ions using microwave energy. Chemosphere 53:1077–1085. doi:10.1016/S0045-6535(03)00572-1

Abreu MM, Matias MJ, Clara M, Magalhães F, Basto MJ (2008) Impacts on water, soil and plants from the abandoned Miguel Vacas copper mine, Portugal. J Geochem Explor 96:161–170. doi:10.1016/j.gexplo.2007.04.012

Adriano DC (1986) Trace elements in the terrestrial environment. Springer, Berlin, 533

Ajmone-Marsan F, Biasioli M (2010) Trace elements in soils of urban areas. Water Air Soil Pollut 213:121–143. doi:10.1007/s11270-010-0372-6

Alexandrovskaya EI, Alexandrovskiy AL (2000) History of the cultural layer in Moscow and accumulation of anthropogenic substances in it. Catena 41:249–259. doi:10.1016/S0341-8162(00)00107-7

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Review. Chemosphere 91:869–881. doi:10.1016/j.chemosphere.2013.01.075

Alloway BJ (2004) Contamination of soils in domestic gardens and allotments: a brief overview. Land Contam Reclam 12:179–187

Alloway BJ (ed) (2013) Heavy metals in soils: trace metals and metalloids, and their bioavailability, vol 22, Environmental pollution series. Springer, Dordrecht, The Netherlands

Alvarenga P, Gonçalves AP, Fernandes RM, de Varenne A, Vallini G, Duarte E, Cunha-Queda G (2008) Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Sci Total Environ 406:43–56. doi:10.1016/j.scitotenv.2008.07.061

Anderson CWN, Brooks RR, Chiarucci A, LaCoste CJ, Leblanc M, Robinson BH, Simcock R, Stewart RB (1999) Phytomining for nickel, thallium and gold. J Geochem Explor 67:407–415. doi:10.1016/S0375-6742(99)00055-2

Andreazza R, Okeke BC, Lambais MR, Bortolon L, de Melo GWB, de Oliveira Camargo FA (2010) Bacterial stimulation of copper phytoaccumulation by bioaugmentation with rhizosphere bacteria. Chemosphere 81:1149–1154. doi:10.1016/j.chemosphere.2010.09.047

Arwidsson Z, Elgh-Dalgren K, von Kronhelm T, Sjöberg R, Allard B, van Hees P (2010) Remediation of heavy metal contaminated soil washing residues with amino polycarboxylic acids. J Hazard Mater 173:697–704. doi:10.1016/j.jhazmat.2009.08.141

Babaoğlu M, Gezgin S, Topal A, Sade B, Dural H (2004) *Gypsophila sphaerocephala* Fenzl ex Tchihat. A boron hyperaccumulator plant species that may phytoremediate soils with toxic B levels. Turk J Bot 28:273–278. doi:10.1590/S0103-90162010000600014

Bade R, Oh S, Shin WS (2012) Assessment of metal bioavailability in smelter-contaminated soil before and after lime amendment. Ecotoxicol Environ Saf 80:299–307. doi:10.1016/j.ecoenv.2012.03.019

Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recycl 11: 41–49. doi:10.1016/0921-3449(94)90077-9

Barcan V (2002) Leaching of nickel and copper from soil contaminated by metallurgical dust. Environ Int 28:63–68. doi:10.1016/S0160-4120(02)00005-3

Barcan V, Kovnatsky E (1998) Soil surface geochemical anomaly around the copper-nickel metallurgical smelter. Water Air Soil Pollut 103:197–218. doi:10.1023/A:1004930316648

Bech J, Poschenrieder C, Llugany M, Barceló J, Tume P, Tobias FJ, Barranzuela JL, Vásquez ER (1997) Arsenic and heavy metal contamination of soil and vegetation around a copper mine in Northern Peru. Sci Total Environ 203:83–91. doi:10.1016/S0048-9697(97)00136-8

Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EA (2003) Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. J Environ Qual 32:432–440. doi:10.2134/jeq2003.4320

Berti WR, Cunningham SD (2000) Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 71–88

Bes C, Mench M (2008) Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilisation. Environ Pollut 156:1128–1139. doi:10.1016/j.envpol.2008.04.006

Besnard E, Chenu C, Robert M (2001) Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soils. Environ Pollut 112:329–337. doi:10.1016/S0269-7491(00)00151-2

Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120. doi:10.1016/j.jenvman.2012.04.002

Biasioli M, Barberis R, Ajmone-Marsan F (2006) The influence of a large city on some soil properties and metals content. Sci Total Environ 356:154–164. doi:10.1016/j.scitotenv.2005.04.033

Birke M, Rauch U (2000) Urban geochemistry: investigations in the Berlin metropolitan area. Environ Geochem Health 22:223–248

Blacksmith Institute (2008a) The world's worst polluted places. Top polluted places 2007. In: Top ten list of the world's most dangerous pollution problems. Blacksmith Institute, New York

Blacksmith Institute (2008b) Top 10. The world's worst pollution problems. Blacksmith Institute, New York

Blaylock MJ (2000) Field demonstrations of phytoremediation of lead-contaminated soils. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, FL, pp 1–12

Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 53–70

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865. doi:10.1021/es960552a

Boojar MMA, Goodarzi F (2007) The copper tolerance strategies and the role of antioxidative enzymes in three plant species grown on copper mine. Chemosphere 67:2138–2147. doi:10.1016/j.chemosphere.2006.12.071

Boyd RS (2012) Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. Plant Sci 195:88–95. doi:10.1016/j.plantsci.2012.06.012

Brierley CL (2008) How will biomining be applied in future? Trans Nonferrous Met Soc China 18:1302–1310. doi:10.1016/S1003-6326(09)60002-9

Brooks RR (ed) (1998) Plants that hyperaccumulate heavy metals. CAB Internat. London

Brooks RR, Robinson BH (1998) The potential use of hyperaccumulators and other plants for phytomining. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York

Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–77. doi:10.1016/0375-6742(77)90074-7

Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. Trends Plant Sci 3:359–362. doi:10.1016/S1360-1385(98)01283-7

Brown SL, Chaney RL, Angle JS, Baker AJM (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soil. J Environ Qual 23:1151–1157

Brun LA, Maillet J, Hinsinger P, Pépin M (2001) Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environ Pollut 111:293–302. doi:10.1016/S0269-7491(00)00067-1

Brun LA, Le Corff J, Maillet J (2003) Effects of elevated soil copper on phenology, growth and reproduction of five ruderal plant species. Environ Pollut 122:361–368. doi:10.1016/S0269-7491(02)00312-3

Buchireddy PR, Bricka RM, Gent DB (2009) Electrokinetic remediation of wood preservative contaminated soil containing copper, chromium, and arsenic. J Hazard Mater 162:490–497. doi:10.1016/j.jhazmat.2008.05.092

Buczkowski R, Kondzielski I, Szymański T (2002) Remediation of soils polluted with heavy metals. (Metody remediacji gleb zanieczyszczonych metalami ciężkimi). UMK Toruń, 95. (in Polish)

Burgstaller W, Schinner F (1993) Leaching of metals with fungi. J Biotechnol 27:91–116

Burt R, Wilson MA, Keck TJ, Dougherty BD, Strom DE, Lindahl JA (2003) Trace element speciation in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana, USA. Adv Environ Res 8:51–67. doi:10.1016/S1093-0191(02)00140-5

Carlon C (ed) (2007) Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonization. European Commission, JRC, Ispra, 306 pp

Casali CA, Moterle DF, Rheinheimer DS, Brunetto G, Corcini ALM, Kaminski J (2008) Copper forms and desorption in soils under grapevine in the Serra Gaúcha of Rio Grande do Sul. R Bras Ci Solo 32:1479–1487

Chandra Sekhar K, Kamala CT, Chary NS, Balaram V, Garcia G (2005) Potential of *Hemidesmus indicus* for the phytoextraction of lead from industrially contaminated soils. Chemosphere 58:507–514. doi:10.1016/j.chemosphere.2004.09.022

Chaney RL, Malik M, Li YM, Brown SL, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8: 279–284

Chaney RL, Angle JS, McIntosh MS, Reeves RD, Li YM, Brewer EP, Chen KY, Roseberg RJ, Perner H, Synkowski EC, Broadhurst CL, Wang S, Baker AJ (2005) Using hyperaccumulator plants to phytoextract soil Ni and Cd. Review. Z Naturforsch C 60:190–198

Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields

commercial phytoextraction and phytomining technologies. Review. J Environ Qual 36:1429–1443. doi:10.2134/jeq2006.0514
Chen H, Cutright T (2001) EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45:21–28. doi:10.1016/S0045-6535(01)00031-5
Chen YX, Lin Q, Luo YM, He YF, Zhen SJ, Yu YL, Wong MH (2003) The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere 50:807–811. doi:10.1016/S0045-6535(02)00223-0
Chen CL, Lo SL, Kuan WH, Hsieh CH (2005) Stabilization of Cu in acid-extracted industrial sludge using a microwave process. J Hazard Mater 123:256–261. doi:10.1016/j.jhazmat.2005.04.014
Cherian S, Oliveira MM (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390. doi:10.1021/es051134l
Chopin EIB, Alloway BJ (2007) Distribution and mobility of trace elements in soils and vegetation around the mining and smelting areas of Tharsis, Ríotinto and Huelva, Iberian Pyrite Belt, SW Spain. Water Air Soil Pollut 182:245–261. doi:10.1007/s11270-007-9336-x
Chung HI (2009) Field applications on electrokinetic reactive pile technology for removal of Cu from in situ and excavated soils. Sci Technol 44:2341–2353. doi:10.5772/54334
Ciccu R, Ghiani M, Serci A, Fadda S, Peretti R, Zucca A (2003) Heavy metal immobilization in the mining-contaminated soils using various industrial wastes. Miner Eng 16:187–192. doi:10.1016/S0892-6875(03)00003-7
Ciura J, Poniedziałek M, Sękara A, Jędrszczyk E (2005) The possibility of using crops as metal phytoremediants. Polish J Environ Stud 14:17–22
Cotter-Howells JD, Caporn S (1996) Remediation of contaminated land by formation of heavy metal phosphates. Appl Geochem 11:335–342. doi:10.1016/0883-2927(95)00042-9
Crecelius EA, Johnson CJ, Hofer GC (1974) Contamination of soils near a copper smelter by arsenic, antimony and lead. Water Air Soil Pollut 3:337–342. doi:10.1007/BF00226464
Cunningham SD, Ow DW (1996) Promises and prospects for phytoremediation. Plant Physiol 110:715–719
Cuske M, Gersztyn L, Karczewska A (2013) The influence of pH on solubility of copper in soils contaminated by copper industry. Civil and Environmental Engineering Reports 11:31–39
De Gregori G, Lobos S, Pinochet H, Potin-Gautier M, Astruc M (2000) Comparative study of copper and selenium pollution in agricultural ecosystems from Valparaiso Region, Chile. Environ Technol 21:307–316. doi:10.1080/09593332108618112
Deng X, Chai L, Yang Z, Tang C, Tong H, Yuan P (2012) Bioleaching of heavy metals from a contaminated soil using indigenous *Penicillium chrysogenum* strain F1. J Hazard Mater 233–234:25–32. doi:10.1016/j.jhazmat.2012.06.054
Dermont G, Bergeron M, Mercier G, Richer-Lafleche M (2008) Soil washing for metal removal: a review of physical/chemical technologies and field applications. J Hazard Mater 152:1–31. doi:10.1016/j.jhazmat.2007.10.043
Dermont G, Bergeron M, Richer-Laflèche M, Mercie G (2010) Remediation of metal-contaminated urban soil using flotation technique. Sci Total Environ 408:1199–1211. doi:10.1016/j.scitotenv.2009.11.036
Derome J (2000) Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilisation. Environ Pollut 107:79–88. doi:10.1016/S0269-7491(99)00132-3
Derome J, Lindroos AJ (1998) Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni smelter, SW Finland. Environ Pollut 99:225–232. doi:10.1016/S0269-7491(97)00185-1
Diatta JB, Grzebisz W, Apolinarska K (2003) A study of soil pollution by heavy metals in the city of Poznań (Poland) using dandelion (*Taraxacum officinale* web) as a bioindicator. EJPAU, Environmental Development 6
Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short-rotation coppice *Salix:* the evidence trail. Environ Int 31:609–613. doi:10.1016/j.envint.2004.10.013
Dradrach A, Szopka K, Karczewska A, Bogacz A (2012) Diversity of total copper content in the vegetation layer of urban lawns in Wrocław. Zeszyty Naukowe UP we Wrocławiu, 588, Rolnictwo CII, pp 35–42 (in Polish)
Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23:97–114. doi:10.1016/j.biotechadv.2004.10.001
Ebbs SD, Kochian LV (1998) Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*). Environ Sci Technol 32:802–806. doi:10.1021/es970698p
Ek AS, Löfgren S, Bergholm J, Qvarfort U (2001) Environmental effects of one thousand years of copper production at Falun, central Sweden. Ambio 30:96–103. doi:10.1023/A:1011112020621
Ensley BD (2000) Rationale for use of phytoremediation. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 3–12
Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde-Geochem 65:29–42. doi:10.1016/j.chemer.2005.06.001
EU (2006) European Commission. Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC. Directive (COM (2006) 232)
Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68:989–1003. doi:10.1016/j.chemosphere.2007.01.062
Faucon MP, Ngoy Shutcha M, Meerts P (2007) Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil. Plant Soil 301:29–36. doi:10.1007/s11104-007-9405-3
Faucon MP, Colinet G, Mahy G, Ngongo Luhembwe M, Verbruggen N, Meerts P (2009) Soil influence on Cu and Co uptake and plant size in the cuprophytes *Crepidorhopalon perennis* and *C. tenuis* (Scrophulariaceae) in SC Africa. Plant Soil 317:201–212. doi:10.1007/s11104-008-9801-3
Fernandes JC, Henriques FS (1991) Biochemical, physiological, and structural effects of excess copper in plants. Bot Rev 57:246–273. doi:10.1007/BF02858564
Fernández-Calviño D, Rodríguez-Suárez JA, López-Periago E, Arias-Estévez M, Simal-Gándara J (2008a) Copper content of soils and river sediments in a winegrowing area, and its distribution among soil or sediment components. Geoderma 145:91–97. doi:10.1016/j.geoderma.2008.02.011
Fernández-Calviño D, Pateiro-Moure M, López-Periago E, Arias-Estévez M, Nóvoa-Muñoz JC (2008b) Copper distribution and acid-base mobilization in vineyard soils and sediments from Galicia (NW Spain). Eur J Soil Sci 59:315–326
Fischer K, Rainer C, Bieniek D, Kettrup A (1992) Desorption of heavy metals from typical soil components (clay, peat) with glycine. Int J Environ Anal Chem 46:53–62. doi:10.1080/03067319208026996
FOREGS (2005) Geochemical atlas of Europe. In: Salminen R (ed) Part 1. Background information, methodology and maps, EuroGeoSurveys. Geological Survey of Finland, Espoo
Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119. doi:10.1016/j.geoderma.2004.01.002
Gałuszka A (2005) The use of microorganisms and plants in metal extracting. Prz Geol 53:858–862

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236. doi:10.1016/S0960-8524(00)00108-5

Ghaderian SM, Ghotbi Ravandi AA (2010) Accumulation of copper and other heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. J Geochem Explor 123:25–32. doi:10.1016/j.gexplo.2012.06.022

Ginocchio R (2000) Effects of a copper smelter on a grassland community in the Puchuncaví Valley, Chile. Chemosphere 41:15–23. doi:10.1016/S0045-6535(99)00385-9

Goecke P, Ginocchio R, Mench M, Neaman A (2011) Amendments promote the development of *Lolium perenne* in soils affected by historical copper smelting operations. Int J Phytoremediation 13:552–566. doi:10.1080/1522652010.495150

Göhre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122. doi:10.1007/s00425-006-0225-0

Goodfellow M, Brown AB, Cai JP, Chun JS, Collins MD (1997) Amycolatopsis japonicum sp. nov, an actinomycete producing (S,S)- N,N-ethylenediaminedisuccinic acid. Syst Appl Microbiol 20:78–84. doi:10.1016/S0723-2020(97)80051-3

Grandlic CJ, Mendez MO, Chorover J, Machado B, Maier RM (2008) Plant growth-promoting bacteria for phytostabilization of mine tailings. Environ Sci Technol 42:2079–2084. doi:10.1021/es072013j

Gray CW, Dunham SJ, Dennis PG, Zhao FJ, McGrath SP (2006) Field evaluation of in situ remediation of a heavy metal contaminated soil using lime and red-mud. Environ Pollut 142:530–539. doi:10.1016/j.envpol.2005.10.017

Grčman H, Velikonja-Bolta Š, Vodnik D, Kos B, Leštan D (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil 235:105–114

Grčman H, Vodnik D, Velikonja-Bolta S, Leštan D (2003) Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. J Environ Qual 32:500–506

Greinert A (1995) Wpływ dodatku iłów do gleby piaskowej na plonowanie roślin oraz sorpcję i przyswajalność Pb, Cd i Zn. Zesz Probl Post Nauk Roln 418b:685–690 (in Polish)

Grudziński W, Matuła M, Sielewiesiuk J, Kernen P (2000) Fitoremediacja—sposób oczyszczania środowiska. Kosmos 49:135–147 (in Polish)

Grzebisz W, Cieśla L, Komisarek J, Potarzycki J (2002) Geochemical assessment of heavy metals pollution of urban soils. Pol J Environ Stud 11:493–499

Gworek B (1993) Wpływ zeolitów na zmniejszenie akumulacji metali ciężkich w roślinach uprawianych na glebach zanieczyszczonych, Rozprawy i Monografie. SGGW, Warszawa, p 167 (in Polish)

Hong S, Candelone JP, Patterson CC, Boruto CF (1996) History of ancient copper smelting pollution during Roman and medieval times recorded in Greenland ice. Science 272:246–248. doi:10.1126/science.272.5259.246

Hu N, Li Z, Huang P, Tao C (2006) Distribution and mobility of metals in agricultural soils near a copper smelter in South China. Environ Geochem Health 28:19–26. doi:10.1007/s10653-005-9007-z

Huang JW, Chen J, Berti WB, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environ Sci Technol 31:800–805. doi:10.1021/es9604828

Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134(1):75–84

Hursthouse A, Tognarelli D, Tucker P, Marsan FA, Martini C, Madrid L, Madrid F, Diaz-Barrientos E (2004) Metal content of surface soils in parks and allotments from three European cities: initial pilot study results. Land Contam Reclam 12:189–196. doi:10.2462/09670513.644

Hutchinson TC, Whitby LM (1977) The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the Sudbury smelting region of Canada. Water Air Soil Pollut 7:421–438. doi:10.1007/BF00285542

ICSG (2013) International copper study group: the world copper factbook 2013. ICSG, Lisbon, Portugal

Ikenaka Y, Nakayama SMM, Muzandu K, Choongo K, Teraoka H, Mizuno N, Ishizuka M (2010) Heavy metal contamination of soil and sediment in Zambia. Afr J Environ Sci Technol 4:729–739

InfoMine (2014) InfoMine's Mining Company & Property Database 2014. www.infomine.com

Jacobson AR, Dousset S, Guichard N, Baveye PC, Andreux F (2005) Diuron mobility through vineyard soils contaminated with copper. Environ Pollut 138:250–259. doi:10.1016/j.envpol.2005.04.004

Jansen S, Broadley MR, Smets E (2002) Aluminium hyperaccumulation in angiosperms: a review of its phylogenetic significance. Bot Rev 68:235–269. doi:10.1663/0006-8101(2002)068[0235:ahiaar]2.0.co;2

Jensen JK, Holm PE, Nejrup J, Larsen MB, Borggaard OK (2009) The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environ Pollut 157:931–937. doi:10.1016/j.envpol.2008.10.024

Jiang LY, Yang XE, He ZL (2004) Growth response and phytoextraction of copper at different levels in soils by *Elsholtzia splendens*. Chemosphere 55:1179–1187. doi:10.1016/j.chemosphere.2004.01.026

Johnson DB (2001) Importance of microbial ecology in the development of new mineral technologies. Hydrometallurgy 59:147–158. doi:10.1016/S0304-386X(00)00183-3

Johnson DB (2010) The biogeochemistry of biomining, Chapter 19. In: Barton LL et al (eds) Geomicrobiology: molecular and environmental perspective. Springer Science & Business Media BV, Dordrecht, The Netherlands, pp 401–426

Kabała C, Singh BR (2001) Fractionation and mobility of copper, lead, and zinc in soil profiles in the vicinity of a copper smelter. J Environ Qual 30:485–492. doi:10.2134/jeq2001.302485x

Kabała C, Medyńska A, Chodak T, Jezierski P, Gałka B (2008) Changes of copper and arsenic concentrations in soils surrounding copper ore tailings impoundment in 12-year long cycle of monitoring investigation. Roczniki Gleboznawcze 59:81–88 (in Polish)

Kabała C, Chodak T, Szerszeń L, Karczewska A, Szopka K, Frątczak U (2009) Factors influencing the concentration of heavy metals in soils of allotment gardens in the city of Wroclaw, Poland. Fresen Environ Bull 18:1118–1124

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants. CRC, Boca Raton, FL

Kabata-Pendias A, Piotrowska M, Witek T (1993) Assessment of soil and plant pollution with heavy metals and sulphur. Guidelines for agriculture, ser. P (53a), IUNG Pulawy

Karami N, Clemente R, Moreno-Jiménez E, Lepp NW, Beesley L (2011) Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J Hazard Mater 191:41–48. doi:10.1016/j.jhazmat.2011.04.025

Karczewska A (1996) Chemical speciation and fate of selected heavy metals in soils strongly polluted by copper smelters. In: Reuther R (ed) Geochemical approaches to environmental engineering of metals, Environmental science. Springer, Berlin, pp 55–79

Karczewska A (2002) Metale ciężkie w glebach zanieczyszczonych emisjami hut miedzi - formy i rozpuszczalność. Rozprawa habilitacyjna. Zeszyty Naukowe AR we Wrocławiu. Rozprawy CLXXXIV, Wydział Rolniczy, Nr 432, Wrocław. (in Polish)

Karczewska A, Bogda A (2006) Heavy metals in soils of former mining areas in the Sudety Mts.—their forms and solubility. Polish J Environ Stud 15:104–110

Karczewska A, Milko K (2010) Effects of chelating agents on Cu, Pb and Zn solubility in polluted soils and tailings produced by copper industry. Ecol Chem Eng A 17:395–403

Karczewska A, Gałka B, Kabała C, Szopka K, Kocan K, Dziamba K (2009a) Effects of various chelators on the uptake of Cu, Pb, Zn and Fe by maize and Indian mustard from silty loam soil polluted by the emissions from copper smelter. Fresen Environ Bull 18:1967–1974

Karczewska A, Gałka B, Kocan K (2009b) A carryover effect of the chelating agents EDTA and EDDS applied to soils on the uptake of copper and iron by maize in the second year of a pot experiment. J Elem 14:693–703

Karczewska A, Kaszubkiewicz J, Jezierski P, Kabała C, Król K (2010) Level of soil contamination with copper, lead and cadmium within a protection zone of copper smelter Legnica in the years 1982 and 2005. Roczniki Gleboznawcze 61:45–51 (in Polish)

Karczewska A, Orłów K, Kabała C, Szopka K, Gałka B (2011) Effects of chelating compounds on mobilization and phytoextraction of copper and lead in contaminated soils. Commun Soil Sci Plant Anal 42:1379–1389. doi:10.1080/00103624.2011.577858

Kelly J, Thornton I, Simpson PR (1996) A study of the influence of anthropogenic activity on the heavy metal content of soils in traditionally industrial and non-industrial areas of Britain. Appl Geochem 11:363–370. doi:10.1016/0883-2927(95)00084-4

KGHM (2013) KGHM Polska Miedź S.A. www.kghm.pl, www.raport-csr.kghm.pl

Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root-soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259. doi:10.1016/j.envexpbot.2009.06.013

Kiikkilä O (2003) Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu–Ni smelter, in SW Finland. Silva Fennica 37:399–415

Kiikkilä O, Perkiömäki J, Barnette M, Derome J, Pennanen T, Tulisalo E, Fritze H (2001) In situ bioremediation through mulching of soil polluted by a copper-nickel smelter. J Environ Qual 30:1134–1143. doi:10.2134/jeq2001.3041134x

Klang-Westin E, Perttu K (2002) Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow. Biomass Bioenergy 23:415–426. doi:10.1016/S0961-9534(02)00068-5

Komárek M, Tlustoš P, Száková J, Chrastný V, Ettler V (2007) The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere 67:640–651. doi:10.1016/j.chemosphere.2006.11.010

Komárek M, Tlustoš P, Száková J, Chrastný V (2008a) The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. Environ Pollut 151:27–38. doi:10.1016/j.envpol.2007.03.010

Komárek M, Száková J, Rohošková M, Javorská H, Chrastný V, Balík J (2008b) Copper contamination of vineyard soils from small wine producers: a case study from the Czech Republic. Geoderma 147:16–22. doi:10.1016/j.geoderma.2008.07.001

Komárek M, Čadková E, Chrastný V, Bordas F, Bollinger J (2010) Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environ Int 36:138–151. doi:10.1016/j.envint.2009.10.005

Koppolu L, Clements LD (2003) Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part I: Preparation of synthetic hyperaccumulator biomass. Biomass Bioenergy 24:69–79. doi:10.1016/S0961-9534(02)00074-0

Kos B, Leštan D (2003) Influence of a biodegradable ([S,S]-EDDS) and nondegradable (EDTA) chelate and hydrogel modified soil water sorption capacity on Pb phytoextraction and leaching. Plant Soil 253:403–411. doi:10.1023/A:1024861725626

Kotrba P, Najmanova J, Macek T, Ruml T, Mackova M (2009) Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnol Adv 27:799–810. doi:10.1016/j.biotechadv.2009.06.003

Kozlov MV (2005) Sources of variation in concentrations of nickel and copper in mountain birch foliage near a nickel-copper smelter at Monchegorsk, north-western Russia: results of long-term monitoring. Environ Pollut 135:91–99. doi:10.1016/j.envpol.2004.10.005

Kozlov MV, Zvereva EL (2007) Industrial barrens: extreme habitats created by non-ferrous metallurgy. Rev Environ Sci Biotechnol 6:231–259. doi:10.1007/s11157-006-9117-9

Krämer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672. doi:10.1007/s002530100631

Kumar PBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Kumpiene J, Ore S, Renella G, Mench M, Lagerkvist A, Maurice C (2006) Assessment of zerovalent iron for stabilization of chromium, copper, and arsenic in soil. Environ Pollut 144:62–69. doi:10.1016/j.envpol.2006.01.010

Kumpiene J, Lagerkvisk A, Maurice C (2007) Stabilization of Pb- and Cu-contaminated soil using coal fly ash and peat. Environ Pollut 145:365–373. doi:10.1016/j.envpol.2006.01.037

Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—a review. Waste Manage 28:215–225. doi:10.1016/j.wasman.2006.12.012

Küpper H, Mijovilovich A, Götz B, Kroneck PMH, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper in higher plants (I): characterisation of copper accumulation, speciation and toxicity in *Crassula helmsii* as a new copper hyperaccumulator. Plant Physiol 151:702–707. doi:10.1104/pp. 109.139717

Kuzovkina YA, Knee M, Quigley MF (2004) Cadmium and copper uptake and translocation in five willow (*Salix* L.) species. Int J Phytoremediation 6:269–287. doi:10.1080/16226510490496726

Labrecque M, Teodorescu TI, Daigle S (1997) Biomass productivity and wood energy of *Salix* species after 2 years growth in SRIC fertilized with wastewater sludge. Biomass Bioenergy 12:409–417. doi:10.1016/S0961-9534(97)00011-1

Lamb DT, Naidu R, Ming H, Megharaj M (2012) Copper phytotoxicity in native and agronomical plant species. Ecotoxicol Environ Saf 85:23–29. doi:10.1016/j.ecoenv.2012.08.018

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120. doi:10.2134/jeq2002.1090

Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522. doi:10.1016/j.envpol.2007.09.015

Lee CSL, Li X, Shi W, Cheung SCN, Thornton I (2006) Metal contamination in urban, suburban, and country park soils of Hong Kong: a study based on GIS and multivariate statistics. Sci Total Environ 356:45–61. doi:10.1016/j.scitotenv.2005.03.024

Lepp NW, Hartley J, Toti M, Dickinson NM (1997) Patterns of soil copper contamination and temporal changes in vegetation in the vicinity of a copper rod rolling factory. Environ Pollut 95:363–369. doi:10.1016/S0269-7491(96)00130-3

Leštan D, Luo C, Li X (2008) The use of chelating agents in the remediation of metal-contaminated soils: a review. Environ Pollut 153:3–13. doi:10.1016/j.envpol.2007.11.015

Li X, Thornton I (2001) Chemical partitioning of trace and major elements in soils contaminated by mining and smelting activities. Appl Geochem 16:1693–1706. doi:10.1016/S0883-2927(01)00065-8

Lim T, Tay J, Wang J (2004) Chelating-agent-enhanced heavy metal extraction from a contaminated acidic soil. J Environ Eng 130:59–66. doi:10.1061/(ASCE)0733-9372(2004)130:1(59)

Linde M, Bengtsson H, Oborn I (2001) Concentrations and pools of heavy metals in urban soils in Stockholm, Sweden. Water Air Soil Pollut 1:83–101. doi:10.1023/A:1017599920280

Løbersli EM, Steinnes E (1988) Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water Air Soil Pollut 37:25–39. doi:10.1007/BF00226477

Lombi E, Hamon RE, Wieshammer G, McLaughlin MJ, McGrath SP (2004) Assessment of the use of industrial by-products to remediate a copper- and arsenic-contaminated soil. J Environ Qual 33:902–910. doi:10.2134/jeq2004.0902

Lottenbach B, Furrer G, Scharli H, Schulin R (1999) Immobilization of zinc and cadmium by montmorillonite compounds. Environ Sci Technol 33:2945–2952. doi:10.1021/es981317q

Lottermoser B (2003) Mine wastes. Characterization, treatment and environmental impacts. Springer, Berlin

Lou L, Shen Z, Li X (2004) The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper-enriched soils. Environ Exp Bot 51:111–120. doi:10.1016/j.envexpbot.2003.08.002

Luo C, Shen Z, Li X (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59:1–11. doi:10.1016/j.chemosphere.2004.09.100

Luo CL, Shen ZG, Li XD (2007) Plant uptake and the leaching of metals during the hot EDDS-enhanced phytoextraction process. Int J Phytoremediation 9:181–196. doi:10.1080/15226510701375986

Ma QY, Logan TJ, Traina SJ (1995) Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks. Environ Sci Technol 29:1118–1126. doi:10.1021/es00004a034

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409(6820):579. doi:10.1038/35054664

Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manage 90:831–837. doi:10.1016/j.jenvman.2008.01.014

Mackie KA, Müller T, Kandel E (2012) Remediation of copper in vineyards. A mini review. Environ Pollut 167:16–26. doi:10.1016/j.envpol.2012.03.023

Madrid F, Liphadzi MS, Kirkham MB (2003) Heavy metal displacement in chelate-irrigated soil during phytoremediation. J Hydrol 272:107–119. doi:10.1016/S0022-1694(02)00258-5

Madrid L, Diaz-Barrientos E, Ruiz-Cortés E, Reinoso R, Biasioli M, Davidson CM, Duarte AC, Grčman H, Hossack I, Hursthouse AS, Kralj T, Ljung K, Otabbong E, Rodrigues S, Urquhart GJ, Ajmone-Marsan F (2006) Variability in concentrations of potentially toxic elements in urban parks from six European cities. J Environ Monit 8:1158–1165. doi:10.1039/B607980F

Maier R (2004) Phytostabilization of mine tailings in the southwestern United States: plant- soil-microbe interactions and metal speciation dynamics, superfund project. Superfund Basic Research Program, University of Arizona

Martens SN, Boyd RS (2002) The defensive role of Ni hyperaccumulation by plants: a field experiment. Am J Bot 89:998–1003. doi:10.3732/ajb.89.6.998

Martley E, Gulson BL, Pfeifer HR (2004) Metal concentrations in soils around the copper smelter and surrounding industrial complex of Port Kembla, NSW, Australia. Sci Total Environ 325:113–127. doi:10.1016/j.scitotenv.2003.11.012

McCall J, Gunn J, Struik H (1995) Photo interpretive study of recovery of damaged lands near the metal smelters of Sudbury, Canada. Water Air Soil Pollut 85:847–852. doi:10.1007/BF00476935

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Review. Curr Opin Plant Biol 3:153–162. doi:10.1016/S1369-5266(99)00054-0

Meers E, Lesage E, Lamsal S, Hopgood M, Vervaeke P, Tack FM, Verloo MG (2005) Enhanced phytoextraction: I. Effect of EDTA and citric acid on heavy metal mobility in a calcareous soil. Int J Phytoremediation 7:129–142. doi:10.1080/16226510590950423

Mejare M, Bulow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Review. Trends Biotechnol 19:67–73. doi:10.1016/S0167-7799(00)01534-1

Mench M, Bes C (2009) Assessment of ecotoxicity of topsoils from a wood treatment site. Pedosphere 19:143–155. doi:10.1016/S1002-0160(09)60104-1

Mench M, Vangronsveld J, Lepp N, Bleeker P, Ruttens A, Geebelen W (2006) Phytostabilisation of metal-contaminated sites, Chapter 5. In: Morel JL et al (eds) Phytoremediation of metal-contaminated soils. Springer, Dordrecht, The Netherlands, pp 109–190

Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schröder P, Vangronsveld J (2010) Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J Soils Sediments 10:1039–1070. doi:10.1007/s11368-010-0190-x

Mendez MO, Maier RM (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Review. Environ Health Perspect 116:278–283. doi:10.1289/ehp.10608

Mesilio L, Farago ME, Thornton I (2003) Reconnaissance soil geochemical survey of Gibraltar. Environ Geochem Health 25:1–8. doi:10.1023/A:1021232412519

Milko S, Koch G, Mesić S, Šparica Miko M, Šparica M, Vreča P (2007) Influence of land use in small karst watersheds on the chemical status of peloid sediments on the eastern Adriatic coast. J Soils Sediments 7:303–312. doi:10.1065/jss2007.10.254

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653. doi:10.1016/j.biotechadv.2011.04.006

Mirlean N, Roisenberg A, Chies JO (2007) Metal contamination of vineyard soils in wet subtropics (southern Brazil). Environ Pollut 149:10–17. doi:10.1016/j.envpol.2006.12.024

Mirlean N, Baisch P, Medeanic S (2009) Copper bioavailability and fractionation in copper contaminated sandy soils in the wet subtropics (southern Brazil). Bull Environ Contam Toxicol 82:373–377. doi:10.1016/j.envpol.2006.12.024

Mleczek M, Mocek A, Magdziak Z, Gąsecka M, Mocek-Płóciniak A (2013) Impact of metal/metalloid-contaminated areas on plant growth. In: Gupta DK (ed) Plant-based remediation processes, vol 35, Soil biology. Springer, Berlin, pp 79–100

Mocek A (2012) Fitoremediacja—metoda nadinterpretowana w zaleceniach do oczyszczania gleb z metali ciężkich. In: Ocena ryzyka zdrowotnego i ekologicznego na terenach rolniczych narażonych na oddziaływanie zanieczyszczeń chemicznych. IUNG—Puławy, pp 7–9. (in Polish)

Mocek A, Owczarzak W, Bartoszewicz A (2001) Rational cultivation of chemically contaminated soils near the copper smelter. Acta Agrophys 56:189–198

Morrell JJ, Hoffman J (2004) Copper, chromium, and arsenic levels in soils surrounding posts treated with chromated copper arsenate (CCA). Wood Fiber Sci 36:119–128

Morton-Bermea O, Hernandez E, Martinez-Pichardo E, Soler-Arechalde AM, Santa-Cruz RL, Gonzalez-Hernandez G, Beramendi-Orosco L, Urrutia-Fucugauchi J (2009) Mexico city topsoils: heavy metals vs. magnetic susceptibility. Geoderma 151:121–125. doi:10.1016/j.geoderma.2009.03.019

Mulligan CN, Cloutier RG (2003) Bioremediation of metal contamination. Environ Monit Assess 84:45–60. doi:10.1023/A:1022874727526

Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207. doi:10.1016/S0013-7952(00)00101-0

Nakajima H, Itoh K, Otake H, Fujimoto K (2011) Spectral properties of the Cu-hyperaccumulating moss *Scopelophila cataractae*. J Photochem Photobiol B 104:467–472. doi:10.1016/j.jphotobiol.2011.06.001

Naresh Kumar R, Nagendran R (2007) Influence of initial pH on bioleaching of heavy metals from contaminated soil employing indigenous *Acidithiobacillus thiooxidans*. Chemosphere 66:1775–1781. doi:10.1016/j.chemosphere.2006.07.091

Naresh Kumar R, Nagendran R (2009) Fractionation behavior of heavy metals in soil during bioleaching with *Acidithiobacillus thiooxidans*. J Hazard Mater 169:1119–1126. doi:10.1016/j.jhazmat.2009.04.069

Nedelkoska TV, Doran PM (2000) Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Miner Eng 13:549–561. doi:10.1016/S0892-6875(00)00035-2

Nieminen TM, Ukonmaanaho L, Shotyk W (2002) Enrichment of Cu, Ni, Zn, Pb and As in an ombrotrophic peat bog near a Cu-Ni smelter in Southwest Finland. Sci Total Environ 292:81–89. doi:10.1016/S0048-9697(02)00028-1

Nikonov V, Goryainova V, Lukina N (2001) Ni and Cu migration and accumulation in forest ecosystems on the Kola Peninsula. Chemosphere 42:93–100. doi:10.1016/S0045-6535(00)00104-1

Ninkov J, Paprić D, Sekulić P, Zeremski-Škorić T, Milić S, Vasin J, Kurjački I (2012) Copper content of vineyard soils at Sremski Karlovci (Vojvodina Province, Serbia) as affected by the use of copper-based fungicides. Int J Environ Anal Chem 92:592–600. doi:10.1080/03067310903428743

Nishikiori T, Okuyama A, Naganawa H, Takita T, Hamada H, Takeuchi T, Aoyagi T, Umezawa H (1984) Production by actinomycetes of (S, S)-N, N0-ethylenediamine–disuccinic acid, an inhibitor of phospholipase. J Antibiot 37:426–427

Nóvoa-Muñoz JC, Queijeiro JMG, Blanco-Ward D, Álvarez-Olleros C, Martínez-Cortizas A, García-Rodeja E (2007) Total copper content and its distribution in acid vineyards soils developed from granitic rocks. Sci Total Environ 378:23–27. doi:10.1016/j.scitotenv.2007.01.027

Nowack B, Rais D, Frey B, Menon M, Schulin R, Günthardt-Goerg MS, Luster J (2006) Influence of metal contamination on soil parameters in a lysimeter experiment designed to evaluate phytostabilization by afforestation. Forest Snow Landsc Res 80:201–211

Odewande AA, Abimbola AF (2008) Contamination indices and heavy metal concentrations in urban soil of Ibadan metropolis, southwestern Nigeria. Environ Geochem Health 30:243–254. doi:10.1007/s10653-007-9112-2

Ordóñez A, Loredo J, DeMiguel E, Charlesworth S (2003) Distribution of heavy metals in the street dusts and soils of an industrial city in Northern Spain. Arch Environ Contam Toxicol 44:160–170. doi:10.1007/s00244-002-2005-6

Page MM, Page CL (2002) Electroremediation of contaminated soils. J Environ Eng 128:208–219. doi:10.1061/-ASCE!0733-9372-2002!128:3-208!

Papoyan A, Kochian LV (2004) Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase. Plant Physiol 136:3814–3823. doi:10.1104/pp. 104.044503

Pasieczna A, Małecka J, Lipnicka T (2003) Atlas of urban soils contamination in Poland. PIG, Warszawa

Paton A, Brooks RR (1996) A re-evaluation of *Haumaniastrum* species as geobotanical indicators of copper and cobalt. J Geochem Explor 56:37–45. doi:10.1016/0375-6742(95)00048-8

Pavlovic M (2011) A review of agribusiness copper use effects on environment. Bulg J Agric Sci 17:491–500

Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS (2005) Phytoremediation and hyperaccumulator plants. In: Tamás MJ, Martinoia E (eds) Molecular biology of metal homeostasis and detoxification. Springer, Berlin, pp 299–340

Peters TH (1995) Revegetation of the copper cliff tailings area. In: Gunn J (ed) Restoration and recovery of an industrial region. Springer, New York, pp 123–133

Pichtel J, Sawyerr HT, Czarnowska K (1997) Spatial and temporal distribution of metals in soils in Warsaw, Poland. Environ Pollut 98:169–174. doi:10.1016/S0269-7491(97)00131-0

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122:151–166. doi:10.1016/j.geoderma.2004.01.005

Pietrzak U, Uren NC (2011) Remedial options for copper-contaminated vineyard soils. Soil Res 49:44–55. doi:10.1071/SR09200

Poschenrieder C, Tolrà R, Barceló J (2006) Can metals defend plants against biotic stress? Trends Plant Sci 11:288–295. doi:10.1016/j.tplants.2006.04.007

Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–701. doi:10.1023/A:1025604627496

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540. doi:10.1016/S0160-4120(02)00152-6

Purvis OW, Pawlik-Skowrońska B, Cressey G, Jones GC, Kearsley A, Spratt J (2008) Mineral phases and element composition of the Cu hyperaccumulator lichen *Lecanora polytropa*. Mineral Mag 72:607–616. doi:10.1180/minmag.2008.072.2.607

Quartacci MF, Irtelli B, Baker AJ, Navari-Izzo F (2007) The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica carinata*. Chemosphere 68:1920–1928. doi:10.1016/j.chemosphere.2007.02.058

Quinn ML (1991) The Appalachian Mountains' Copper Basin and the concept of environmental susceptibility. Environ Manage 15:179–194. doi:10.1007/BF02393849

Rajkumar M, Ae N, Prasad MN, Freitas H (2010) Potential of siderophore producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149. doi:10.1016/j.tibtech.2009.12.002

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181. doi:10.1016/j.plantsci.2010.08.016

Raskin I, Ensley BD (2000) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 15–31

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226. doi:10.1016/S0958-1669(97)80106-1

Rawlings DE, Johnson DB (eds) (2007) Biomining. Springer, Heidelberg

Redente EF, Richards JL (1997) Effects of lime and fertilizer amendments on plant growth in smelter impacted soils in Montana. Arid Soil Res Rehabil 11:353–366. doi:10.1080/15324989709381488

Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York

Reichman S (2002) The responses of plants to metal toxicity a review focusing on copper, manganese and zinc. Australian Minerals & Energy Environment Foundation, Melbourne

Reijnders L (2003) Recovery of dissipated copper and the future of copper supply. Resour Conserv Recy 38:59–66. doi:10.1016/S0921-3449(02)00148-9

Rimmer DL, Vizard CG, Pless-Mulloli T, Singleton I, Air VS, Keatinge ZA (2006) Metal contamination of urban soils in the vicinity of a municipal waste incinerator: one source among many. Sci Total Environ 356:207–216. doi:10.1016/j.scitotenv.2005.04.037

Rodríguez N, Menéndez N, Tornero J, Amils R, de la Fuente V (2005) Internal iron biomineralization in *Imperata cylindrica*, a perennial grass: chemical composition, speciation and plant localization. New Phytol 165:781–789. doi:10.1111/j.1469-8137.2004.01264.x

Romić M, Romić D, Dolanjski D, Stričević I (2004) Heavy metal accumulation in topsoils from the wine-growing regions. Part 1. Factors which control retention. Agric Conspec Sci 69:1–10

Römkens P, Bouwman L, Japenga J, Draaisma C (2002) Potential and drawbacks of chelate-enhanced phytoremediation of soils. Environ Pollut 116:109–121. doi:10.1016/S0269-7491(01)00150-6

Roszyk E, Szerszeń L (1988) Accumulation of heavy metals in the arable layer of soils of the sanitary protection zone in the vicinity of copper metallurgic plants. Part 1: Legnica. Part 2: Głogów. Roczniki Gleboznawcze 39:135–141, 147–156. (in Polish)

Ruby MV, Davis A, Nicholson A (1994) *In situ* formation of lead phosphates in soils as a method to immobilize lead. Environ Sci Technol 28:646–654. doi:10.1021/es00053a018

Rusjan D, Strlič M, Pucko D, Korošec-Koruza Z (2007) Copper accumulation regarding the soil characteristics in Sub-Mediterranean vineyards of Slovenia. Geoderma 141:111–118. doi:10.1016/j.geoderma.2007.05.007

Ruttens A, Colpaert JV, Mench M, Boisson J, Carleer R, Vangronsveld J (2006) Phytostabilization of a metal contaminated sandy soil. II: Influence of compost and/or inorganic metal immobilizing soil amendments on metal leaching. Environ Pollut 144:533–539. doi:10.1016/j/envpol/2006.01.038

Sáinz A, Grande JA, De la Torre ML (2004) Characterisation of heavy metal discharge into the Ria of Huelva. Environ Int 30:557–566. doi:10.1016/j.envint.2003.10.013

Salomons W (1995) Environmental impacts of metals derived from mining activities: processes, prediction, prevention. J Geochem Explor 52:5–23. doi:10.1016/0375-6742(94)00039-E

Salt DE, Prince RC, Pickering IJ (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109:1427–1433. doi:10.1104/pp. 109.4.1427

Santibáñez C, Verdugo C, Ginocchio R (2008) Phytostabilization of copper mine tailings with biosolids: implications for metal uptake and productivity of *Lolium perenne*. Sci Total Environ 395:1–10. doi:10.1016/j.scitotenv.2007.12.033

Sas-Nowosielska A, Kucharski R, Pogrzeba M, Malkowski E (2008) Soil remediation scenarios for heavy metal contaminated soil. In: Soil chemical pollution, risk assessment, remediation and security. Springer, Dordrecht, The Netherlands, pp 301–307

Sauvé S, Cook N, Hendershot WH, McBride MB (1996) Linking plant tissue concentrations and soil copper pools in urban contaminated soils. Environ Pollut 94:153–157. doi:10.1016/S0269-7491(96)00081-4

Schwab P, Zhu D, Banks MK (2007) Heavy metal leaching from mine tailings as affected by organic amendments. Bioresour Technol 98:2935–2941. doi:10.1016/j.biortech.2006.10.012

Seeley M, Wells CS, Wannamaker EJ, Mattuck RL, Ren S, Beck BD (2013) Determining soil remedial action criteria for acute effects: the challenge of copper. Regul Toxicol Pharmacol 65:47–59. doi:10.1016/j.yrtph.2012.10.011

Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41:168–214. doi:10.1080/10643380902718418

Shi W, Shao H, Li H, Shao M, Du S (2009) Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J Hazard Mater 170:1–6. doi:10.1016/j.jhazmat.2009.04.097

Smolińska B, Król K (2012) Leaching of mercury during phytoextraction assisted by EDTA, KI and citric acid. J Chem Technol Biotechnol 87:1360–1365. doi:10.1002/jctb.3826

Soler-Rovira P, Madejón E, Madejón P, Plaza C (2010) *In situ* remediation of metal-contaminated soils with organic amendments: role of humic acids in copper bioavailability. Chemosphere 79:844–849. doi:10.1016/j.chemosphere.2010.02.054

Song J, Zhao FJ, Luo YM, McGrath SP, Zhang H (2004) Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. Environ Pollut 128:307–315. doi:10.1016/j.envpol.2003.09.019

Spiak Z, Gediga K (2009) Usefulness of selected mineral wastes for reclamation of copper industry dumping site. Environ Protect Eng 35:79–88

Spurgeon DJ, Hopkin SP (1996) The effects of metal contamination on earthworm populations around a smelting works: quantifying species effects. Appl Soil Ecol 4:147–160. doi:10.1016/0929-1393(96)00109-6

Srivastava M, Ma LQ, Santos JAG (2006) Three new arsenic hyperaccumulating ferns. Sci Total Environ 364:24–31. doi:10.1016/j.scitotenv.2005.11.002

Stolarski M, Szczukowski S, Tworkowski J, Klasa A (2008) Productivity of seven clones of willow coppice in annual and quadrennial cutting cycles. Biomass Bioenergy 32:1227–1234. doi:10.1016/j.biombioe.2008.02.023

Suèr P, Gitye K, Allard B (2003) Speciation and transport of heavy metals and macroelements during electroremediation. Environ Sci Technol 37:177–181. doi:10.1021/es010226h

Sun YB, Sun GH, Zhou QX, Xu YM, Wang L, Liang XF, Sun Y, Qing X (2011) Induced-phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with Marvel of Peru (*Mirabilis jalapa* L.). Plant Soil Environ 57:364–371

Šyc M, Pohořelý M, Kameníková P, Habart J, Svoboda K, Punčochář M (2012) Willow trees from heavy metals phytoextraction as energy crops. Biomass Bioenergy 37:106–113. doi:10.1016/j.biombioe.2011.12.025

Szerszeń L, Karczewska A, Roszyk E, Chodak T (1991) Rozmieszczenie Cu, Pb i Zn w profilach gleb przyległych do hut miedzi. Roczniki Gleboznawcze XLII 3–4:199–206 (in Polish)

Szerszeń L, Chodak T, Karczewska A (1993) Areal, profile and time differentiation of heavy metal content in soils in the vicinity of copper smelters in LGOM, Poland. In: Eijsackers HJP, Hamers T (eds) Integrated soil and sediment research: a basis for proper protection. Kluwer, Dordrecht, The Netherlands, pp 279–281

Szerszeń L, Chodak T, Kabała C (2004) Zmiany zawartości miedzi, ołowiu i cynku w glebach w rejonie hut miedzi Głogów i Legnica w latach 1972–2002. Roczniki Gleboznawcze 55:195–205 (in Polish)

Tandy S, Schulin R, Nowack B (2006) Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration. Environ Sci Technol 40:2753–2758

Tappero R, Peltier E, Gräfe M, Heindel K, Ginder-Vogel M, Livi KJT, Rivers ML, Marcus MA, Chaney RL, Sparks DL (2007) Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. New Phytol 175:641–654. doi:10.1111/j.1469-8137.2007.02134.x

Terry N, Banuelos G (eds) (2000) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, FL

Thornton I (1996) Impacts of mining on the environment; some local, regional and global issues. Appl Geochem 11:355–361. doi:10.1016/0883-2927(95)00064-X

Townsend T, Solo-Gabriele H, Tolaymat T, Stook K, Hosein N (2003) Chromium, copper, and arsenic concentrations in soil underneath CCA-treated wood structures. Soil Sediment Contam 12:779–798. doi:10.1080/10588330331878528

USGS (2013) U.S. Geological Survey, Mineral commodity summaries 2013. U.S. Geological Survey, 198 p

Valix M, Usai F, Malik R (2001) Fungal bioleaching of low grade laterite ores. Miner Eng 14:197–203. doi:10.1016/S0892-6875(00)00175-8

van Herwijen R, Hutchings TR, Al-Tabbaa A, Moffat AJ, Johns ML, Ouki SK (2007) Remediation of metal contaminated soil with mineral-amended composts. Environ Pollut 150:347–354. doi:10.1016/j.envpol.2007.01.023

Van Nevel L, Mertens J, Oorts K, Verheyen K (2007) Phytoextraction of metals from soils: How far from practice? Environ Pollut 150: 34–40

Vangronsveld J, Colpaert JV, Van Tichelen KK (1996) Reclamation of a bare industrial area contaminated by non-ferrous metals: physico-chemical and biological evaluation of the durability of soil treatment and revegetation. Environ Pollut 94:131–140. doi:10.1016/S0269-7491(96)00082-6

Verdugo C, Sánchez P, Santibáñez C, Urrestarazu P, Bustamante E, Silva Y, Gourdon D, Ginocchio R (2010) Efficacy of lime, biosolids, and mycorrhiza for the phytostabilization of sulfidic copper tailings in Chile: a greenhouse experiment. Int J Phytoremediation 13:107–125. doi:10.1080/15226510903535056

Virkutyte J, Sillanpaa N, Latostenmaa P (2002) Electrokinetic soil remediation—critical overview. Sci Total Environ 289:97–121. doi:10.1016/S0048-9697(01)01027-0
Voglar GE, Leštan D (2010) Solidification/stabilisation of metals contaminated industrial soil from former Zn smelter in Celje, Slovenia, using cement as a hydraulic binder. J Hazard Mater 178:926–933. doi:10.1016/j.jhazmat.2010.02.026
Volk TA, Abrahamson LP, Nowak CA, Smart LB, Tharakan PJ, White EH (2006) The development of short-rotation willow in the northeastern United States for bioenergy and bioproducts, agroforestry and phytoremediation. Biomass Bioenergy 30:715–727. doi:10.1016/j.biombioe.2006.03.001
Wang H, Shan XQ, Wen B, Zhang S, Wang ZJ (2004) Responses of antioxidative enzymes to accumulation of copper in a copper hyperaccumulator of *Commoelina communis*. Arch Environ Contam Toxicol 47:185–192. doi:10.1007/s00244-004-2222-2
Wang F, Lin X, Yin R (2005) Heavy metal uptake by arbuscular mycorrhizas of *Elsholtzia splendens* and the potential for phytoremediation of contaminated soil. Plant Soil 269:225–232. doi:10.1007/s11104-004-0517-8
Wang FY, Lin XG, Yin R (2007) Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. Pedobiologia 51:99–109. doi:10.1016/j.pedobi.2007.02.003
Watling HR (2006) The bioleaching of sulphide minerals with emphasis on copper sulphides—a review. Hydrometallurgy 84:81–108. doi:10.1016/j.hydromet.2006.05.001
Wei SH, Zhou QX, Wang X (2005) Cadmium-hyperaccumulator *Solanum nigrum* L. and its accumulating characteristics. Huan Jing Ke Xue 26:167–171
Wei C, Wang C, Yang L (2009) Characterizing spatial distribution and sources of heavy metals in the soils from mining-smelting activities in Shuikoushan, Hunan Province, China. J Environ Sci 21:1230–1236. doi:10.1016/S1001-0742(08)62409-2
Wenger K, Gupta SK, Furrer G, Schulin R (2002) Zinc extraction potential of two common crop plants, *Nicotiana tabacum* and *Zea mays*. Plant Soil 242:217–225. doi:10.1023/A:1016253821174
Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408. doi:10.1007/s11104-008-9686-1
Wenzel WW, Unterbrunner R, Sommer P, Pasqualina S (2003) Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. Plant Soil 249:83–96. doi:10.1023/A:1022516929239
Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254. doi:10.1016/j.copbio.2009.02.012
Wightwick A, Mollah M, Smith J, MacGregor A (2006) Sampling considerations for surveying copper concentrations in Australian vineyard soils. Aust J Soil Res 44:711–717. doi:10.1071/SR05135
Wightwick AM, Salzman SA, Reichman SM, Allinson G, Menzies NW (2013) Effects of copper fungicide residues on the microbial function of vineyard soils. Environ Sci Pollut Res 20:1574–1585. doi:10.1007/s11356-012-1114-7
Wilcke W, Müller S, Kanchanakool N, Zech W (1998) Urban soil contamination in Bangkok: heavy metal and aluminium partitioning in topsoils. Geoderma 86:211–228. doi:10.1016/S0016-7061(98)00045-7
Wilson B, Pyatt FB (2007a) Heavy metal dispersion, persistence and bioaccumulation around an ancient copper mine situated in Anglesey, UK. Ecotoxicol Environ Saf 66:224–231. doi:10.1016/j.ecoenv.2006.02.015
Wilson B, Pyatt FB (2007b) Heavy metal bioaccumulation by the important food plant, *Olea europaea* l., in an ancient metalliferous polluted area of Cyprus. Bull Environ Contam Toxicol 78:390–394. doi:10.1007/s00128-007-9162-2
Wilson-Corral V, Anderson C, Rodriguez-Lopez M, Arenas-Vargas M, Lopez-Perez J (2011) Phytoextraction of gold and copper from mine tailings with *Helianthus annuus* L. and *Kalanchoe serrata* L. Miner Eng 24(13):1488–1494. doi:10.1016/j.mineng.2011.07.014
Winterhalder K (1996) Environmental degradation and rehabilitation of the landscape around Sudbury, a major mining and smelting area. Environ Rev 4:185–224. doi:10.1139/a96-011
Winterhalder K (1999) Landscape degradation by smelter emissions near Sudbury, Canada, and subsequent amelioration and restoration. In: Innes JL, Oleksyn J (eds) Forest dynamics in heavily polluted regions. IUFRO Task Force on Environmental Change 2000. Report No. 1, pp 87–119
Winterhalder K (2000) Reclamation of smelter-damaged lands. Chapter 33. In: Barnhisel RI, Darmody RG, Daniels WL (eds) Reclamation of drastically disturbed lands, vol 41, Agronomy monograph. American Society of Agronomy, Madison, WI, pp 819–853
Wong CS, Li X, Thornton I (2006) Urban environmental geochemistry of trace metals. Environ Pollut 142:1–16. doi:10.1016/j.envpol.2005.09.004
Wu J, Hsu F, Cunningham S (1999) Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. Environ Sci Technol 33:1898–1904. doi:10.1021/es9809253
Wu L, Li H, Luo YM, Christie P (2004) Nutrients can enhance phytoremediation of copper-polluted soil by Indian mustard. Environ Geochem Health 26:331–335. doi:10.1023/B:EGAH.0000039598.24033.4e
Yan S, Ling QC, Bao ZY (2007) Metals contamination in soils and vegetables in metal smelter contaminated sites in Huangshi, China. Bull Environ Contam Toxicol 79:361–366. doi:10.1007/s00128-007-9219-2
Yang SX, Deng H, Li MS (2008) Manganese uptake and accumulation in a woody hyperaccumulator, *Schima superba*. Plant Soil Environ 54(10):441–446
Yruela I (2005) Copper in plants. Braz J Plant Physiol 17:145–156. doi:10.1590/S1677-04202005000100012
Zagury GJ, Samson J, Deschênes L (2003) Occurrence of metals in soil and ground water near chromated copper arsenate-treated utility poles. J Environ Qual 32:507–514. doi:10.2134/jeq2003.5070
Zhang X-H, Liu J, Huang H-T, Chen J, Zhu Y-N, Wang D-Q (2007) Chromium accumulation by the hyperaccumulator plant *Leersia hexandra* Swartz. Chemosphere 67:1138–1143. doi:10.1016/j.chemosphere.2006.11.0
Zhang G, Lin Y, Wang M (2011) Remediation of copper polluted red soils with clay materials. J Environ Sci 23:461–467. doi:10.1016/S1001-0742(10)60431-7
Zheng YM, Chen TB, He JZ (2008) Multivariate geostatistical analysis of heavy metals in topsoils from Beijing, China. J Soils Sediments 8:51–58. doi:10.1065/jss2007.08.245
Zhou DM, Deng CF, Cang L (2004) Electrokinetic remediation of a Cu contaminated red soil by conditioning catholyte pH with different enhancing chemical reagents. Chemosphere 56:265–273. doi:10.1016/j.chemosphere.2004.02.033
Zhu LY, Pilon-Smits EA, Jouanin L, Terry N (1999) Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol 119:73–80. doi:10.1104/pp.119.1.73
Zou Z, Qiu R, Zhang W, Dong H, Zhao Z, Zhang T, Wei X, Cai X (2009) The study of operating variables in soil washing with EDTA. Environ Pollut 157:229–236. doi:10.1016/j.envpol.2008.07.009
Zwoździak J, Zwoździak A (1982) Trace metal behaviour in the vicinity of a copper smelter. Int J Environ Stud 1:35–41. doi:10.1080/00207238208709962

# 13 Phytoremediation of the Metalloid Selenium in Soil and Water

Zhilin Wu, Gary S. Bañuelos, Xuebin Yin, Zhiqing Lin, Norman Terry, Ying Liu, Linxi Yuan, and Miao Li

## 13.1 Introduction

Heavy metals and metalloids, such as selenium (Se), are released into the environment by mining industry, and agriculture, threatening environmental and human health (Bañuelos 2001). Due to the acute toxicity of Se, there is an urgent need to develop low-cost, effective, and sustainable methods to remove it from the environment or to detoxify it. Plant-based approaches, such as phytoremediation, are relatively inexpensive since they are performed in situ and are solar-driven (Bañuelos et al. 2002). In this review, we discuss specific advances in plant-based approaches for the remediation of contaminated water and soil. Dilute concentrations of Se can be removed from large volumes of wastewater by constructed wetlands. Plants play an important but indirect role in that they supply fixed carbon and other nutrients to rhizosphere microbes responsible for the uptake and detoxification of contaminants. We discuss the potential of constructed wetlands for use in remediating Se in agricultural drainage water and industrial effluent, as well as concerns over its potential ecotoxicity.

Plants play a more direct role in remediation of upland soil. Plants may be used to accumulate Se in their harvestable biomass (phytoextraction). Plants can also convert and release Se in a volatile form (phytovolatilization) (Pilon-Smits and LeDuc 2009). We discuss how genetic engineering has been used to develop plants with enhanced efficiency for Se of phytoextraction and phytovolatilization. Se-hyperaccumulating plants and microbes with unique abilities to tolerate, accumulate, and detoxify metals and metalloids represent an important reservoir of unique genes that could be transferred to fast-growing plant species for enhanced Se of phytoremediation (Zhu et al. 2009). Improved analytical techniques are being used to elucidate the mechanisms by which plants detoxify Se. This knowledge is crucial for optimizing new genetic engineering strategies. Finally, new strategies are required to improve the acceptability of using genetically engineered plants for remediation projects.

Z. Wu
Key Laboratory of Agri-Food Safety of Anhui Province, School of Plant Protection, Anhui Agriculture University, Hefei 230036, Anhui, China

Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Suzhou 215123, Jiangsu, China

G.S. Bañuelos
USDA-ARS, 9611 South Riverbend Avenue, Parlier, California 93648, USA

X. Yin • L. Yuan (✉)
Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Suzhou 215123, Jiangsu, China

School of Earth and Space Sciences, University of Science and Technology of China, Hefei 230026, China
e-mail: yuanli@mail.ustc.edu.cn

Z. Lin
Department of Biological Sciences & Environmental Sciences Program, Southern Illinois University Edwardsville, Edwardsville, Illinois 62026, USA
e-mail: zhlin@siue.edu

N. Terry
Department of Plant and Microbial Biology, College of Natural Resources, UC Berkeley, CA 94720-3102, USA
e-mail: nterry@berkeley.edu

Y. Liu
Suzhou Advanced Lab for Selenium and Human Health, Jiangsu Bio-Engineering Research Centre of Selenium, Suzhou Institute for Advanced Study, University of Science and Technology of China, Suzhou 215123, Jiangsu, China

M. Li, Ph.D. (✉)
Key Laboratory of Agri-Food Safety of Anhui Province, School of Plant Protection, Anhui Agriculture University, Hefei 230036, Anhui, China
e-mail: ahlimiao@163.com

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## 13.2 Plant-Based Approaches to Se Remediation

### 13.2.1 Constructed Wetlands

Constructed wetlands have been used as a low-cost treatment to remove a wide range of waterborne contaminants from polluted waters such as municipal wastewater and effluents from electricity-generating facilities and oil refineries in the world (Azaizeh et al. 2006). They comprise a complex ecosystem of plants, microbes, and sediment that together act as a biogeochemical filter, efficiently removing dilute contaminants from very large volumes of wastewater. The anoxic environment and organic matter production in wetlands promote biological and chemical processes that transform contaminants to immobile or less toxic forms (Lin et al. 2010). Plants support microbially mediated transformations of contaminants by supplying fixed-carbon as an energy source for bacteria and by altering the chemical environment in their rhizosphere (Azaizeh et al. 2006; Lin et al. 2010). Plants also take up and accumulate metals and metalloids in their tissues (Azaizeh et al. 2006; Lin et al. 2010). At this point, Se can be metabolized to nontoxic and/or volatile forms, which may escape the local ground ecosystem by release to the atmosphere (Azaizeh et al. 2006; Lin et al. 2002, 2010; Shardendu et al. 2003).

An experimental wetland was constructed at the Tulare Lake Drainage District (TLDD) in the San Joaquin Valley (Calif.) in 1996. Its purpose was to evaluate the potential of constructed wetlands for the removal of Se from agricultural irrigation drainage water. Ten individual cells were tested, either unvegetated or vegetated singly or with a combination of sturdy bulrush, cattail, etc. (Lin et al. 2002, 2010; Shardendu et al. 2003). On average, the wetland cells removed 69 % of the total Se mass from the inflow. Vegetated wetland cells removed Se more efficiently than the unvegetated cell, without significant differences among vegetated cells (Lin et al. 2002, 2010; Shardendu et al. 2003). An important objective of the TLDD wetland project was to determine if Se concentrations in drainage water could be reduced to less than 2 lg/L before disposal into evaporation ponds, the overall goal being to minimize toxic effects of Se on aquatic biota and waterfowl in the ponds. Although the Se concentrations in the outflow were significantly lower than those in the inflow for all cells, the goal of 2 lg Se per liter in the outflow was not reached (Lin et al. 2002, 2010; Shardendu et al. 2003).

Microcosm experiments provide an initial means of evaluating the Se remediation potential of a constructed wetland with a greater degree of experimental control, less cost, and substantially reduced environmental risk than a study of the wetland itself. Such a microcosm study was used to evaluate the potential of constructed wetlands to remediate effluent containing highly toxic selenocyanate (SeCN) generated by a coal gasification plant (Lin et al. 2002, 2010; Shardendu et al. 2003). The concentrations of Se were several orders of magnitude higher than those normally treated by constructed wetlands. The microcosms removed 79 % Se mass, significantly reducing the toxicity of the effluent. Because cattail (*Typhia latifolia* L.) showed no growth retardation when supplied with the contaminated wastewater, constructed wetlands planted with these species show particular promise for remediating this highly toxic effluent. Although constructed wetlands offer a less expensive alternative to other water-treatment methods, the approach needs to be optimized to enhance efficiency and reproducibility, and reduce ecotoxic risk. Most of the contaminants removed from the waste-stream are immobilized in the sediment. For example, in the microcosm experiment discussed above, the sediment contained 63 % of the Se while only 2–4 % was accumulated in plant tissue (Lin et al. 2002, 2010; Shardendu et al. 2003). In the TLDD wetland, 41 % of the supplied Se left the wetland; the remaining 59 % was retained in the wetland cell, partitioned between the surface sediment (0–20 cm; 33 %), organic detrital layer (18 %), fallen litter (2 %), standing plants (<1 %), and standing water (<1 %) (Lin et al. 2002, 2010; Shardendu et al. 2003). The Se in the agricultural drainage water entering the TLDD wetland was predominantly in the form of selenate (95 %); it was reduced in sediment to a mixture of elemental Se (45 %), organic Se (40 %), and selenite (15 %) (Lin et al. 2002, 2010; Shardendu et al. 2003). Although elemental Se is essentially nontoxic, some selenite and some species of organic Se are more toxic than selenate. There is concern that, since Se concentrations in the organically rich surface sediments increased over time, that this Se could eventually enter the aquatic food chain and exert ecotoxic effects.

### 13.2.2 Se of Phytovolatilization

One very important way of increasing the efficiency of Se removal and decreasing Se ecotoxicity of wetlands is to enhance Se volatilization by plants and microbes. Because of the chemical similarity of sulfur (S) and Se, plants and microbes are able to take up inorganic and organic forms of Se and metabolize them to volatile forms via the S assimilation pathway. Biological volatilization has the advantage of removing Se from a contaminated site in relatively nontoxic forms, such as dimethylselenide (DMSe), which is 500–700 times less toxic than SeOÀ2 or SeOÀ2 (Lin et al. 2002, 2010; Shardendu et al. 2003). Although the volatilized Se may eventually be redeposited in other areas, this is not a problem in California where much of the state is deficient in Se with respect to the nutrition of animals, which require Se in low concentrations (Lin et al. 2002, 2010; Shardendu et al. 2003).

The extent of Se volatilization is highly dependent upon a number of environmental factors, such as the composition of the microbial community, choice of macrophytes, Se speciation, organic matter amendment, and other physiochemical conditions (Lin et al. 2002, 2010; Shardendu et al. 2003). Selenium volatilization rates increase with increasing ambient temperature (Lin et al. 2002, 2010; Shardendu et al. 2003). Not only do higher temperatures increase the vapor pressure of volatile DMSe, they also stimulate the metabolic activity of plants and microbes. The chemical form of Se present in the inflow also affects the extent of Se volatilization (Lin et al. 2002, 2010; Shardendu et al. 2003). This is because biological metabolism of Se from inorganic forms, the predominant Se forms in most waste streams, to volatile DMSe is slowed by certain rate-limiting enzymatic steps. For example, Se-removal is more efficient from selenite-dominated water than selenate-dominated water (Lin et al. 2002; Bañuelos et al. 1997), because the reduction of selenate to selenite is often a rate-limiting step. Certain plant and microbe species may not have the same rate limitations on Se metabolism as others. For instance, microbes living in the rhizosphere of plant, the highest volatilizing cell, appear to efficiently metabolize Se such that 77 % of the Se was present in organic forms. Selenium volatilization may be enhanced through managing hydrological conditions, judicious choice of plant species, altering carbon availability to promote microbial activity, or seeding with microbes and microalgae (Lin et al. 2002; Bañuelos et al. 1997). Another possible approach is to genetically manipulate microbes, algae, or plants to increase their output of volatile Se.

## 13.3 Strategies for Enhancing the Phytoremediation of Se

### 13.3.1 Genetic Engineering

Recent research has shown that genetic modification of plants can increase their phytoremediation efficiency (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). Identifying candidate genes for transfer and/or overexpression is critical. One useful approach is to overexpress enzymes catalyzing rate-limiting steps; for example, ATP sulfurylase (APS), which facilitates the reduction of selenate to selenite, is rate-limiting with respect to the production of reduced, organic Se compounds (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). Indian mustard plants overexpressing APS have increased tolerance and accumulation of selenium (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). However, APS Indian mustard does not volatilize more Se than wild type (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). This is likely due to additional downstream rate-limiting steps in the S/Se assimilation pathway. Indeed, Se volatilization rates from Indian mustard are similar from selenocysteine (SeCys) and selenite, while volatilization from selenomethionine (SeMet) is many fold faster (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). This suggests the involvement of a rate-limiting step in the synthesis of SeMet from SeCys. To test this hypothesis, Indian mustard plants overexpressing cystathionine-c-synthase (CGS) were developed. The CGS Indian mustard had enhanced tolerance to selenite and volatilized Se two to three times faster than wild type, while at the same time accumulating less Se in roots and shoots (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004).

### 13.3.2 Chloroplast Engineering

After all the work involved in identifying key genes, transforming plants, and evaluating Se of phytoremediation potential in laboratory and greenhouse experiments, there are still regulatory barriers to overcome in getting transgenic plants in the field, remediating contaminated sites. Such constraints have spurred researchers to innovate new methods of creating transgenic plants that will be more palatable to the public and pose less potential risk of hybridizing with nearby plants or adversely affecting wildlife. One such technique is the use of chloroplast transformation, the use of which prevents the escape of transgenes via pollen to related weeds and crops (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). This method was recently used to stably integrate the bacterial merAB operon into the chloroplast genome of tobacco. The resulting plants were substantially more resistant to highly toxic organic mercury, in the form of phenylmercuric acetate, than wild type (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004). Previously, all attempts to genetically engineer plants with improved phytoremediation had been based on transformation of the nuclear genome. Other important advantages of chloroplast transformation include the fact that codon optimization is not required to improve expression of bacterial transgenes, very high levels of transgene expression (up to 46 % w/w of total protein), absence of gene silencing, absence of positioning effect, ability to express multiple genes in a single transformation event, and sequestration of foreign proteins in the organelle, preventing adverse interactions with cytoplasm (Bañuelos et al. 2005, 2007; LeDuc et al. 2006; Van Huysen et al. 2004).

### 13.3.3 Se of Hyperaccumulator

Some plants naturally hyperaccumulate metals, meaning that they are able to accumulate metals to ppm levels in the order of thousands in their shoots. Hyperaccumulating plants have been identified for a number of metals (Freeman and Bañuelos

2011; Valdez Barillas et al. 2012; Freeman et al. 2012). The phytoremediation efficiency of most metal hyperaccumulators is limited by their slow growth rate and low biomass. Using genetic engineering we should be able to enhance phytoremediation potential by transforming fast-growing host plants with key genes from natural hyperaccumulators.

One such gene is selenocysteine methyltransferase (SMT), cloned from the Se hyperaccumulator Astragalus bisulcatus (Freeman and Bañuelos 2011; Valdez Barillas et al. 2012; Freeman et al. 2012). SMT converts the amino acid SeCys to the nonprotein amino acid (MetSeCys). By doing so, it diverts the flow of Se from the Se amino acids that may otherwise be incorporated into protein, leading to alterations in enzyme structure and function and toxicity (Freeman and Bañuelos 2011; Valdez Barillas et al. 2012; Freeman et al. 2012). Transgenic plants overexpressing SMT show enhanced tolerance to Se, particularly selenite, and produced three to sevenfold more biomass than wild type and threefold longer root lengths (Freeman and Bañuelos 2011; Valdez Barillas et al. 2012; Freeman et al. 2012). The SMT plants accumulated up to fourfold more Se than wild type, with higher proportions in the form of MetSeCys. Additionally, SMT Arabidopsis and SMT Indian mustard volatilized Se two to three times faster when treated with SeCys and selenate, respectively.

## 13.4 Use of Plant-Microorganisms in the Remediation of Se

The diversity and adaptability of microorganisms allows them to thrive in harsh, toxic environments where higher plants are unable to grow. As such, microbes represent a potential reservoir of important genes involved in metal detoxification. Highly efficient phytoremediating plants could be generated that overexpress microbial genes (Schmidt et al. 2013; Vickerman et al. 2004). Many such microorganisms have been found, but much remains to be learned at the molecular level. One promising strategy to elucidate microbial hypertolerance and hyperaccumulation mechanisms is to compare natural cultures with adapted cultures. The genetic and biochemical basis for this adaptation is an interesting target for genetic engineering. For example, a single-celled freshwater microalgae (*Chlorella* sp.) is interesting because of its ability to efficiently reduce selenate (Schmidt et al. 2013; Vickerman et al. 2004). In fact, in just 24 h, 87 % of the selenate accumulated had been converted to intermediate organic compounds. This capacity to efficiently reduce Se may have evolved in microalgae because their large surface-to-volume ratio means that their Se uptake rates can be relatively high while space available for storage of toxic Se compounds is small. Since high rates of accumulation have toxic effects on long-term development, the ability to convert selenate to DMSe could be a big advantage (Schmidt et al. 2013; Vickerman et al. 2004). The potential of these microalgae for bioremediation is limited, however, by the fact that uptake of selenate is strongly inhibited by the presence of sulfate in the medium. Without sulfate, the *Chlorella* sp. was able to remove 90 % of supplied selenate through accumulation and volatilization. These high rates were not observed in the presence of 1 mM sulfate, where only 1.8 % of Se was volatilized. Without sulfur, the Chlorella had 2.6 times higher sulfate transporter activity, which most likely leads to the higher rates of selenate uptake. It has previously been observed that sulfate deprivation can lead to increased activity of enzymes involved in sulfate uptake and reduction (Schmidt et al. 2013; Vickerman et al. 2004). However, since the action of selenate reduction does not appear rate-limiting in this microalga, transforming plants with the Chlorella ATP sulfurylase gene may be a useful means to increase Se volatilization rates in higher plants.

### 13.4.1 Se of Analytical Techniques

The successful use of genetic engineering to optimize plants for Se of phytoremediation depends on a thorough knowledge of the uptake and metabolism of Se of interest. Elucidating the genetic and biochemical basis for metal/metalloid tolerance and accumulation strategies is often hampered by the difficulty in determining the levels of, and positively identifying, intermediate metabolites and complexes (Montes-Bayón et al. 2002; Carvalho et al. 2001; Bañuelos et al. 2012). Fortunately, technologies are being developed and improved that should shed new light on these metabolic pathways. For example, recent work with HPLC-ICP-MS and HPLC-ESI-MS has identified selenomethylmethionine (SeMM) as the predominant Se species in *Brassica juncea* roots supplied with SeMet (Montes-Bayón et al. 2002; Carvalho et al. 2001; Bañuelos et al. 2012). This work provides chemical evidence for the view that Se-Met is methylated to SeMM (Montes-Bayón et al. 2002; Carvalho et al. 2001; Bañuelos et al. 2012). Since roots are the primary site of Se volatilization, cleavage of SeMM appears to directly produce volatile DMSe. Similar techniques have also shown promise in elucidating the fate of As in plants, which is less well understood. In a recent study, HPLC-ICP-MS was used to analyze As metabolites in As-treated Indian mustard. Arsenic species were found bound to thiols. The ESI-Q-TOF results strongly suggest the presence of As bound to PC2, PC3, and PC4 (Montes-Bayón et al. 2002; Carvalho et al. 2001; Bañuelos et al. 2012).

## 13.5 Conclusions

Recent research has shown that phytoremediation can be an effective method for removing and detoxifying heavy metals and metalloids such as Se from contaminated soil and water. The identification of unique genes from natural Se hyperaccumulators and their subsequent transfer to fast-growing species is another promising approach as demonstrated with SMT transgenic plants. Microbial genomes may provide another reservoir of candidate genes for use in genetic engineering strategies. Advances in optimizing plants for phytoremediation will depend on gaining new knowledge about the fate and transport of Se in plants and innovative technologies to improve the acceptability of transgenic organisms for phytoremediation.

## References


Azaizeh H, Salhani N, Sebesvari Z, Shardendu S, Emons H (2006) Phytoremediation of selenium using subsurface-flow constructed wetland. Int J Phytoremediation 8(3):187–198

Bañuelos GS (2001) The green technology of selenium phytoremediation. Biofactors 14(1–4):255–260

Bañuelos GS, Ajwa HA, Wu L, Guo X, Akohoue S, Zambrzuski S (1997) Selenium-induced growth reduction in Brassica land races considered for phytoremediation. Ecotoxicol Environ Saf 36(3):282–287

Bañuelos GS, Lin ZQ, Wu L, Terry N (2002) Phytoremediation of selenium-contaminated soils and waters: fundamentals and future prospects. Rev Environ Health 17(4):291–306

Bañuelos G, Terry N, Leduc DL, Pilon-Smits EA, Mackey B (2005) Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. Environ Sci Technol 39(6):1771–1777

Bañuelos G, LeDuc DL, Pilon-Smits EA, Terry N (2007) Transgenic Indian mustard overexpressing selenocysteine lyase or selenocysteine methyltransferase exhibit enhanced potential for selenium phytoremediation under field conditions. Environ Sci Technol 41(2): 599–605

Bañuelos GS, Walse SS, Yang SI, Pickering IJ, Fakra SC, Marcus MA, Freeman JL (2012) Quantification, localization, and speciation of selenium in seeds of canola and two mustard species compared to seedmeals produced by hydraulic press. Anal Chem 84(14):6024–6030

Carvalho KM, McGettigan MJ, Martin DF (2001) GC/MS analysis of volatile organic selenium species produced during phytoremediation. J Environ Sci Health A Tox Hazard Subst Environ Eng 36(7): 1403–1409

Freeman JL, Bañuelos GS (2011) Selection of salt and boron tolerant selenium hyperaccumulator Stanleya pinnata genotypes and characterization of Se phytoremediation from agricultural drainage sediments. Environ Sci Technol 45(22):9703–9710

Freeman JL, Marcus MA, Fakra SC, Devonshire J, McGrath SP, Quinn CF, Pilon-Smits EA (2012) Selenium hyperaccumulator plants Stanleya pinnata and Astragalus bisulcatus are colonized by Se-resistant, Se-excluding wasp and beetle seed herbivores. PLoS One 7(12):505–516

LeDuc DL, AbdelSamie M, Móntes-Bayon M, Wu CP, Reisinger SJ, Terry N (2006) Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. Environ Pollut 144(1):70–76

Lin ZQ, de Souza M, Pickering IJ, Terry N (2002) Evaluation of the macroalga, muskgrass, for the phytoremediation of selenium-contaminated agricultural drainage water by microcosms. J Environ Qual 31(6):2104–2110

Lin ZQ, Terry N, Gao S, Hussein H, Ye ZH (2010) Vegetation changes and partitioning of selenium in 4-year-old constructed wetlands treating agricultural drainage. Int J Phytoremediation 12(3):255–267

Montes-Bayón M, Yanes EG, Ponce de León C, Jayasimhulu K, Stalcup A, Shann J, Caruso JA (2002) Initial studies of selenium speciation in Brassica juncea by LC with ICPMS and ES-MS detection: an approach for phytoremediation studies. Anal Chem 74(1):107–113

Pilon-Smits EA, LeDuc DL (2009) Phytoremediation of selenium using transgenic plants. Curr Opin Biotechnol 20(2):207–212

Schmidt R, Tantoyotai P, Fakra SC, Marcus MA, Yang SI, Pickering IJ, Bañuelos GS, Hristova KR, Freeman JL (2013) Selenium biotransformations in an engineered aquatic ecosystem for bioremediation of agricultural wastewater via brine shrimp production. Environ Sci Technol 47(10):5057–5065

Shardendu, Salhani N, Boulyga SF, Stengel E (2003) Phytoremediation of selenium by two helophyte species in subsurface flow constructed wetland. Chemosphere 50(8):967–973

Valdez Barillas JR, Quinn CF, Freeman JL, Lindblom SD, Fakra SC, Marcus MA, Gilligan TM, Alford ÉR, Wangeline AL, Pilon-Smits EA (2012) Selenium distribution and speciation in the hyperaccumulator Astragalus bisulcatus and associated ecological partners. Plant Physiol 159(4):1834–1844

Van Huysen T, Terry N, Pilon-Smits EA (2004) Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine-gamma-synthase. Int J Phytoremediation 6(2):111–118

Vickerman DB, Trumble JT, George GN, Pickering II, Nichol H (2004) Selenium biotransformations in an insect ecosystem: effects of insects on phytoremediation. Environ Sci Technol 38(13):3581–3586

Zhu YG, Pilon-Smits EA, Zhao FJ, Williams PN, Meharg AA (2009) Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. Trends Plant Sci 14(8):436–442

# 14 Phytoremediation Using Microbial Communities: I

Mohammad Miransari

## 14.1 Introduction

The presence of heavy metals in the soil, which are usually resulted by anthropogenic activities (Schachtschabel et al. 1992), can be unfavorable to the environment plant growth. Different strategies have been used to remediate the adverse effects of heavy metals on the environment and plant growth including the use of tolerant plants such as hyperaccumulators and the use of soil microbes such arbuscular mycorrhizal (AM) fungi, plant growth-promoting rhizobacteria (PGPR) (Miransari 2011a; 2014), and endophytic microbes (Shen et al. 2013).

Different combinations of bioremediation strategies may be used including the single use of hyperaccumulators, the single or combined use of microbial strains and species, and the combined use of plants and tolerant plants such as hyperaccumulators with their symbiotic or nonsymbiotic microbes. Under any contaminated conditions the right plant and microbial species must be selected, tested, and used (Rajkumar et al. 2009). Miransari (2011a) suggested that if the ability of hyperaccumulators is improved by their association with soil microbes such as mycorrhizal fungi, it can be more likely to remediate the polluted soils. It is because some of the hyperaccumulators are not able to develop a symbiotic association with their host plants, which have been attributed to the production of some exudates from the host plant roots. Accordingly, if the symbiotic microbe is able to inoculate the host plant and if such a potential is improved by using different techniques, the remediation ability of the host plant improves.

Different techniques are used by tolerant plants to handle the stress of heavy metals. For example, plants can stabilize the metal in the rhizosphere, can increase its solubility, and may also absorb high rate of the metal and accumulate it in their vacuoles. However, it may be a more effective method if the alleviating ability of the host plant is improved by their association with mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), and endophytic bacteria, which are usually found in different parts of their host plants such as roots and the aerial parts. Accordingly, some of the latest findings related to the use of soil microbes for the bioremediation of polluted areas are presented.

## 14.2 Heavy Metals

Heavy metals (53 elements) are categorized based on their density (Holleman and Wiberg 1985). Some of the heavy metals such as iron (Fe), copper (Cu), zinc (Zn), and nickel (Ni) are required for plant growth and crop production. However, at high concentration, they adversely affect plant growth. There are different functions for heavy metals in plant including their role in the redox reactions, catalyzing enzymatic activities, as electron carriers and their presence in the structure of DNA and RNA (Zenk 1996).

The adverse effects of heavy metals on the growth of plant is by influencing the functionality of enzymes and hence protein structure. They may also substitute a required element in the cellular structure of different plant tissues or different biochemical reactions. The activity, functionality, and permeability of plasma membrane are affected by heavy metals. The oxidative stress of heavy metals can also adversely affect plant growth by the production of reactive oxygen species (Sajedi et al. 2010, 2011).

Using different mechanisms, plants must be able to keep ion homeostasis in their tissues by detoxifying the adverse effects of heavy metals so that they can function properly (Clemens 2001). For example, heavy metals are chelated by organic products and cellular membranes are able to bind heavy metals. The presence of products such as metallothioneins and phytochelatins, inside cell, with high affinity for

M. Miransari, Ph.D. (✉)
AbtinBerkeh Ltd., Company, #37, Nazer Alley, Malek Ave., Isfahan, 815474311, Iran
e-mail: miransari1@gmail.com

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the absorption of heavy metals, can control their cellular concentration by their transfer to the vacuoles as they cross the tonoplast (Hall 2002).

## 14.3 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal (AM) fungi are among the soil fungi, which are able to establish symbiotic association with most terrestrial plants. In such a symbiosis the fungal spores are able to germinate, in the presence of the host plant, and produce an extensive hyphal network. However, it has been indicated that even if the host plant is not present, the fungal spores are able to germinate but do not proceed with the next stages of symbiosis (Miransari 2010).

The extensive hyphal network can significantly increase the absorbing capacity of the host plant for water and nutrients. The network also produces two important organelles called vesicles and arbuscules. The former is a vacuolated organelle, which is able to store high rate of elements and different biochemicals. Such a property can be especially useful to the growth of host plant under stress. For example, when the plant is subjected to salt stress, to alleviate the stress and keep a suitable rate of $K^+/Na^+$, the fungi store a high rate of $Na^+$ and $Cl^-$ in their vesicles. The arbuscules are branched like structures, which are the interface for the exchange of nutrients with the host plant roots and hence can significantly increase the nutrient-absorbing potential of the host plant (Audet and Charest 2007; Daei et al. 2009).

The extensive network of fungal hyphae is able to significantly increase the absorbing potential of the host plant by growing around the host plant roots, in the zones and micropores, where even the finest root hairs are not able to grow and absorb water and nutrients. Such a property can especially be useful under stress, because the increased uptake of water and nutrients from a higher volume of soil alleviates the adverse effects of stress on the growth of the host plant (Miransari et al. 2008). The alleviating effects of AM fungi under stresses such as heavy metals are presented in the following.

## 14.4 Mycorrhizal Plant Under Stress

Mycorrhizal fungi can favorably affect plant growth under different conditions including stress by the following mechanisms: (1) increasing plant water and nutrient uptake, (2) improving the structure of soil by the production of glomalin or adherence of soil particles, (3) interacting with the other soil microbes, (4) production of different biochemicals, (5) affecting plant systemic required resistance, (6) activating different plant genes, and (7) controlling the unfavorable effects of pathogens (Gaur and Adholeya 2004; Gonzalez-Chavez et al. 2004; Hildebrandt et al. 2006).

Under stress usually mycorrhizal plants can perform more efficiently than non-mycorrhizal plants due to the favorite effects of the fungi, as previously mentioned on the growth of the host plant. However, the fungal alleviating effects are determined by different factors, such as the fungal and the host plant species, the properties of the soil and climate, etc. (Miransari 2010).

It has been indicated that if the fungal species are isolated from stress conditions, they will be more efficient to alleviate the stress (Daei et al. 2009). The interactions between the fungi and the other soil microbes in the root rhizosphere are also an important parameter affecting the fungal performance. Accordingly, there are some negative and positive interactions, which may enhance or decrease the alleviating potential of mycorrhizal fungi on the growth of the host plant under stress (Miransari 2011b).

Mycorrhizal fungi with their unique abilities are able to alleviate the stress of heavy metals on the growth of the host plant by the following mechanisms. The fungi are able to: (1) absorb high rate of heavy metals in their hyphae, (2) influence the availability of heavy metals in the rhizosphere, (3) affect plant growth and hence the absorption of heavy metals, and (4) activate the related stress genes in the host plant (Kaldorf et al. 1999; Khan 2005, 2006; Miransari 2011a, b).

Hyperaccumulators as tolerant plants to the stress of heavy metals are able to absorb and accumulate high rate of heavy metals in their tissues, while their growth remains unaffected. However, the important point is how to improve the ability of hyperaccumulators to absorb higher rate of heavy metals. Usually the hyperaccumulators, which are from the Brassicaceae family, are not host to mycorrhizal fungi, with the exception of species such as *T. praecox*, which are able to develop symbiosis with mycorrhizal fungi (Pawlowska et al. 2000).

If the symbiotic ability of *T. praecox* with the fungi increases, the plant can be used more efficiently under heavy metal stress. According to Vogel-Mikus et al. (2006), *T. praecox* developed symbiotic association with mycorrhizal fungi and increased the uptake of nutrients by the host plant; however, it decreased the absorption of heavy metals by the host plant. This indicates the both alleviating effects of the fungi on the pollution of soil and on the growth of the host plant.

It is important to select the right fungal species, so that the process of bioremediation can be performed more efficiently. Usually if the inoculum is selected from the stress areas, it will be able to more efficiently handle the stress. Such species have developed mechanisms with time, which can make them tolerate the stress and hence develop a symbiotic association with the host plant (Khan 2005).

Although mycorrhizal fungi are able to alleviate the stress of heavy metals in association with their host plants, high level of stress can adversely affect the improving abilities of the fungi. For example, Chen et al. (2003) indicated that under non-stressed conditions *Glomus caledonium* was able to colonize the host plant roots at a rate of more than 70 %; however at concentration of 300 and 600 mg/kg Zn, the colonization rate was 50 %. Similarly, Duponnois et al. (2006) indicated that root colonization by mycorrhizal fungi decreased from 60 to 20 % under Cd pollution of 560 mg/kg. However, the positive interactions between mycorrhizal fungi and florescent pseudomonas increased the colonization rate from 32 to 45 % (Miransari 2011a).

The following indicate how heavy metals may be translocated by the fungal hyphae to the root tissues: (1) The cellular wall or the fungal vacuoles may accumulate the heavy metals, (2) siderophore and glomalin may sequester the heavy metals in the root apoplasm or in the soil (Gonzalez-Chavez et al. 2004), (3) phytochelatins or metallothioneins may deposit heavy metals in the cells of fungi or plant, and (4) the metal transporters of plasmalemma or tonoplast in both symbionts may translocate heavy metals from cytoplasm (Galli et al. 1994; Schutzendubel and Polle 2002).

In the roots of mycorrhizal plants, the heavy metal content is subject to change indicating the role of mycorrhizal fungi on the expression of the related genes at transcription and translation levels (Repetto et al. 2003; Ouziad et al. 2005). The expression of *GintZnT1* in the hyphae of *G. intraradices* indicates its alleviating role on the stress of excess Zn (Gonzalez-Guerrero et al. 2005). Under the stress of Cd and Cu, the expression of *GintABC1* transporters and its detoxification effects in the fungal hyphae indicate how such a gene may affect the alleviation of stress (Gonzalez-Guerrero et al. 2006). Under the contamination of excess Zn, the expression of the related genes resulted in the production of glutathione S-transferase, which is a Zn transporter and can alleviate the oxidative stress (Smith et al. 2004; Hildebrandt et al. 2006).

## 14.5 Mechanisms of Stress Alleviation

There are different mechanisms by which the plant can survive the stress including the plant properties or its association with the soil microbes such as mycorrhizal fungi, PGPR, and endophytic microbes. As previously mentioned there are hyperaccumulator plants, which are able to absorb and accumulate high rate of heavy metals in their tissues, while their growth remains unaffected. Most of such plants are not able to establish a symbiotic association with mycorrhizal fungi because of their root products. However, there are a few family species, which are able to be in symbiotic association with the fungi (Assuncao et al. 2001). If such kind of ability is improved, for example, through their symbiosis with the fungi, it would be possible to increase the remediation potential of the host plant under stress.

Hyperaccumulators have some genes, which are expressed under the stress of heavy metals and make the plant localize the heavy metals in its tissues, without affecting its growth. Under stress plants use different mechanisms to alleviate the stress. Usually plants will not accumulate high rate of heavy metals and just hyperaccumulators are able to absorb high concentration of heavy metals and handle the stress. Among the plant species just 0.2 % including 450 species (most of them Ni accumulators) have the ability of hyperaccumulating heavy metals and hence are called hyperaccumulators (Miransari 2011a).

Among the species of *Thlaspi* family, the hyperaccumulators of Ni, Zn, Cd, and Pb include 23, 10, 3, and 1 plant species, respectively. *T. praecox*, *T. caerulescens*, and *T. goesingense* are the three species of Zn hyperaccumulators (Vogel-Mikus et al. 2005; 2008). However, *T. caerulescens* is the most well-known hyperaccumulators of Zn with the ability to grow in the soils with serpentine including Zn, Cd, Co, Pb, Ni, Cr and absorb up to 30,000 and 1,000 mg/kg of Zn and Cd, respectively, and its growth is not affected (Assuncao et al. 2001). Based on a dry weight, the accumulation of Zn, Cd, and Pb by this species is up to 1.5, 0.6, and 0.4 %, respectively. There are some specific metal transporters, which enable the host plant to absorb and transfer high rates of heavy metals to different plant tissues (Vogel-Mikus et al. 2005; Pongrac et al. 2007). Plant cellular vacuoles of *T. caerulescens* have a high ability to absorb Zn at high concentration (Kupper et al. 1999).

Usually hyperaccumulators are able to absorb heavy metals at high concentration using the following mechanisms: (1) production of organic molecules including organic acids, nicotinamide, glutathione, cysteine, histidine, and other thiols, which are able to bind heavy metals and form organometallic complexes (Kupper et al. 2004), (2) the transport ability, (3) potential of compartmentation, and (4) the transfer of such products to the cellular vacuoles (Kupper et al. 1999).

The weak point about hyperaccumulators is that they are not able to produce high rate of biomass. Hence, a method, which may result in higher production of hyperaccumulator biomass, may be of practical use and increase the efficiency of such plants for bioremediation. However, the strength point about hyperaccumulators is the presence of transporters such as for Zn (*ZNT1*) with a high affinity for heavy meals that enable the plant to transfer Zn across the plasma membrane of root cell, in the xylem, and eventually to the leaf. The related plant genes must be expressed so that the transporter can accumulate high rate of Zn in plant (Pence et al. 2000).

The Zn transporter genes in *T. caerulescens* including ZNT1 and ZNT2 as well as ZTP1 (similar to ZAT of Arabidopsis) were isolated by Assuncao et al. (2001). Compared with *T. arvense*, the high expression of such genes in *T. caerulescens* indicates that the latter is a heavy metal hyperaccumulator. The Zn hyperaccumulating of *T. goesingense* is related to the presence of a protein called TgMTP1 in plant cellular membrane. The high concentration of this protein in the vacuolar membrane of the plant shoot can significantly increase the transport of heavy metals to the vacuoles and hence increases plant hyperaccumulating ability and tolerance under stress. Zn concentration determines the activity of the protein to accumulate high Zn concentration in vacuoles (Gustin et al. 2009).

Different parameters such as pH, temperature, and the ability of *T. caerulescens* to produce organic acids by its roots determine plant ability to hyperaccumulate heavy metals. For example, at pH 5–6 and during winter, the plant is able to absorb higher rate of heavy metals. The plant is also able to find heavy metals in the rhizosphere more efficiently, compared with non-hyperaccumulating plants. It is because the production of organic products by the roots of *T. caerulescens* can increase the availability of heavy metals in the soil (Pence et al. 2000; McGrath et al. 2006; Milner and Kochian 2008).

Indicating the mechanisms, which can make the plant hyperaccumulate heavy metals, can be useful for the enhanced ability of hyperaccumulators under stress and for the important process of biofortification (Hanikenne et al. 2008; Verbruggen et al. 2009). Briefly, the processes of hyperaccumulation by tolerant plants include the following: plant uptake by roots, xylem loading and unloading, chelating for detoxification, vacuolar uptake, homeostasis, etc. (Verbruggen et al. 2009).

By the bioremediation processes including extracting, degrading, rhizofiltering, stabilizing, and volatilizing, the pollutants such as heavy metals are collected from the soil. By the extracting process, the heavy metals are absorbed from the soil by the harvestable parts of the plant. By the degrading processes, the pollutants are decomposed by plant and microbes. The rhizofiltering process results in the absorption of heavy metals from the wastewaters. The stabilization process is by decreasing the mobility of heavy metals and their subsequent immobilization as a result of microbial and root activities. Plant roots are also able to volatilize pollutants to the atmosphere through the process of volatilization (Khan 2005). The extraction process is the absorption of pollutants by different plant tissues. However, it is more effective if the concentration of heavy metals, for example, is not higher than a certain amount (Khan 2006).

## 14.6 PGPR, Endophytic Microbes, and Stress of Heavy Metals

With respect to the disadvantages of non-biological methods used for the remediation of polluted environments, such as expenses, and being environmentally non-recommendable, the use of biological methods, which is usually the use of hyperaccumulators and/or microbes, has been tested and proved to be useful on the remediation of polluted environments (Glick 2003; Kuiper et al. 2004; McGrath et al. 2006).

Usually under the stress of heavy metals, microbes can help the host plant to alleviate the stress by: (1) increasing the metal mobility by the production of biosurfactants (Herman et al. 1995), organic acids (Di Smine et al. 1998), and siderophores and (2) enhancing plant growth by mycorrhizal fungi (Khan 2006) or PGPR (Zhuang et al. 2007). Under the stress of heavy metal, plants transfer most of heavy metals to their roots so that the aerial parts can function more efficiently. Plant species, morphology, and physiology are also among the parameters affecting plant efficiency under the stress of heavy metals (Audet and Charest 2007).

In the combined use of microbes and plants to remediate the environment from pollutants, the carbon (C) source is supplied by plant roots to the microbial population for the absorption and/or degradation of pollutants including heavy metals. The amount of C which is supplied by the host plant and utilized by the microbes is equal to at least 40 % of the photosynthates produced by plant roots (Lynch and Moffat 2005).

PGPR and endophytic microbes are also able to alleviate the stress of heavy metals on the growth of their host plants using the following mechanisms:

(1) Binding heavy metals by producing organic products and their subsequent increased availability and detoxification (Dobbelaere et al. 2003; Dimkpa et al. 2009; Rajkumar et al. 2009).
(2) They can also adjust the concentration of heavy metals in the soil by affecting plant growth (Lebeau et al. 2008).
(3) The production of enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase by PGPR, which is able to catalyze ACC (ethylene prerequisite) to α-ketobutyrate and ammonium (Glick 2003).
(4) Auxin production by PGPR increases the bioavailability of heavy metals and their subsequent uptake by plant (Zaidi et al. 2006).

The endophytic microbes are able to colonize the internal parts of their host plant. In such kind of association, plant properties do not look symbiotic and plant growth is not also adversely affected but the physiological alteration enhances plant growth. Such physiological effects include the production of different products such as osmolytes, the changed stomata activity, the decreased potential of membrane, and the affected rate of phospholipids in the membrane (Sheng et al. 2008).

PGPR and endophytic microbes can affect the process of bioremediation of heavy metals by affecting the bioextraction and biostabilization processes by plant. By increasing the bioavailability of heavy metals, the process of bioextraction by plant increases and hence higher rate of heavy metals is absorbed by the host plant. However, the process of biostabilization can affect the translocation of heavy metals in plant by decreasing their availability in plant roots (Rouch et al. 1995; Dimkpa et al. 2009; Rajkumar et al. 2010).

Interestingly, the endophytic bacteria including gram-negative and gram-positive have been isolated from the tissues of hyperaccumulator plants under the stress of heavy metals showing tolerance to the stress. This can be due to the fact that high concentration of heavy metals in the tissues can make the endophytic bacteria adapt to the stress.

The following mechanisms may affect the results of heavy metal bioaugmentation by the soil microbes: (1) the properties of metals, (2) the type of experiment (field, greenhouse, or laboratory), (3) the conditions of experiment, (4) microbial species and strains, and (5) plant species. However, the effectiveness of bioaugmentation process is determined by the following: (1) root and shoot growth affecting the process of bioextraction and microbial activities, and (2) the properties of soil affecting the bioavailability of heavy metals and hence their uptake by plant (Lebeau et al. 2008).

The bioavailability of heavy metals is important affecting their uptake by plant. Parameters including the properties of soil, climate, heavy metal, and plant affect the bioavailability of plants. There is a wide range of PGPR, which can be used for the bioremediation of soil polluted with heavy metals including *Pseudomonas*, *Rhizobium*, *Burkholderia*, *Agrobacterium*, *Alcaligenes* (*Ralstonia*), *Azospirillum*, *Serratia*, *Arthrobacter*, *Bacillus*, and *Azotobacter* (Glick 2003, 2010).

As previously mentioned the use of endophytic microbes including fungi and bacteria is also useful for the bioremediation of polluted areas. For example, Shen et al. (2013) investigated the role of the endophytic fungi *Peyronellaea* under the stress of heavy metals on the growth of maize (*Zea mays* L.) in a polluted soil. They indicated that the use of endophytic fungi increased maize tolerance to the stress of heavy metals; however, the isolation of the fungi from a contaminated site did not affect their efficiency on the alleviation of stress related to non-contaminated sites. The absorption and accumulation of heavy metal by the plant was also affected by the fungi. They found that the origin of the fungi may not affect their efficiency on the alleviation of heavy metal stress.

## 14.7 Conclusion and Future Perspectives

There is a wide range of soil microbes, which can be used for the bioremediation of polluted soils such as arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria, and endophytic microbes. Different mechanisms are used by the soil microbes to alleviate the stress of heavy metals affecting the bioremediation of soil or plant growth. The important point about treating heavy metals in the soil is their bioavailability, which is affected by both microbial and plant activities. The use of appropriate microbe and plant strain and species is of great significance for the process of bioremediation. The use of plants, which are hyperaccumulators, can significantly affect bioremediation; however, if the symbiotic association between hyperaccumulators and the microbes can be improved, the efficiency of bioremediation may also increase. The strategies, which are used for bioremediation, must have the following properties: (1) economically and environmentally sustainable, (2) useable in a large area, (3) repeatable, and (4) applicable to a combination of heavy metals. The important point of selecting microbes from stress areas and the interactions between them and with the host plant can also affect the results of bioremediation. Future research may focus on: (1) using the right combination of soil microbes, (2) how the applicability of microbes under different conditions of heavy metal stress may increase, (3) how the response of the host plants under stress may be improved, and (4) how the growth of plants including hyperaccumulators may increase under stress.

## References

Assuncao AGL, Martins P, Folter S, Vooijs R, Schat H, Aarts G (2001) Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 24:217–226

Audet P, Charest C (2007) Heavy metal phytoremediation from a meta-analytical perspective. Environ Pollut 147:231–237

Chen B, Li XL, Tao HQ, Christie P, Wong MH (2003) The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere 50:839–846

Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486

Daei G, Ardakani M, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Di Simine CD, Sayer JA, Gadd GM (1998) Solubilization of zinc phosphate by a strain of Pseudomonas fluorescens isolated from a forest soil. Biol Fertil Soils 28:87–94

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Duponnois R, Kisa M, Assigbetse K, Prin Y, Thioulouse J, Issartel M, Moulin P, Lepage M (2006) Fluorescent pseudomonads occurring in *Macrotermes subhyalinus* mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Sci Total Environ 370:391–400

Galli U, Schüepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plant 92:34–368

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86:528–534

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323

Gonzalez-Guerrero M, Azcon-Aguilar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol N (2005) Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130–140

Gonzalez-Guerrero M, Azcon-Aguilar C, Ferrol N (2006) GintABC1 and GintMT1 are involved in Cu and Cd homeostasis in Glomus intraradices. Abstracts of the 5th international conference on Mycorrhiza, Granada, Spain

Gustin J, Loureiro M, Kim D, Na G, Tikhonova M, Salt D (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. Plant J 57:1116–1127

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11
Hanikenne M, Talke I, Haydon M, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U (2008) Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. Nature 453:391–395
Herman D, Artiola J, Miller R (1995) Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. Environ Sci Technol 29:2280–2285
Hildebrandt U, Ouziad F, Marner FJ, Bothe H (2006) The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. FEMS Microbiol Lett 254:258–267
Holleman A, Wiberg E (1985) Lehrbuch der anorganischen chemie. Wiley, Berlin
Kaldorf M, Kuhn A, Schroder W, Hildebrandt U, Bothe H (1999) Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J Plant Physiol 154:718–728
Khan A (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364
Khan AG (2006) Mycorrhizoremediation an enhanced form of phytoremediation. J Zhejiang Univ Sci B 7:503–514
Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 17:6–15
Kupper H, Zhao F, McGrath S (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–311
Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PHM (2004) Tissue- and age-dependent differences in the complexation of cadmium and zinc in the cadmium/zinc hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by X-ray absorption spectroscopy. Plant Physiol 134:748–757
Lebeau T, Braud A, Jezequel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: A review. Environ Pollut 153:497–522
Lynch J, Moffat A (2005) Bioremediation – prospects for the future application of innovative applied biological research. Ann Appl Biol 146:217–221
McGrath SP, Lombi E, Gray CW, Caille N, Dunham SJ, Zhao FJ (2006) Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis* halleri. Environ Pollut 141:115–125
Milner M, Kochian L (2008) Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Ann Bot 102:3–13
Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2008) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biol Biochem 40:1197–1206
Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stresses. Plant Biol 12:563–569
Miransari M (2011a) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653
Miransari M (2011b) Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl Microbiol Biotechnol 89:917–930, Review article
Miransari M (2014) Plant growth promoting rhizobacteria. J Plant Nutr 37:2227–2235
Ouziad F, Hildebrandta U, Schmelzerb E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol 162:634–649
Pawlowska TE, Chaney RL, Chin M, Charavat I (2000) Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. Appl Environ Microbiol 66:2526–2530
Pence N, Larsen P, Ebbs S, Letham D, Lasat M, Garvin D, Eide D, Kochian L (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci U S A 97:4956–4960
Pongrac P, Vogel-Mikus K, Kump P, Necemer M, Tolrà R, Poschenrieder C, Barceló J, Regvar M (2007) Changes in elemental uptake and arbuscular mycorrhizal colonization during the life cycle of Thlaspi praecox Wulfen. Chemosphere 69:1602–1609
Rajkumar M, Ae N, Prasad M, Freitas H (2009) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149
Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149
Repetto O, Bestel-Corre G, Dumas-Gaudot E, Berta G, Gianinazzi-Pearson V, Gianinazzi S (2003) Targeted proteomics to identify cadmium-induced protein modifications in *Glomus mosseae-inoculated* pea roots. New Phytol 157:555–567
Rouch DA, Lee TOB, Morby AP (1995) Understanding cellular responses to toxic agents: a model for mechanisms-choice in bacterial resistance. J Ind Microbiol 14:132–141
Sajedi NA, Ardakani MR, Rejali F, Mohabbati F, Miransari M (2010) Yield and yield components of hybrid corn (*Zea mays* L.) as affected by mycorrhizal symbiosis and zinc sulfate under drought stress. Physiol Mol Biol Plants 16:343–351
Sajedi NA, Ardakani MR, Madani H, Naderi A, Miransari M (2011) The effects of selenium and other micronutrients on the antioxidant activities and yield of corn (*Zea mays* L.) under drought stress. Physiol Mol Biol Plants 17:215–222
Schachtschabel P, Blume H-P, Brummer G, Hartge K-H, Schwertmann U (1992) Lehrbuch der bodenkunde. Ferdinand Enke, Stuttgart
Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365
Shen M, Liu L, Li D, Zhou W, Zhou Z, Zhang C, Luo Y, Wang H (2013) The effect of endophytic *Peyronellaea* from heavy metal-contaminated and uncontaminated sites on maize growth, heavy metal absorption and accumulation. Fungal Ecol 6:539–545
Sheng XF, He LY, Wang QY, Ye HS, Jiang C (2008) Effects of inoculation of biosurfactant producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium amended soil. J Hazard Mater 155:17–22
Smith AP, DeRidder BP, Guo WJ, Seeley EH, Regnier FE, Goldsbrough PB (2004) Proteomic analysis of Arabidopsis glutathione S-transferases from benoxacor- and copper treated seedlings. J Biol Chem 279:26098–26104
Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776
Vogel-Mikus K, Drobne D, Regvar M (2005) Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ Pollut 133:233–242
Vogel-Mikus K, Pongrac P, Kump P, Necemer M, Regvar M (2006) Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. Environ Pollut 139:362–371
Vogel-Mikus K, Regvar M, Mesjasz-Przybylowicz J, Przybylowicz W, Simcic J, Pelicon P, Budnar M (2008) Spatial distribution of cadmium in leaves of metal hyperaccumulating *Thlaspi praecox* using micro-PIXE. New Phytol 179:712–721
Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64:991–997
Zenk MH (1996) Heavy metal detoxification in higher plants: a review. Gene 179:21–30
Zhuang X, Chen J, Shim H, Bai Z (2007) New advances in plant growth promoting rhizobacteria for bioremediation. Environ Int 33:406–413

# 15 Phytoremediation Using Microbial Communities: II

Elizabeth Temitope Alori

## 15.1 Introduction

Phytoremediation is a novel green technology that uses specialized plants and associated soil microbes to remove, destroy, sequester or reduce the concentrations or toxic effects of contaminant in polluted environment especially soil and water. It refers to a group of plant-based technologies that use either naturally occurring or genetically engineered plants to clean contaminated environments. This technology depends on the ability of both the plant and associated microorganisms to adapt to or survive in high-metal environments. Polluted soil poses a severe problem to both ecosystem health and land development. Soil pollution threatens the health of human, plant and animal. Soil pollution can spread to other parts of the natural environment because soil is at the confluence of many natural systems. For instance, groundwater that percolates through a polluted soil can carry soil contaminants into streams, rivers, wells and drinking water. Plants growing on polluted soil may contain harmful levels of pollutants that can be passed on to the animals and people that eat them. Dust blown from polluted soil can be inhaled directly by passers-by. Additionally, polluted soil renders valuable open land unusable for parks, recreation or commercial development. The fact that both soil minerals and soil pollutants carry small electric charges that cause each to bond with each other makes polluted soil very hard to clean. A range of technologies such as fixation, leaching, soil excavation, chemical treatment, vitrification, electrokinetics and landfill of the top contaminated soil, bioventing, thermal desorption, soil vapour extraction, biopiles, etc., have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution (Haque et al. 2008). Excavation of polluted soil for off-site treatment or disposal is labour intensive, consumes a lot of time and requires the use of heavy machinery hence very expensive (Danh et al. 2009). Therefore, cheaper on-site, or in situ, remediation techniques have recently become the focus of research. One of the most interesting and promising of these in situ techniques is phytoremediation. Using plants to remediate soil pollution comprised of two components, one by the root-colonizing microbes and the other by plants themselves which absorb, accumulate, translocate, sequester and detoxify toxic compounds to non-toxic metabolites. Plants frequently lack metabolic capacity for the degradation of many pollutants hence the need to utilize degradation ability of soil organisms. Metal tolerance of plants is generally increased by symbiotic, root-colonizing, arbuscular mycorrhizal fungi (AMF), through metal sequestration in the AMF hyphae. More also excretion of the glycoprotein glomalin by AMF hyphae can form complex metals in the soil. Exposure of plants to microorganisms within the rhizoplane protects the plants from the toxic effect of the contaminants and also takes part in phytoremediation. Resistant plants can thrive on sites that are too toxic for other plants to grow. They in turn give the microbial processes the boost they need to remove organic pollution more quickly from the soil.

The mechanism responsible for the phytoremediation of contaminated soil has been proved to be as a result of increase in microbial activity. Organic toxins, those that contain carbon such as the hydrocarbons found in gasoline and other fuels, can be broken down by microbial processes. Soil fungi, for example, improve phytoremediation ability of plants by increasing the absorptive area of the roots of plants. The efficiency of *Tithonia diversifolia* and *Helianthus annuus* in remediating soils contaminated with zinc and lead nitrates could be improved by introducing mycorrhizal fungi in order to increase the absorptive area of the roots of these plants (Adesodun et al. 2010). Plants on the other hand play a key role in determining the size and health of soil microbial populations. All plant roots secrete organic materials that can be used as food for microbes, and this creates a healthier, larger, more diverse and active

E.T. Alori, M.Sc., Ph.D. (✉)
Department of Crop and Soil Science, Landmark University, Omu Aran, Kwara State, Nigeria
e-mail: aloritope@yahoo.com

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microbial population, which in turn causes a faster breakdown of pollutants. Phytoremediation reduces contaminant levels through microbial degradation in the rhizosphere. Phytoremediation systems increase the catabolic potential of rhizosphere soil by altering the functional composition of the microbial community (Siciliano et al. 2003). Plants, through their "rhizosphere effects", support hydrocarbon-degrading microbes that assist in phytoremediation in the root zone (Nie et al. 2009). For example, root activities in perennial ryegrass and alfalfa increase the number of rhizobacteria capable of petroleum degradation in the soil (Kirk et al. 2005). In turn, healthy microbial communities enhance soil nutrient availability to the plants (Wenzel 2009). Phytoremediation process can also be enhanced by the addition of specific inocula of microorganism to contaminated soils (bioaugmentation). Also, plants that are relatively tolerant to various environmental contaminants are often stunted in the presence of the contaminant. Therefore, plant growth-promoting microorganisms can be added to the roots of plants to remedy this situation. The best bioaugmentation performance can be achieved by the use of microorganisms that are already present in the soil, since indigenous microorganisms are well adjusted to their own environment. Inoculating plants with genetically engineered strains of bacteria that degrade a specific contaminant has shown promising results. Biostimulation, a process which involves manipulating the nutrient and pH levels of the soil to increase microbial populations, can also be used to amplify the population of soil organism responsible for biodegradation. Hence, fertilizers can be used together with bioaugmentation to facilitate degradation of pollutants.

## 15.2 Advantages of Phytoremediation Using Microbes

1. Low-cost: It is less expensive than alternative engineering-based solutions such as soil excavation, incineration or land filling of the contaminated materials.
2. Aesthetically pleasing and appealing to the public. Trees and smaller plants used in phytoremediation make a site more attractive, reduce noise and improve surrounding air quality.
3. Site use and remediation can occur simultaneously.
4. In situ approach: It treats the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.
5. Environmentally friendly: Poses no health risk to neither plant, human nor animal.
6. Enhance soil nutrient availability to the plants.
7. It takes advantage of natural plant processes and requires less equipment and labour than other methods since plants do most of the work.
8. Saves energy since the site can be cleaned up without digging up and hauling soil or pumping groundwater.
9. Trees and smaller plants used in phytoremediation help control soil erosion.
10. Creates a more fertile soil as soil organic matter is increased as a result of root secretions and falling stems and leaves.
11. Phytoremediation does not degrade the physical or chemical health of the soil as compared to soil excavation method that removes the organic-matter-rich topsoil and, because of the use of heavy machinery, compacts the soil that is left behind.
12. Its by-product can find a range of other uses. Some of the plants used for phytoremediation produce metabolites or phenolic compounds that are of commercial value in the pharmaceutical industry.
13. The roots of plants used create pores through which water and oxygen can flow.

## 15.3 Limitations of Phytoremediation Using Microbes

1. A long time period is required for remediation. It is a slow process that may take many growing seasons before an adequate reduction of pollution is achieved, whereas soil excavation and treatment clean up the site quickly. Multiple metal-contaminated soils require specific metal accumulator species and therefore require a wide range of research prior to the application. The cadmium/zinc model hyperaccumulator *Thlaspi caerulescens*, for example, is sensitive towards copper (Cu) toxicity, which is a problem in remediation of Cd/Zn from soils in the presence of Cu by application of this species.
2. Scientific understanding of mechanisms is still limited; this is because the technique is still in its infancy state.
3. Hyperaccumulators can be a pollution hazard themselves. For instance, animals can eat the hyperaccumulators and cause the toxins to enter the food chain. If the concentration of contaminant in the plants is high enough to cause toxicity, there must be a way to segregate the plants from humans and wildlife, which may not be an easy task.

## 15.4 Environmental Contaminants

The following compounds have been reported as contaminants in soil and water:

Pesticides; explosives; oil; heavy metal such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), uranium (U), vanadium (V) and wolfram (W); polychlorinated biphenyls; polycyclic aromatic hydrocarbons (PAHs); chlorinated solvents; xenobiotics; munitions; semi-coke solid wastes (which contain several organic and inorganic compounds such as oil products, asphaltenes, phenols, PAHs, sulphuric compounds); oil shale; and organic synthetic compounds.

## 15.5 Factors that Affect Phytoremediation

Certain factors affect the uptake, distribution and transformation of contaminants. Some of these factors include the following:

1. Level of contamination: They work best where contaminant levels are low because high concentrations may limit plant growth and take too long to clean up.
2. Plant species used for phytoremediation: Certain plants are better at removing contaminants than others. This may be due to differences in root exudate patterns, differences in root architecture as well as differences in genetic composition of the plant. Tall fescue with fibrous root system, for example, increases the potential of soil microbial community to degrade hydrocarbons, whereas rose clover with a coarse, woody root system decreases it (Siciliano et al. 2003). Plants used for phytoremediation must be able to tolerate the types and concentrations of contaminants present. They also must be able to grow and survive in the local climate. Chemical, physical and microbiological plants with low biomass yield and reduced root systems do not support efficient phytoremediation and most likely do not prevent the leaching of contaminants into the aquatic system.
3. Depth of contamination: Small plants like ferns and grasses have been used where contamination is shallow. Because tree roots grow deeper, trees such as poplars and willows are used for hydraulic control or to clean up deeper soil contamination and contaminated groundwater.
4. Plant growth and development stage. Phytoremediation is most effective during the vegetative growth stages of plants. Plant vegetative growth stage is the most important phase for phytoremediation (Nie et al. 2011).
5. Type and properties of inoculum used for bioaugmentation.
6. Soil condition: Soil abiotic and biotic factors may determine the survival and activity of the introduced microorganisms (Juhanson et al. 2007). Some of the abiotic factors include temperature, soil pH, soil organic matter, soil moisture, cation exchange capacity, etc.
7. Bioavailability of contaminant to the microbial community is another factor influencing biodegradation of pollutants.
8. Age of the contaminants.
9. Physical and chemical properties of the contaminant. Contaminants that are soluble in water may pass by the root system without being accumulated.
10. Climatic factors. Plant survival and growth are adversely affected by extreme climatic factors.
11. Toxicity of soil.
12. Bioavailability of contaminant to plants. Metal that is tightly bound to the organic portions of the soil may not be available to plants.
13. Contaminant source.

## 15.6 Phytoremediation Strategies

These technologies to be discussed below are based on the plant's ability to absorb, accumulate, sequester and detoxify toxic metals:

1. *Hydraulic control*: In this process of phytoremediation, plants act like a pump, drawing the groundwater up through their roots to keep it from moving. It reduces the movement of contaminated groundwater towards clean areas off-site.
2. *Phytoaccumulation* (*phytoextraction*)**:** Plants absorb, accumulate and transport pollutants from the soil to aboveground plant parts (shoots). Removing the metals is as simple as pruning or cutting the plant aboveground mass. Plants, and their associated soil microbes, can release chemicals that act as biosurfactants in the soil that increase the uptake of contaminants. The aboveground plant parts rich in accumulated metal can be easily and safely processed by drying, ashing or composting. The plants used in a phytoextraction scheme should ideally have large biomass production and accumulate high concentration of metals in the aboveground portions (Adesodun et al. 2010). Over 500 plant species (101 families) and approximately 0.2 % of angiosperms have been reported to possess metal hyperaccumulation ability (Krämer 2010).
3. *Phytostabilization* involves the use of plants to reduce the mobility and bioavailability of contaminants in soil either through precipitation or adsorption onto roots. Plants adsorb contaminants onto their roots where microorganisms that live in the soil break down the adsorbed contaminants to less harmful chemicals. Mycorrhizal association, for example, is known to inhibit transport of metallic cations into plant roots. Some plant species such as *Combretum* and *Rhus* (Anacardiaceae) have the ability of in situ stabilization of some metals (Regnier et al. 2009; Mokgalaka-Matlala et al. 2010).
4. *Phytodegradation* is the breaking down of contaminants into less toxic substances in the soil through the activities of microorganisms in the rhizosphere of plant roots or externally through metabolites produced by plants. For instance, exudates (peptides) from the bacterium *Pseudomonas putida* can decrease cadmium (Cd) toxicity in plants. Natural exudates such as siderophores, organic acids and phenolics released by the roots of certain plants can form complexes (chelates) with metals in the rhizosphere.

**Table 15.1** Phytoremediation strategies of various groups of contaminants

| Technology | Action on contaminants | Main type of contaminant | Vegetation |
|---|---|---|---|
| Phytostabilization | Retained in situ | Organics and metals | Cover maintained |
| Phytodegradation | Attenuated in situ | Organics | Cover maintained |
| Phytovolatilization | Removed | Organics and metals | Cover maintained |
| Phytoextraction | Removed | Metals | Harvested repeatedly |
| Phytofiltration | Retained in situ | Metals | Cover maintained |

Metals such as the toxic Cr(lll) can be converted to the much less toxic Cr(Vl) by enzymes found on the roots of wetland plants. During detoxification plants release glutathione conjugates into the rhizosphere where they could be metabolized by microbes (Schroder et al. 2007).

5. *Phytovolatization* involves use of plants to take up certain contaminants and then converts them into gaseous forms that vaporize into the atmosphere. This process is driven by the evapotranspiration of plants. Plants that have high evapotranspiration rate are sought after in phytovolatilization. Organic contaminants, especially volatile organic compounds (VOCs), are passively volatilized by plants. For example, hybrid poplar trees have been used to volatilize trichloroethylene (TCE) by converting it to chlorinated acetates and $CO_2$. Metals such as Se can be volatilized by plants through conversion into dimethylselenide [Se $(CH_{3)2}$]. Genetic engineering has been used to allow plants to volatilize specific contaminants. For example, the ability of the tulip tree *(Liriodendron tulipifera*) to volatilize methyl-Hg from the soil into the atmosphere (as HgO) was improved by inserting genes of modified *E. coli* that encode the enzyme mercuric ion reductase.
6. *Phytofiltration***:** This involves rhizofiltration where contaminants such as metals are precipitated within the rhizosphere. Metal plaque forms typically on the roots of wetland plants through the release of oxygen via the parenchyma of roots. Iron oxides, for example, can precipitate along with other metals into the metal plaque. Metal plaque on roots acts as a reservoir for active iron ($Fe^{2+}$), which in turn increases the tolerance of plants to other toxic metals (Table 15.1).

The ability of the plants to degrade or metabolize xenobiotic pollutants can be improved by transferring genes from organisms (bacteria, fungi, plant and animals) which have potential for degradation and mineralization of xenobiotic pollutants. That is, catabolic genes essential for the degradation of contaminants are boosted in a plant resulting in enhanced phytoremediation. The plants which received the genes are called transgenic plants. The genes are introduced into the candidate plants using *Agrobacterium*-mediated or direct DNA method of gene transfer. Phytoremediation process in plant can also be improved by constructing plants with enhanced secretion of enzymes capable of degrading xenobiotics into the rhizosphere (Gerhardt et al. 2009) (Tables 15.2 and 15.3).

**Table 15.2** General processes affecting rhizoremediation

| Processes | Effects |
|---|---|
| Root exudates | Microbial growth stimulation |
| $O_2$—redox reaction | Microbial growth stimulation |
| $CO_2$—soil pH plant chelators and biosurfactants | Contaminant bioavailability |
| $H^+$ & $OH^-$—soil pH acid/base reaction | Contaminant bioavailability |
| Microbial enzymes | Plant growth |
| Ion uptake | Plant growth |
| Microbial chelator—plant nutrient delivery | Plant growth |

## 15.7 Phytoremediators

PHYTOREM database (complied by Environment Canada) estimates that more than 750 plant species worldwide have potential for phytoremediation (Sarma 2011). Some of these include:

*Bromus hordeaceus*, *Festuca arundinacea*, *Trifolium fragiferum*, *Trifolium hirtum*, *Vulpia microstachys*, *Bromus carinatus*, *Elymus glaucus*, *Festuca rubra*, *Hordeum californicum*, *Leymus triticoides*, *Nassella pulchra*, *Combretum* sp., *Rhus* sp., *Phragmites australis*, *Alyssum corsicum*, *Alyssum murale*, mustard greens, *Helianthus annuus*, *Agrostis tenuis*, *Thlaspi caerulescens* (alpine pennycress), *Brassica juncea* (Indian mustard), *Liriodendron tulipifera* (yellow poplar) and *Nicotiana glauca* (Table 15.4).

## 15.8 Phytoremediation Traits

Plant adaptation and responses to contaminated environments depend on many physiological, molecular, genetic and ecological traits. Indicators of a plant's phytoremediation potential include the following:

1. Tolerance to high pH and salinity.
2. Tolerance to extreme drought and waterlogged conditions.
3. Good rooting system and adequate depth of root zone. Fibrous rooting system provides large surface area for root-soil contact.
4. High level of tolerance with respect to the contaminant known to exist at the site.
5. High growth rate and biomass yield.

**Table 15.3** Some examples of microorganisms used for phytoremediation

| Plants | Microbes | Contaminants | References |
|---|---|---|---|
| Sugar beets | *Pseudomonas* sp. | PCBs | Villacieros et al. 2005 |
| *Thlaspi goesingense* | *Methylobacterium* sp. | Nickel | Idris et al. 2004 |
| Rock cress | *Pseudomonas* sp. | PCBs | Narasimhan et al. 2003 |
| Alfalfa | *Pseudomonas* sp. | PCBs | Brazil et al. 1995 |
| Wheat | *Pseudomonas* sp. | TCE | Yee et al. 1998 |
| *Thlaspi goesingense* | *Sphingomonas* sp. | Nickel | Idris et al. 2004 |
| Wild rye | *Pseudomonas* sp. | Chlorobenzoic acid | Siciliano and Germida 1998 |
| Pea | *Pseudomonas* sp. | 24-D | Germaine et al. 2006 |
| Popular | *Pseudomonas* sp. | MTBE, TCE, BTEX | Germaine et al. 2004; Moore et al. 2006 |
| Pea | *Pseudomonas* sp. | Naphthalene | Germaine et al. 2009 |
| Barmultra grass | *Pseudomonas* sp. | Naphthalene | Kuiper et al. 2004 |
| Barley | *Pseudomonas* sp. | Phenanthrene | Anokhina et al. 2004 |
| Common reed | *Sinorhizobium meliloti* P221 | Phenanthrene | Golubev et al. 2009 |
| Switch grass | Indigenous degraders | PCBs | Chekol et al. 2004 |
| Red clover, ryegrass | Indigenous degraders | 24-D | Shaw and Burns 2004 |
| Ryegrass | Indigenous degraders | PCPs | He et al. 2005 |
| White mustard | Indigenous degraders | Petroleum hydrocarbon | Liste and Prutz 2006 |
| Hybrid poplar | Indigenous degraders | BTEX, toluene | Barac et al. 2009 |
| English oak, common ash | Indigenous degraders | TCE, toluene | Weyens et al. 2009 |
| Birch | Indigenous degraders | PAHs | Sipilä et al. 2008 |
| Altai wild rye, tall wheat grass | Indigenous degraders | Petroleum hydrocarbon | Phillips et al. 2009 |
| Corn | *Gordonia* sp. S2Rp-17 | Diesel | Hong et al. 2011 |
| Yellow lupine | *Burkholderia cepacia* | Toluene | Barac et al. 2009 |
| Poplar | *Burkholderia cepacia* | Toluene | Taghavi et al. 2005 |
| Barley | *Burkholderia cepacia* | 24-D | Jacobsen, 1997 |
| Wheat | *Azospirillum lipoferum* spp. | Crude oil | Muratova et al. 2005; Shaw and Burns 2004 |
| Tall fescue grass | *Azospirillum brasilense Cd* | PAHs | Huang et al. 2004 |
| Tall fescue grass | *Enterobacter cloacae* CAL2 | PAHs | Huang et al. 2004 |
| Poplar | *Methylobacterium populi* BJ001 | TNT, RDX, HMX | Van Aken et al. 2004a; Van Aken et al. 2004b |

*PAH* polyaromatic hydrocarbon; *TCE* trichloroethylene; *PCB* polychlorinated biphenyl; *MTBE* methyl tert-butyl ether; *BTEX* benzene, toluene, ethylbenzene and xylenes; *24-D* 2,4-dichlorophenoxyacetic acid; *PCP* pentachlorophenol; *TNT* 2,4,6-trinitrotoluene; *RDX* hexahydro-1,3,5-trinitro-1,3,5-triazine; *HMX* octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

**Table 15.4** Some examples of phytoremediators and contaminants

| Plants | Metals | References |
|---|---|---|
| *Arabis gemmifera* | Cd and Zn | Kubota and Takenaka 2003 |
| *Crotalaria dactylon* | Ni and Cr | Saraswat and Rai 2009 |
| *Thlaspi caerulescens* | Cd and Zn | Kupper and Kochian 2010 |
| *Pelargonium* sp. | Cd | Dan et al. 2002 |
| *Arabidopsis halleri* | Cd | Kupper et al. 2000 |
| *Crotalaria juncea* | Ni and Cr | Saraswat and Rai 2009 |
| *Thlaspi caerulescens* | Cd, Pb and Zn | Banasova and Horak 2008 |
| *Brassica napus* | Cd | Selvam and Wong 2009 |
| *Arabidopsis thaliana* | Zn and Cd | Saraswat and Rai 2009 |
| *Thlaspi caerulescens* | Zn, Cd and Ni | Assuncao and Schat 2003 |
| *Pistia stratiotes* | Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn | Odjegba and Fasidi 2004 |
| *Chengiopanax sciodophylloides* | Mn | Mizuno et al. 2008 |
| *Pteris vittata* | As | Dong 2005 |
| *Sedum alfredii* | Pb and Zn | Sun et al. 2005 |
| *Tamarix smyrnensis* | Cd | Manousaki et al. 2008 |
| *Potentilla griffithii* | Zn and Cd | Hu et al. 2009 |
| *Rorippa globosa* | Cd | Sun et al. 2010 |

6. High level of tolerance to waterlogging and extreme drought condition.
7. High level of accumulation, translocation and uptake potential of contaminant.
8. Habitat preference of plant, e.g. terrestrial aquatic or semiaquatic.

## 15.9 Effects of the Metals on the Phytoremediators

Plants that have been successfully used as phytoremediators were able to tolerate, accumulate or translocate the metals by reasons of the following effects of the metals on the plants:

1. The plant physiology: Metals affect the physiology of plants either by promoting or inhibiting the growth of the plant. Some develop metal tolerance characteristics through apoplastic or symplastic detoxification mechanisms (Pilon-Smits et al. 2009). Some are absorbed from soil solution through passive transport. Hg, for example, may preferentially bind with sulphur- and nitrogen-rich ligands (amino acids) and enter inside the cells. Cd can induce changes in lipid profile (Ouariti et al. 1997) and can also affect the enzymatic activities associated with membranes such as the $H^+$ ATPase (Fodor et al. 1995).
2. Biomass production of the plants that have been successfully used as phytoremediators.

## 15.10 Responses of Microbial Communities to Phytoremediation

Different plant species have different effects on microorganisms in the soil. For instance, *Alyssum corsicum*, *Alyssum murale* and *Brassica juncea* (Ni hyperaccumulators) have been reported to increase both the population and biomass of soil microorganisms. By absorbing nickel from the soil and excreting root exudates, the plants reduced nickel toxicity and improved the living environment of the microbes (Cai et al. 2007). Phytoremediation increased the number of phenol-degrading bacteria as well as metabolic diversity of microbial community in semi-coke polluted soil (Truu et al. 2003). Perennial ryegrass supports a general increase in microbial activity and numbers in the rhizosphere, some of which have catabolic activity towards petroleum hydrocarbons in petroleum-contaminated soil. Alfalfa, on the other hand, seems to specifically increase the number of microorganisms capable of degrading more complex hydrocarbons (Kirk et al. 2005). Plant-dependent changes in microbial functionality are the result of some form of communication between the associated microorganisms and the plant. For example, bacterial products, such as lumichrome, stimulate root respiration and thereby increase the availability of root exudates for bacteria (Phillips et al. 2009).

## 15.11 Sources of Environmental Pollution

1. Increased toxic waste from increased population
2. Anthropogenic activities such as agriculture
3. Metal purification procedure, which includes mining, smelting and the tailings from industries

## References


Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA (2010) Phytoremediation potentials of Sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with Zinc and Lead nitrates. Water Air Soil Pollut 207:195–201. doi:10.1007/s 11270-009-0128-3

Anokhina TO, Kochetkov VV, Zelenkova NF, Balakshina VV, Boronin AM (2004) Biodegradation of phenanthrene by *Pseudomonas* bacteria bearing rhizospheric plasmids in model plant: microbial associations. Appl Biochem Microbiol 40:568–572. doi:10.1023/B:ABIM.0000046992.01220.35

Assuncao AGL, Schat H (2003) *Thlaspi caerulescens* an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360. doi:10.1046/j.1469-8137.20000820.x

Banasova V, Horak O (2008) Heavy metal content in *Thlaspi caerulescens* J.et C. presl growing on metalliferous and non-metalliferous soils in Central Slovakia. Int J Environ Pollut 33:133–145. doi:10.1504/IJEP.2008.019388

Barac T, Weyens N, Oeyen L, Taghavi S, van der Lelie D, Dubin D, Spliet M, Vangronveld J (2009) Field note: hydraulic containment of BTEX plume using poplar trees. Int J Phytoremediation 11:416–424. doi:10.1080/15226510802655880

Brazil GM, Kenefick L, Callanan M, Haro A, de Lorenzo V, Dowling DN, O'Gara F (1995) Construction of a rhizosphere pseudomonad with potential to degrade polychlorinated biphenyls and detection of bph gene expression in the rhizosphere. Appl Environ Microbiol 61:1946–1952, aem.asm.org/content/61/5/1946

Cai X, Qiu R, Chen G, Zeng X, Fang X (2007) Response of microbial communities to phytoremediation of nickel contaminated soils. Front Agric China 1:289–295. doi:10.1007/s11703-007-0049-0

Chekol T, Vough LR, Chaney RL (2004) Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect. Environ Int 30:799–804. doi:10.1016/j.envint.2004.01.008

Dan TV, Krishnaraj S, Saxena PK (2002) Cadmium and nickel uptake and accumulation in scented geranium (*Pelargonium* sp. Frensham). Water Air Soil Pollut 137:355–364. doi:10.1023/A:1015590007901

Danh LT, Truong P, Mammucari R, Tran T, Foster N (2009) Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. Int J Phytorem 11:664–691. doi:10.1080/15226510902787302

Dong R (2005) Molecular cloning and characterization of a phytochelatin synthase genes, PvPCS1 from *Pteris vittata* L. J Ind Microbiol Biotechnol 32:527–533. doi:10.1007/s10295-005-0234-1

Fodor E, Szabo-Nagy A, Erdei L (1995) The effects of cadmium on the fluidity and H+ATPase activity of plasma membrane from sunflower and wheat roots. J Plant Physiol 147:87–92. doi:10.1016/S0176-1617

Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176:20–30. doi:10.1016/jplantsci.2008.09.014

Germaine K, Keogh E, Cabellos GG, Borremans B, van der Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell CD, Ryan D, Dowling DN (2004) Colonisation of poplar trees by *gfp* expressing bacterial endophytes. FEMS Microbiol Ecol 48:109–118. doi:10.1016/j.femsec.20012.009

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310. doi:10.1111/j.1574-6941.2006.00121.x

Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234. doi:10.1111/j.1574-6968.2009.01637.x

Golubev S, Schelud'ko A, Muratova A, Makarov O, Turkovskaya O (2009) Assessing the potential of rhizobacteria to survive under Phenanthrene pollution. Water Air Soil Pollut 198:5–16. doi:10.1007/s11270-008-9821-x

Haque N, Peralta-Videa JR, Jones GL, Gill TE, Gardea-Torresdey JL (2008) Screening the phytoremediation potential of desert broom (*Baccharis sarothroides* Gray) growing on mine tailings in Arizona, USA. Environ Pollut 153:362–368. doi:10.1016/j.envpol.2007.08.024

He Y, Xu JM, Tang CX, Wu YP (2005) Facilitation of pentachlorophenol degradation in the rhizosphere of ryegrass (Lolium perenne I). Soil Biol Biochem 37:2017–2024. doi:10.1016/j.soilbio.2005.0002

Hong S, Kim D, Baek S, Kwon S, Samson RA (2011) Taxonomy of Eurotium species isolated from meju. J Microbiol 49(4):669–674. doi:10.1007/s12275-011-0376-y

Hu PJ, Qiu RL, Senthilkumar P, Jiang D, Chen ZW, Tang YT, Liu FJ (2009) Tolerance accumulation and distribution of zinc and cadmium in hyperaccumulator *Potentilla griffithii*. Environ Exp Bot 66:317–325. doi:10.1016/j.envexpbot.2009.02.014

Huang X, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004) A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Pollut 130:465–476. doi:10.1016/j.envpol 20009.031

Idris A, Inane B, Hassan MN (2004) Overview of waste disposal and landfills/dumps in Asian countries. J Mater Cy Waste Manag 6:104–110. doi:10.1007/s10163-004-0117-y

Jacobsen CS (1997) Plant protectionand rhizosphere colonization of barley by seed inoculated herbicide degrading *burkholderia (pseudomonas) cepacia* DBOL (pRO101) in 2, 4-D contaminated soil. Plant Soil 189:139–144. doi:10.1023/A:1004296615446

Juhanson J, Truu J, Heinaru E, Heinaru A (2007) Temporal dynamics of microbial community in soil during phytoremediation field experiment. J Environ Engineering Lands Manag 14:213–220. doi:10.1080/16486897.2007.9636933

Kirk JL, Klironomos JN, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum-contaminated soil. Environ Pollut 133:455–465. doi:10.1016/j.envpol.2004.06.002

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534. doi:10.1146/annurev-arplant-042809-112156

Kubota H, Takenaka C (2003) *Arabis gemmifera* is a hyperaccumulator of Cd and Zn. Int J Phytorem 5:197–220. doi:10.1080/713779219

Kuiper I, Bloemberg GV, Lugtenberg BJJ (2001) Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. Mol Plant Microbe Interact 14:1197–1205. doi:10.1094/MPMI.2001.14.10.1197

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 17:6–15. doi:10.1094/MPMI.2004.17.1.6

Kupper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84. doi:10.1007/s004250000366

Kupper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges population). New Phytol 185:114–129. doi:10.1111/j.1469-8137.2009.03051.x

Liste HH, Prutz I (2006) Plant performance, Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges population) bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere 62:1411–1420. doi:10.1016/j.chemosphere.2005.05.018

Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N (2008) Phytoextraction and phytoextraction of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non saline and saline soils. Environ Res 106:326–332. doi:10.1016/jenvres.2007.04.004

Mizuno T, Hirano K, Kato S, Obata H (2008) Cloning of ZIP family metal transporter genes from the manganese hyperaccumulator plant *Chengiopanax sciadophylloides* and its metal transport and resistance abilities in yeast. Soil Sci Plant Nutr 54:86–94. doi:10.1111/j.1747-0765.2007.00206.x

Mokgalaka-Matlala NS, Regnier TC, Combrinck S, Weiersbye IM (2010) Selection of tree species as assets for mine Phytoremediation using the genus *Rhus* (Anacardiaceae) as a model. In: Fourie AB, Tibbett M (eds) Proceedings of the 5th International Conference on Mine Closure 2010, Australian Centre for Geomechanics, Perth, Australia, p 343–350

Moore FP, Barac T, Borrernans B, Oeyen L, Vangronsveld J, van der Lie D, Cambell CD, Moore ERB (2006) Endophytic bacterial diversity in poplar trees growing on BTEX contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29:539–556. doi:10.1016/j.syapm.2005.11.012

Muratova AY, Turkovskaya OV, Antonyuk LP, Makarov OE, Pozdnyakova LI, Ignatov VV (2005) Oil-oxidizing potential of associative rhizobacteria of the genus *Azospirillum*. Microbiology 74:210–215. doi:10.1007/s11021-005-0053-4

Narasimhan K, Basheer C, Bajic VB, Swarup S (2003) Enhancement of plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. Plant Physiol 132:146–153. doi:10.1104/pp.102.016295

Nie M, Zhang XD, Wang JQ, Jiang LF, Yang J et al (2009) Rhizosphere effects on soil bacterial abundance and diversity in the Yellow River Deltaic ecosystem as influenced by petroleum contamination and soil salinization. Soil Biol Biochem 41:2535–2542. doi:10.1016/j.soilbio.2009.09.012

Nie M, Wang Y, Yu J, Xiao M, Jiang L et al (2011) Understanding plant-microbe interactions for phytoremediation of petroleum-polluted soil. PLoS One 6:e17961. doi:10.1371/journal.pone.0017961

Odjegba VJ, Fasidi IO (2004) Accumulation of trace elements by *Pistia stratiotes:* implications for phytoremediation. Ecotoxicology 13:637–646. doi:10.1007/s10646-003-4424-1

Ouariti O, Boussama N, Zarrouk M, Cherif A, Ghorbali MH (1997) Cadmium and copper induced changes in tomato membranes lipids. Phytochemistry 45:1343–1350. doi:10.1016/S0031-9422(97)00159-3

Phillips LA, Greer CW, Farrell RE, Germida JJ (2009) Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatment. Appl Soil Ecol 42(1):9–17. doi:10.1016/j.apsoil.2009.01.002

Pilon-Smits EAH, Quinn CF, Tapken W, Malagoni M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12:267–274. doi:10.1016/j.pbi.2009.04.009

Regnier TC, Kouekam CR, Leonard CM, Combrink S, Mokgalaka NS, Weiersbye IM (2009) Chemical analysis and potential use of the tree *Combretum erythrophyllum* grown on gold and uranium mine tailings seepage. In: Fourie A, Tibbett M (eds) Proceedings of the 4th International Conference on Mine Closure, Perth, Australia, p 539–547

Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138. doi:10.3923/jest.2011.118.138

Saraswat S, Rai JPN (2009) Phytoextraction potential of six plant species grown in multimetal contaminated soil. Chem Ecol 25:1–11. doi:10.1080/02757540802657185

Schroder P, Scheer CE, Diekmann F, Stampfl A (2007) How plants cope with foreign compounds: translocation of xenobiotic glutathione conjugates in roots of barely (*Hordeum vulgare*). Environ Sci Pollut Res Int 14:114–122

Selvam A, Wong JWC (2009) Cadmium uptake potential of Brassica napus cocropped with Brassica parachinensis and Zea mays. J Hazard Mater 167:170–178. doi:10.1016/j.jhazmat.2008.12.103

Shaw LJ, Burns RG (2004) Enhanced mineralization of [U-C-14]2,4-dichlorophenoxyacetic acid in soil from the rhizosphere of Trifolium pratense. Appl Environ Microbiol 70:4766–4774. doi:10.1128/AEM. 70.8.4766-4774.2004

Siciliano SD, Germida JJ (1998) Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environ Rev 6:65–79. doi:10.1139/a98-005

Siciliano SD, Germida JJ, Banks K, Greer CW (2003) Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. Appl Environ Microbiol 69:483–489. doi:10.1128/AEM 69:483-489.2003

Sipilä TP, Keskinen AK, Åkerman ML, Fortelius C, Haahtela K, Yrjälä K (2008) High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of I.E.3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. ISME J 2:968–981. doi:10.1038/ismej.2008.50

Sun Q, Ye ZH, Wang XR, Wong MH (2005) Increase of glutathione in mine population of sedum alfredii: a Zn hyperaccumulator and Pb accumulator. Phytochemistry 66:2549–2556, 10.10s16/j.phytochem.2005.08.012

Sun R, Jin C, Zhou Q (2010) Characteristics of cadmium accumulation and tolerance in *Rorippa globosa* (Turez) Thell., a species with some characteristics of cadmium hyperaccumulation. Plant Growth Regul 61:67–74. doi:10.1007/s10725-010-9451-3

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. Appl Environ Microbiol 71:8500–8505. doi:10.1128/AEM.71.12.8500-8505.2005

Truu J, Talpsep E, Velder E, Heinaru E, Heinaru A (2003) Enhanced biodegradation of oil-shale chemical industry solid wastes by phytoremediation and bioaugmentation. Oil Shale 20:421–428

Van Aken B, Peres CM, Doty SL, Yoon JM, Schnoor JL (2004a) Methylobacterium populi sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (Populus deltoides x nigra DN34). Int J Syst Evol Microbiol 54:1191–1196. doi:10.1099/ijs. 0.02796-0

Van Aken B, Yoon JM, Schnoor JL (2004b) Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (*Populus deltoides x nigra* DN34). Appl Environ Microbiol 70:505–517

Villacieros M, Whelan C, Mackova M, Molgaard J, Sánchez-Contreras M, Lloret J, Aguirre de Cárcer D, Oruezábal RI, Bolaños L, Macek T, Karlson U, Dowling DN, Martín M, Rivilla R (2005) Polychlorinated biphenyl rhizoremediation by Pseudomonas fluorescens F113 derivatives, using a Sinorhizobium meliloti nod system to drive bph gene expression. Appl Environ microbio 71:2687–2694

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408. doi:10.1007/s11104-008-9686-1

Weyens N, Taghavi S, Barac T, van der Lelie D, Boulet J, Artois T, Carleer R, Vangronsveld J (2009) Bacteria associated with oak and ash on a TCE-contaminated site: characterization of isolates with potential to avoid evapotranspiration of TCE. Environ Sci Pollut Res 16:830–843. doi:10.1007/s11356-009-0154-0

Yee DC, Maynard JA, Wood TK (1998) Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monooxygenase constitutively. Appl Environ Microbiol 64:112–118

# 16 Effects of Biosolids on the Transpiration Rate of Rainbow Pink (*Dianthus chinensis*) Grown in Cadmium-Contaminated Soils

Hung-Yu Lai, Bo-Ching Chen, and Zueng-Sang Chen

## 16.1 Introduction

Heavy metal (HM) contamination of soil is a major environmental problem all over the world. Phytoextraction, a cost-effective and environmentally friendly technique, was evidenced to be a feasible technique to remove HM from contaminated soils using herbaceous plants (McGrath and Zhao 2003; Lin et al. 2010; Lai et al. 2010) and woody plants (Dickinson and Pulford 2005; Mertens et al. 2006; Li et al. 2010; Fan et al. 2011). Over 450 species of hyperaccumulators, plants that can accumulate a high concentration of HM without damage, have been discovered in the past 20–30 years (Lin et al. 2010). Among them, rainbow pink (*Dianthus chinensis*) was validated to accumulate high concentrations of cadmium (Cd) in the shoots when growing in artificial Cd-contaminated soils (Lin et al. 2010).

In order to shorten the period needed for decontamination, different chemical agents were applied to promote the efficiency of phytoextraction (Lombi et al. 2001; Komárek et al. 2010). However, they had negative effects on soil quality, activity of microorganisms (Römkens et al. 2002; Ultra et al. 2005), and activity of enzymes (Epelde et al. 2008). Furthermore, the resulting increase in the mobility of the HMs may amplify their vertical movement in soils and result in groundwater contamination (Wu et al. 2004; Lai and Chen 2007). The chemical-enhanced phytoextraction is thus not a feasible way from a sustainability standpoint.

The transpiration rate (TR) plays an important role in determining the accumulation of HM by plants, and higher TR generally leads to higher shoot Cd levels (Liu et al. 2010). Pulford and Watson (2003) also reported that the potential candidates for phytoextraction must be high biomass, have deep root systems, and have excellent TR. Cadmium accumulation in plants is mainly driven by transpiration; however, the elevated Cd concentrations in soil decreased the TR and photosynthetic rates and further decreased the biomass of plants (Shi and Cai 2009). Nevertheless, forgoing negative effect on biomass is not beneficial for phytoextraction. Because the HMs in soils are partially water soluble, the accumulation of HM has a close relationship with the TR of plants (Salt et al. 1995). One can therefore improve the TR and biomass of plants by the management of fertilization, and the accumulation of HMs will increase accordingly.

According to US Environmental Protection Agency in 40 CFR Part 503, biosolids (BS) are defined as sewage sludge that is beneficially reused (US EPA 2000) and could raise the dry weight (DW) of soybeans (Vieira 2001) and pak choi (Lu et al. 2012). Relative to control without applying BS, the Cd concentrations in the edible parts of pak choi grown in the Cd-contaminated soils amended with BS also increased (Lu et al. 2012). Experimental results of forgoing studies show that BS can promote both the biomass and the accumulation of Cd which may decline the period needed in phytoextraction. If BS are applied to HM-contaminated soils, the growth and accumulation of HMs in plants theoretically increase. A pot experiment was thus conducted in this study to assess the effects of applying different amounts of BS on the leaf area (LA) of rainbow pink. The TRs of rainbow pink grown in various treatments were determined to understand their relationship with LA.

## 16.2 Materials and Methods

### 16.2.1 Collection of Soil Samples and Analysis

Soil samples were collected from the surface soil (0–15 cm) of MingDao University's organic farm. The BS, extracted sludge after thickening, digestion, and dewatering, was taken

H.-Y. Lai, Ph.D. • B.-C. Chen, Ph.D.
Department of Post-Modern Agriculture, MingDao University, No.369, Wenhua Rd., Peetow, Changhua 52345, Taiwan

Z.-S. Chen, Ph.D. (✉)
Department of Agricultural Chemistry, National Taiwan University, No.1, Sec.4, Roosevelt Rd., Taipei 10617, Taiwan
e-mail: soilchen@ntu.edu.tw

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from a wastewater treatment plant in central Taiwan. Basic properties of the soil and BS were analyzed after pretreatment, including pH value (w/v = 1/1; Thomas 1996), electrical conductivity in the saturation extraction ($EC_e$; Rhoades 1996), water-holding capacity (WHC; Gardner 1986), organic carbon (OC; Nelson and Sommers 1996), and cation exchange capacity (CEC; Thomas 1982). The total content of nitrogen (N), available phosphate (P), and available potassium (K) in the BS was also analyzed according to Bremner (1996), Kuo (1996), and Helmke and Sparks (1996), respectively.

### 16.2.2 Preparation of Cd-Contaminated Soils and Pot Experiment

A solution of $Cd(NO_3)_2 \cdot 4H_2O$ was sprayed on the soil to achieve a target Cd concentration of 0 (Cd-CK), 10 (Cd-10), 20 (Cd-20), and 40 mg Cd $kg^{-1}$ (Cd-40). The artificial Cd-contaminated soils were further uniformly mixed with 0, 2, and 5 % (w/w) sieved BS. A pot experiment was conducted in a 25 °C phytotron (110 μmol $s^{-1}$ $m^{-2}$; day/night = 12/12 h) at MingDao University with three replicates. A 1-kg mixture of soil and BS was added to a square pot, and one seedling of rainbow pink (*D. chinensis*) was planted. The water content of the soil was controlled at 80 % of WHC by weighting and adding deionized water every 2–3 days.

### 16.2.3 Determination of TR, LA, and Cd Fractions

Thirty-five days after transplanting, the water content of the soil was raised to 100 % of WHC, and the surface of each pot was sealed with aluminum foils to avoid evaporation. The total weight of each pot was determined 24 h later, and the TR was calculated. The leaves of rainbow pink were harvested 40 days after transplant and then divided into roots, leaves, and other parts. Forgoing organs were first flushed with tap water, followed by deionized water and oven dried at 65 °C for 72 h, and then determined the DW. To determine the LA, the dry leaves were arranged on weighted A4 paper and then photostated. The blacked parts were cut, weighted, and then the LA was further calculated using the mass ratio between them.

Except for those properties analyzed, a sequential extraction was conducted to separate the Cd concentration in different fractions. Tessier et al. (1979) used exchangeable (F-I), carbonate bound (F-II), Fe/Mn oxide bound (F-III), organic matter bound (F-IV), and residual (F-V) fractions to determine the presence of HM in the soil. Briefly, the sequential extraction procedure involved the extraction of 1.0 g of each soil sample with 8 mL of 1 M $MgCl_2$ (1 h at pH 7.0) for F-I, 8 mL of 1 M NaOAc (5 h at pH 5.0) for F-II, 15 mL of 0.04 M $NH_2OH \cdot HCl$ in 25 % HOAc (5 h at 96 °C) for F-III, and 12 mL of 30 % $H_2O_2$ + 9 mL of 0.02 M $HNO_3$ (5 h at 85 °C) + 4 mL of 2 M $NH_4OAc$ (30 min at 25 °C) for F-IV. The residual soil sample after extraction was further digested with aqua regia to obtain F-V. The concentrations of Cd in the extracts of F-I to F-V were determined with a flame atomic absorption spectrophotometer (PerkinElmer AAnalyst 200). After digestion with aqua regia, the total concentration ($C_{total}$) of Cd in the soil samples was determined, and the recovery percentage (PR) was calculated. Data was acceptable once the PR was in the levels of 100 ± 10 %.

$$PR(\%) = \frac{\sum\left(C_{I} + C_{II} + C_{III} + C_{IV} + C_{V}\right)}{C_{total}}$$

where $C_{total}$ is the total Cd concentration (mg $kg^{-1}$) and

$C_I$, $C_{II}$, $C_{III}$, $C_{IV}$, and $C_V$ are the Cd concentrations (mg $kg^{-1}$) in F-I, F-II, F-III, F-IV, and F-V, respectively.

### 16.2.4 Statistical Analysis

The differences between the LA and the TR in the different treatments were detected using analysis of variance (ANOVA), followed by the LSD (least significant difference) test. The level of significance was set as $p = 0.05$.

## 16.3 Results and Discussion

### 16.3.1 Soil Properties

The pH of the tested soil was 7.43 with moderate $EC_e$ (1.66 dS $m^{-1}$) and OC (1.09 %). After artificially spiking, the total concentration of Cd in the Cd-10, Cd-20, and Cd-40 was 10.5 ± 0.9, 18.3 ± 4.2, and 39.9 ± 1.9 mg $kg^{-1}$, respectively. The biosolid had a lower pH value (5.57), but a higher $EC_e$ (4.25 dS $m^{-1}$), OC (29.8 %), and CEC (2.19 $cmol_{(+)}$ $kg^{-1}$), compared with the soil. The total content of N, available P, and available K in the BS was 430 ± 14, 904 ± 12, and 690 ± 0 mg $kg^{-1}$, respectively.

Table 16.1 shows the effects of the application of different amounts of BS on the soil properties. Relative to the control and for most of the treatments, the application of BS decreased the pH and raised the $EC_e$ levels in the soils. For some treatments, the $EC_e$ significantly increased to 2.1–2.6 dS $m^{-1}$. Higher $EC_e$ decreases the water potential and is negative for the growth of plants. The effect of the application of BS on the $EC_e$ of soil should be taken into account to avoid the occurrence of saline soil ($EC_e \geqq 4$ dS $m^{-1}$). However, even the highest application rate of BS, the $EC_e$ was still in the acceptable levels. Because of the high content of OC in BS, the OC contents of soils increased significantly from 1.0–1.7 (BS-0 %) to 1.6–2.2 % (BS-2 %) and 1.7–2.4 % (BS-5 %). A higher content of OC has a beneficial effect on improving the structure of soil as the aeration and drainage

**Table 16.1** Effects of the application of biosolid on the soil properties

| | Soil properties | | | |
|---|---|---|---|---|
| | pH | $EC_e$ (dS $m^{-1}$) | OC (%) | CEC ($cmol_{(+)}\,kg^{-1}$) |
| | Cd-CK | | | |
| BS-0 % | 7.43 ± 0.11 a | 1.66 ± 0.17 b | 1.09 ± 0.04 c | 0.65 ± 0.03 a |
| BS-2 % | 7.36 ± 0.04 a | 1.97 ± 0.23 b | 1.70 ± 0.18 b | 0.66 ± 0.08 a |
| BS-5 % | 7.29 ± 0.09 a | 2.56 ± 0.21 a | 2.36 ± 0.30 a | 0.71 ± 0.05 a |
| | Cd-10 | | | |
| BS-0 % | 7.18 ± 0.15 a | 1.59 ± 0.18 b | 1.23 ± 0.11 b | 0.74 ± 0.01 a |
| BS-2 % | 7.07 ± 0.11 a | 1.80 ± 0.23 b | 1.60 ± 0.30 a | 0.69 ± 0.10 a |
| BS-5 % | 7.04 ± 0.09 a | 2.20 ± 1.10 a | 1.79 ± 0.30 a | 0.72 ± 0.04 a |
| | Cd-20 | | | |
| BS-0 % | 7.24 ± 0.18 b | 2.02 ± 0.59 a | 1.47 ± 0.18 b | 0.61 ± 0.03 c |
| BS-2 % | 7.65 ± 0.18 a | 1.90 ± 0.25 a | 2.11 ± 0.49 a | 0.71 ± 0.05 b |
| BS-5 % | 7.52 ± 0.12 ab | 2.22 ± 0.13 a | 2.18 ± 0.41 a | 0.82 ± 0.06 a |
| | Cd-40 | | | |
| BS-0 % | 7.49 ± 0.17 a | 1.82 ± 0.57 a | 1.61 ± 0.32 b | 0.71 ± 0.06 a |
| BS-2 % | 7.68 ± 0.05 a | 1.64 ± 0.24 a | 1.60 ± 0.26 b | 0.72 ± 0.06 a |
| BS-5 % | 7.38 ± 0.26 a | 2.11 ± 0.26 a | 2.34 ± 0.34 a | 0.76 ± 0.05 a |

Different letter indicates significantly different between BS for the same Cd treatment at $p < 0.05$. Replicates ($n$) = 3

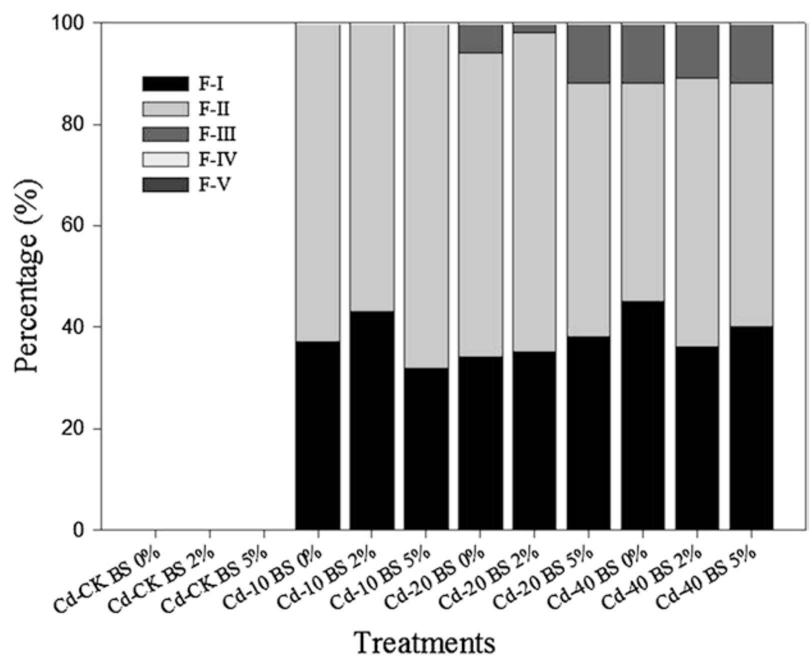


**Fig. 16.1** Effects of the application of biosolid on the fractions of Cd in the soils

will be enhanced, thereby improving the growth of the plants. Although the extractable P after the pot experiment was not determined, according to the properties of BS and in an ideal situation, the concentrations of extractable P will reach 45 mg $kg^{-1}$ after the application of 5 % of BS which is regarded as a very rich level (>34 mg $kg^{-1}$) according to Jones (2001). There was no significant difference for most of the treatments on the CEC of soils.

### 16.3.2 Effects of BS on the Cd Fractions and DW

Results of sequential extraction showed that the added Cd was primarily present in the F-II for all the treatments and occupied approximately 56 % of the total concentration (Fig. 16.1). Approximately 38 % of the added Cd was detected in the F-I, followed by 6 % in the F-III, and was not detectable in the F-IV or F-V. Possibly resulting from the decreases in the pH of the soil, the application of BS affected the concentration of Cd in the F-II. However, because the availability of Cd in the contaminated soil was partly controlled by the period of contamination and the fractions of HMs may be changed to less phytoavailable speciation over time (Martinez and Motto 2000; Wang et al. 2003). The Cd in the artificially spiked soil used in this study had higher availability, and most of the Cd added to the soil was in the F-I and F-II, which had a relatively higher mobility and availability compared with the other fractions.

The DW of the roots and the shoots of rainbow pink were not significantly affected by the application of different proportions of BS (Fig. 16.2). The increase of Cd concentration in the soils to about 40 mg $kg^{-1}$ also did not significantly affect the DW, and the DW of the shoots of rainbow pink were approximately twofold greater than the roots. Forgoing phenomena evidenced that rainbow pink can tolerate the toxicity of added Cd which is in agreement with Lai and Chen (2004). Relative to the BS-0 %, the LA of rainbow pink grown in different treatments significantly increased with the treatment of BS, and the increases reached 1–10 % and 10–30 % with the treatments of BS-2 % and BS-5 %, respectively (Fig. 16.3). The experimental results of this study were contrary to those of Shi and Cai (2009) who revealed that elevated Cd concentrations in soil decreased the LA of peanut. Accordingly and compared with the control, the TR

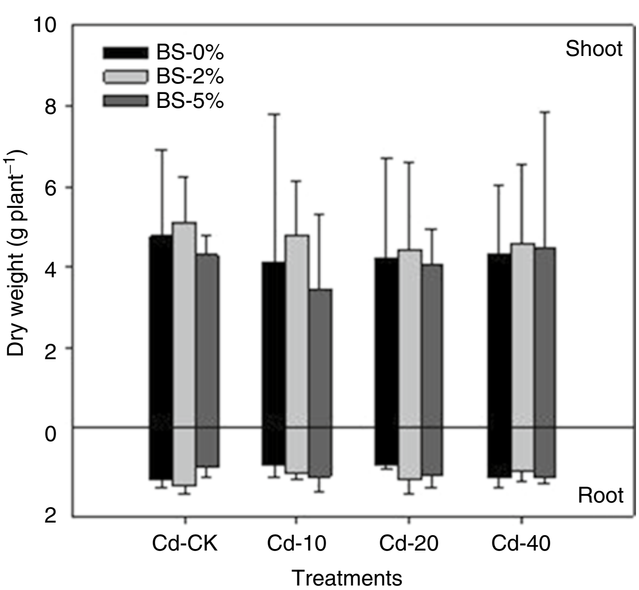


**Fig. 16.2** Effects of the application of biosolid on the dry weight of rainbow pink grown in the Cd-contaminated soils

increased 3–8 % and 8–16 % with the treatments of BS-2 % and BS-5 %, respectively, although there were no significant differences between them (Fig. 16.4).

There were no significant differences in the DW of rainbow pink with treatments of Cd at CK to 40 mg $kg^{-1}$. The application of BS did not significantly affect the DW of the roots and shoots of rainbow pink (Fig. 16.2). Concerning the toxic symptoms of Cd, the plants showed visible necrosis and whitish-brown chlorosis under Cd stress. The insignificant decrease of biomass in plants growing in HM-contaminated soils is one of the characteristics of a hyperaccumulator as opposed to a normal plant (Wei and Zhou 2004). The experimental results of this study showed that the DW of rainbow pink were not significantly changed at 10–40 mg Cd $kg^{-1}$, thereby revealing its strong tolerance to Cd stress.

### 16.3.3 Relationship Between LA and TR

If the LA was further linear regressed with the TR, there were well relationships between them for most of the treatments (Fig. 16.5). The experimental results of this study reveled that the application of BS promotes the LA, and consequently, the TR of rainbow pink grown in artificially Cd-contaminated soils. Shi and Cai (2009) planted peanut in a mixture of sand and perlite and additionally supplemented with $CdCl_2$. They found that the elevated Cd concentrations decreased the biomass and LA of peanut and infer that the xerophytic feathers in leaf structure led to a decrease in TR and photosynthetic rate and further decreased the biomass of plants. The phytoextraction efficiency is determined by the total removal of HM by plant which is the product of biomass and the accumulated concentration. Plants with huge biomass and can accumulated high concentration of HMs will shorten the period needed in decontamination. However, they are always opposite and with an exponential decay relationship under the stress of HMs (Wei et al. 2012) because the stress of Cd inhibited the growth of plant.

Experimental results of this study evidenced that the application of BS had positive effect on enhancing the biomass and LA of rainbow pink grown in Cd-contaminated soils and subsequently the TR. If most of the Cd in soils was existed in water soluble or exchangeable fractions as the status in this study, the efficiency of phytoextraction would be enhanced when amending Cd-contaminated soils with BS. Many previous studies used chemical agents to promote the accumulation of HM and thus to shorten the period in decontamination; however, chemical-enhanced phytoextraction was evidenced to have negative effects on the microorganisms (Römkens et al. 2002) and

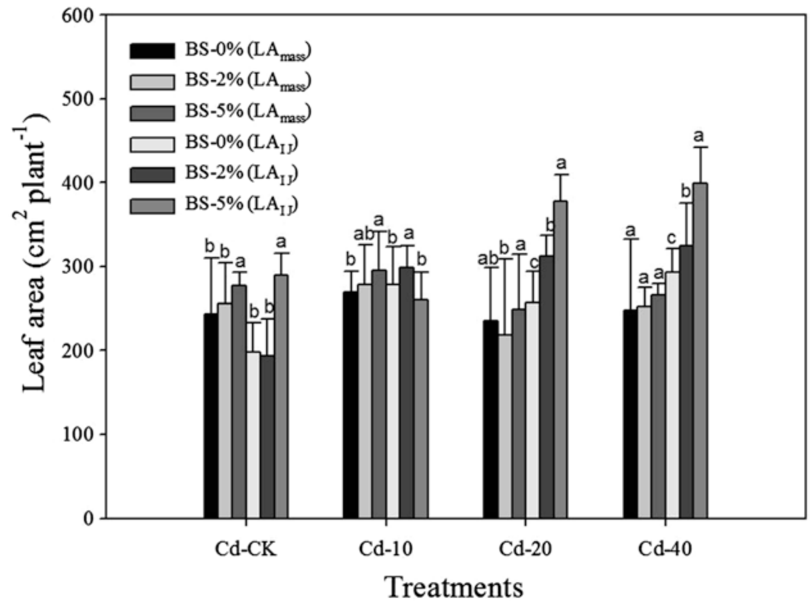


**Fig. 16.3** Effects of the application of biosolid on the leaf area (LA) of rainbow pink grown in the Cd-contaminated soils. Different letter indicates significantly different between BS for the same Cd treatment at $p<0.05$. Replicates $(n)=3$

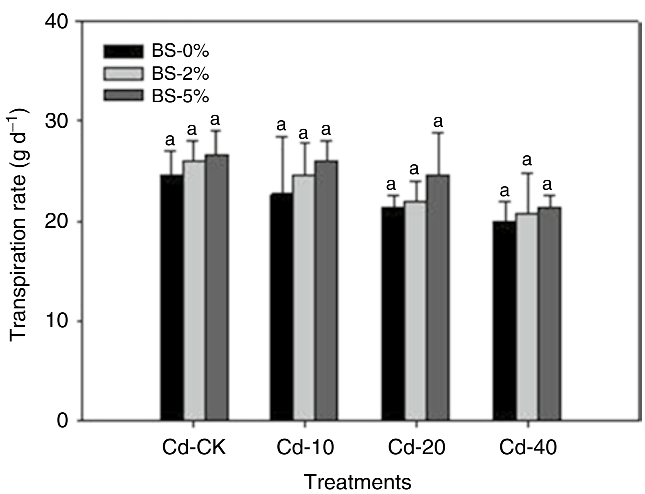


**Fig. 16.4** Effects of the application of biosolid on the transpiration rate (TR) of rainbow pink grown in the Cd-contaminated soils. Different letter indicates significantly different between BS for the same Cd treatment at $p<0.05$. Replicates $(n)=3$

soil quality (Lai 2015). The primary advantages of phytoremediation, economic and environmentally friendly, will disappear because most of the chemical agents are costly.

To increase the feasibility of phytoremediation, there were more and more studies that have been conducted to promote the efficiency of phytoremediation by using mycorrhizal fungi (Leung et al. 2006, 2010; Wong et al. 2007; Wu et al. 2011). The other methods include the use of woody plants with huge biomass (Pulford and Watson 2003; Dickinson and Pulford 2005; Mertens et al. 2006; Li et al. 2009; Pietrini et al. 2010; Zhivotovsky et al. 2011) and the application of chicken manure to promote the accumulation and growth of plants (Das and Maiti 2009). Biosolids are the precipitation from wastewater treatment plants, and landfills are the major disposal method in Taiwan; however, this method seems to be unfeasible in the future results from the deficient land resources in Taiwan. Because the BS is enriched in organic matter and nutrients, the reuse of BS was evidenced to raise the dry weight of soybeans (Vieira 2001). Experimental results of this study also provided strong evidences.

## 16.4 Conclusions

The dry weight of rainbow pink was not significantly affected by the treatments of Cd and BS. Amendments of BS increased the LA and therefore the TR of rainbow pink, especially the BS-5 %. There was a positive linear relationship between the LA and the TR. In order to enhance the efficiency of phytoextraction, further studies are proposed to investigate the relationship between LA, TR, and the accumulation of Cd by rainbow pink.

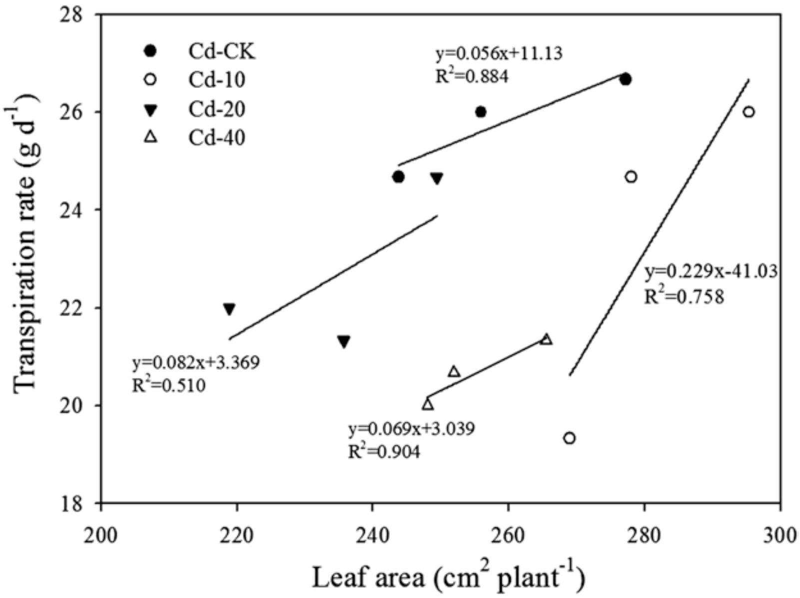


**Fig. 16.5** Linear relationships between the leaf area (LA) and the transpiration rate (TR) of rainbow pink grown in the Cd-contaminated soils

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## References


Bremner JM (1996) Nitrogen-total. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods. SSSA, Inc. and ASA, Inc., Madison, WI

Das M, Maiti SK (2009) Growth of *Cymbopogon citratus* and *Vetiver zizanioides* on Cu mine tailings amended with chicken manure and manure-soil mixtures: a pot scale study. Int J Phytoremediation 11:651–666

Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ Int 31:609–661

Epelde L, Hernández-Allica J, Becerril JM, Blanco F, Garbisu G (2008) Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci Total Environ 401:21–28

Fan KC, Hsi HC, Chen CW, Lee HL, Hseu ZY (2011) Cadmium accumulation and tolerance of mahogany (*Swietenia macrophylla*) seedlings for phytoextraction applications. J Environ Manage 92:2818–2822

Gardner WH (1986) Water content. In: Klute A, Campbell GS, Nielsen DR, Jackson RD, Mortland MM (eds) Methods of soil analysis, Part 1, Physical and mineralogical methods, 2nd edn. ASA, Inc. and SSSA, Inc., Madison, WI

Helmke PA, Sparks DL (1996) Lithium, sodium, potassium, rubidium, and cesium. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods. SSSA, Inc. and ASA, Inc., Madison, WI

Jones TB Jr (2001) Laboratory guide for conducting soil test and plant analysis. CRC Press LLC, Boca Raton, FL

Komárek M, Vanêk A, Mrnka L, Sudová R, Száková J, Tejnecký V, Chrastný V (2010) Potential and drawbacks of EDDS-enhanced phytoextraction of copper from contaminated soils. Environ Pollut 158:2428–2438

Kuo S (1996) Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods. SSSA, Inc. and ASA, Inc., Madison, WI

Lai HY, Chen ZS (2004) Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. Chemosphere 55:421–430

Lai HY, Chen ZS (2007) Effects of multi-dose EDTA application on the phytoextraction of Cd, Zn, and Pb by rainbow pink (*Dianthus chinensis*) in contaminated soil. Desalination 210:236–247

Lai HY, Juang KW, Chen ZS (2010) Large-area experiment on uptake of metals by twelve plants growing in soils contaminated with multiple metals. Int J Phytoremediat 12:785–797

Lai HY (2015) Negative effects of chelants on soil qualities of five soil series. Int J Phytoremediation 17:242–248

Leung HM, Ye ZH, Wong MH (2006) Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils. Environ Pollut 139:1–8

Leung HM, Wu FY, Cheung KC, Ye ZH, Wong MH (2010) The effect of arbuscular mycorrhizal fungi and phosphate amendment on arsenic uptake, accumulation and growth of *Pteris vittata* in As-contaminated soil. Int J Phytoremediat 12:384–403

Li JT, Liao B, Dai ZY, Zhu R, Shu WS (2009) Phytoremediation of Cd-contaminated soil by carambola (*Averrhoa carambola*) in field trails. Chemosphere 76:1233–1239

Li JT, Liao B, Lan CY, He ZH, Baker AJM, Shu WS (2010) Cadmium tolerance and accumulation in cultivars of a high biomass tropical tree (*Averrhoa carambola*) and its potential for phytoextraction. J Environ Qual 39:1262–1268

Lin CC, Lai HY, Chen ZS (2010) Bioavailability assessment and accumulation by five garden flower species grown in artificially cadmium-contaminated soils. Int J Phytoremediat 12:454–467

Liu X, Peng K, Wang A, Lian CL, Shen ZG (2010) Cadmium accumulation and distribution in populations of *Phytolacca Americana* L. and the role of transpiration. Chemosphere 78:1136–1141

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30:1919–1926

Lu CJ, Chiu YL, Chen HL, Lai HY (2012) Effects of sludge and pH adjustment on Cd speciation in soil and growth and Cd accumulation in Pak Choi. Soil Sediment Contam 21:510–524

Martinez CE, Motto HL (2000) Solubility of lead, zinc and copper added to mineral soils. Environ Pollut 107:153–158

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282

Mertens J, Vervaeke P, Meers E, Tack FMF (2006) Seasonal changes of metals in willow (*Salix* sp.) stands for phytoremediation on degraded sediment. Environ Sci Technol 40:1962–1968

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Page AL, Sparks DL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods. ASA, Inc. and SSSA, Inc., Madison, WI

Pietrini F, Zacchini M, Iori V, Pietrosanti L, Bianconi D, Massacci A (2010) Screening of poplar clones for cadmium phytoremediation using photosynthesis biomass and cadmium content analysis. Int J Phytoremediat 12:105–120

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540

Rhoades JD (1996) Salinity: electrical conductivity and total dissolved solids. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods, 2nd edn. ASA, Inc. and SSSA, Inc., Madison, WI

Römkens P, Bouwman L, Japenga J, Draaisma C (2002) Potentials and drawbacks of chelate-enhanced phytoremediation of soils. Environ Pollut 116:109–121

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109:1427–1433

Shi GG, Cai QS (2009) Leaf plasticity in peanut (*Arachis hypogaea* L.) in response to heavy metal stress. Environ Exp Bot 67:112–117

Tessier A, Gampbell P, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844–851

Thomas GW (1982) Exchangeable cation. In: Page AL et al (eds) Methods of soil analysis, Part 2, Chemical and microbiological properties, vol 9, Agronomy monograph. ASA, Inc. and SSSA, Inc., Madison, WI

Thomas GW (1996) Soil pH and soil acidity. In: Page AL, Sparks DL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis, Part 3, Chemical methods. ASA, Inc. and SSSA, Inc., Madison, WI

Ultra VU Jr, Yano A, Iwasaki K, Tanaka S, Mei KY, Sakurai K (2005) Influence of chelating agent addition on the copper distribution and microbial activity in soil and copper uptake by brown mustard (*Brassica juncea*). Soil Sci Plant Nutr 51:193–202

US EPA (2000) Biosolids technology fact sheet: land applied of biosolids. EPA 832-F-00-064

Vieira RF (2001) Sewage sludge effects on soybean growth and nitrogen fixation. Biol Fertil Soils 34:196–200

Wang CX, Mo Z, Wang H, Wang ZJ, Cao ZH (2003) The transportation, time-dependent distribution of heavy metals in paddy crops. Chemosphere 50:717–772

Wei JL, Lai HY, Chen ZS (2012) Chelator effects on bioconcentration and translocation of cadmium by hyperaccumulators, *Tagetes patula* and *Impatiens walleriana*. Ecotoxicol Environ Safe 84:173–178

Wei SH, Zhou QX (2004) Identification of weed species with hyperaccumulative characteristics of heavy metals. Prog Nat Sci 14:1259–1265

Wong CC, Wu SC, Kuek C, Khan AG, Wong MH (2007) The role of mycorrhizae associated with vetiver grown in Pb/Zn-contaminated soils: greenhouse study. Restor Ecol 15:60–67

Wu LH, Luo YM, Xing XR, Christie P (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. Agric Ecosyst Environ 102:307–318

Wu SC, Wong CC, Shu WS, Khan AG, Wong MH (2011) Mycorrhizo-remediation of lead/zinc mine tailing using vetiver: a filed study. Int J Phytoremediat 13:61–74

Zhivotovsky OP, Kuzovkina JA, Schulthess CP, Morris T, Pettinelli D, Ge M (2011) Hydroponic screening of willows (*Salix* L.) for lead tolerance and accumulation. Int J Phytoremediat 13:75–94

# 17 Phytoremediation and the Electrokinetic Process: Potential Use for the Phytoremediation of Antimony and Arsenic

Nazaré Couto, Paula Guedes, Alexandra B. Ribeiro, and Dong-Mei Zhou

## 17.1 Introduction

### 17.1.1 Soil and Mining Activities

Soil is a natural resource, non-renewable at a human scale and with important environmental and socio-economic functions. It is a complex living body with dynamics resulting from the interaction amongst the lithosphere, hydrosphere, biosphere and atmosphere representing an interface between organisms (biosphere and geosphere), water (atmosphere and hydrosphere), air and rock. The solid phase of soil presents mineral and organic phases, whereas pore space holds water or air. Soil has an important role in nutrient cycling, ecosystem productivity as well as carbon and hydrologic cycle being, at the same time, a matrix for plant growth and habitat for multiple species. Soil is also a source of nutrients and organic waste recycling.

Mining and smelting industries produce huge amounts of waste and tailings which get deposited at the surface. The degraded soils and the waste rocks and tailings are often very unstable and will become sources of pollution as they have a high concentration of heavy metals and other toxic chemicals. When exposed to environmental conditions, these materials give rise to the oxidation of the remaining sulphides, through several reactions (chemical, electrochemical and biological), and form ferric hydroxides and sulphuric acid combined in acidic mine drainage (Soucek et al. 2000). For these reasons, mining activities will have a direct effect on the loss of cultivated land, forest or grazing land and the overall loss of production (Wong 2003). The indirect effects will include air and water pollution and siltation of rivers that will eventually lead to the loss of biodiversity, amenity and economic wealth (Bradshaw 1993).

Pollution that occurs as a consequence of hard rock mining persists for hundreds of years after the cessation of mining operations (Nagajyoti et al. 2010). Hence, areas impacted by former mining activities could remain nowadays severely polluted even if vestiges of such operations are not really apparent.

Lead, cadmium, copper, zinc, nickel, chromium and arsenic are the metals/metalloids most frequently reported to have the highest impact on organisms (Vamerali et al. 2010) including nervous, cardiovascular, renal and gastrointestinal disorders as well as cancers.

Antimony is a toxic bioaccumulative element with similar chemical and toxicological properties to arsenic, and moderate levels of them may lead to harmful environmental effects. Thus, arsenic, antimony and their compounds are considered to be priority pollutants by the US EPA and the EU (Filella et al. 2002).

Arsenic is found associated with many types of mineral deposits, especially with those including sulphide mineralisation (Alloway 1995), and the Iberian Pyrite Belt (IPB, SW Iberian Peninsula, from Portugal to Spain) is one of the most outstanding massive sulphide provinces in the world since it contains more than 80 deposits and about 1,700 Mt of reserves (Sáez et al. 1999). The long-term and intense exploitation has produced a large amount of sulphide-rich wastes that, in contact with precipitation water, are oxidised generating highly pollutant acid mine drainage, this phenomenon being responsible for the presence of acidity and metalloids in the soils and superficial water of the area.

N. Couto, Ph.D. (✉) • P. Guedes, M.Sc.
CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica 2829-516, Portugal

Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
e-mail: md.couto@fct.unl.pt

A.B. Ribeiro, Ph.D.
CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica 2829-516, Portugal

D.-M. Zhou, Ph.D.
Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

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China is the highest producer of Sb with approximately 89.8 % of the world's share, *Index 2011* (http://www.indexmundi.com/en/commodities/minerals/antimony/antimony_t9.html. Accessed 24 July 2013), and this results in large amount of this metalloid being released to the environment (Wilson et al. 2010; Flynn et al. 2003). Only in 2007, over $9.45\times10^5$ tons of waste water, $6.72\times10^5$ tons of mining and smelting residue and $1.50\times10^3$ tons of arsenic–alkali residue were discharged into the nearby environment ((He et al. 2012) and references therein).

### 17.1.2 Arsenic and Antimony as Environmental Contaminants

Antimony and arsenic are metalloids belonging to group 15 of the periodic table, and they both occur naturally in the environment at trace levels. Due to their identical $s^2p^3$ outer orbital electron configuration, Sb and As present the same range of oxidation states in environmental systems occurring in four oxidation states: +V, +III, 0 and −III. More commonly, they occur as oxides, hydroxides or oxoanions either in the +V state in relatively oxic environments (antimonates and arsenates) or in the −III state in anoxic environments (Wilson et al. 2010).

Arsenic has long been recognised as a toxic element (Azcue and Nriagu 1994), but the understanding of Sb toxicity and environmental behaviour is more limited (Wilson et al. 2010; Filella et al. 2009). Chemical similarities between the two metalloids have prompted concerns over the enrichment of Sb in many environments, and it is often considered that it behaves similarly to As, but not always with justification (Casiot et al. 2007). The toxicities of Sb and As in the environment strongly depend upon the speciation (Filella et al. 2002) being the inorganic forms considered more toxic than organic forms and predominate over organic forms in most environmental systems (Ellwood and Maher 2002). The general order of toxicity for Sb species is given as organoantimonials (e.g. methylated species) < antimonates (Sb (V)) < antimonites (Sb (III)) (Filella et al. 2002), which is similar to As: organoarsenicals (e.g. ethylated species) < arsenates (As (V)) < arsenites (As (III)) (Yamauchi and Fowler 1994).

Both metalloids can be strongly retained in soils (Flynn et al. 2003), and the extent of sorption influences the mobile and bioavailable fraction and consequently the extent of plant accumulation. The biogeochemistry of metalloids, e.g. arsenic, can be influenced by soil properties as the content of Fe, Al, Ca and P, soil organic matter and cation exchange capacity (CEC) (Sarkar and Datta 2006). In fact, several factors influence As and Sb retention in soil, but soil pH has an important influence as does the occurrence of co-occurring and competing ions (Wilson et al. 2010).

Background As and Sb concentrations are important to define the levels of soil contamination and establish remediation goals. In mining areas, the concentrations of these metalloids are, as expected, much higher than, that is, in urban areas. Concentrations of Sb and As in non-contaminated soils are typically below 10 mg $kg^{-1}$ (Wilson et al. 2010). In mining areas the soil concentrations of these metalloids can be increased up to three orders of magnitude (Hiller et al. 2012; Okkenhaug et al. 2011). If dealing with, for example, medicinal plant species (Vaculík et al. 2013), these impacted places with Sb and As are of high concern as metalloids can enter the food chain. Currently, the World Health Organization has set the acceptable daily intake for As at 2 mg $day^{-1}$ kg of body $weight^{-1}$ (WHO 1989) and the tolerable daily intake for Sb at 6 mg $day^{-1}$ kg of body $weight^{-1}$ (WHO 2003).

The high metalloid content may pose a greater risk to human health in highly contaminated areas, so research and management/remediation strategies need to be considered in these regions.

## 17.2 Phytoremediation of Metals and Metalloids

### 17.2.1 The Concept

Biological remediation can be, classically, divided into bio- and phytoremediation techniques that rely on the capabilities of plants and/or microorganisms to eliminate or accumulate contaminants from a matrix. Phytoremediation degrades, sequesters and/or removes contaminants from different environmental matrices with plants handling contaminants without affecting topsoil. Several advantages are intrinsically linked with the technology as it is a non-destructive natural option that does not disrupt the environment nor damage the soil structure, being highly accepted by the community. Additionally, it is cost-effective, does not need extensive labour or specialised equipment and can be applied anywhere (since the land is suitable for plant growing), and the establishment of vegetation prevents metal leaching and erosion of contaminated soils. Limitations associated with phytoremediation are related with climatic and geological determinants, scope of application, removal rates as well as extremes of environmental toxicity, which are normally variable amongst plant species.

Plants enclose the ability to remove/alter contaminants from environmental matrixes and detoxify by different mechanisms, such as phytoextraction (or phytoaccumulation), phytofiltration, phytostabilisation, phytovolatilisation, phytodegradation, rhizodegradation and phytodesalination (Ali et al. 2013). Phytoextraction focuses on the uptake of contaminants by roots and further translocation to and accumulation in above-ground biomass leading to a long-term cleanup of soil. Plant shoots enclosing metals/metalloids can be harvested and further disposed or reused for environmental applications (e.g. biomass production, phytomining).

Phytoextraction can be performed employing hyperaccumulators, (1) plants that produce low amount of above-ground biomass but accumulate metals to a greater extent or (2) plants that accumulate metals at less extent but produce more above-ground biomass with a total accumulation being comparable with hyperaccumulators (Ali et al. 2013).

The extent of metal mobility in soil is related with its chemical composition and sorption properties. After entering in soil, a percentage will reversibly bind to clay surfaces; some will precipitate with other inorganic phases such as phosphates or carbonates, namely, in the presence of alkaline pH, some will specifically adsorb onto solid surfaces; and some will bind to organic colloids such as humic and fulvic acids (Antoniadis et al. 2006). Retention of metals by soil is usually electrostatic, with ions with a positive charge being associated with negatively charged sites on the soil and vice versa. Soil CEC reflects the potential to retain these ions as it indicates a number of negative charges *per* unit mass.

The strong binding to soil particles or precipitation will cause insoluble metals/metalloids that will be mostly unavailable for uptake (Lasat 2002; Sheoran et al. 2011). Thus, the efficiency and extent of phytoextraction will be dependent of contaminant bioavailability for uptake, speciation and efficiency of each plant species regarding metal uptake (ability to intercept, absorb and accumulate contaminants) (Ali et al. 2013; Bech et al. 2012; Bhargava et al. 2012; Saifullah et al. 2009) as plants have specific responses to metal/metalloid tolerance, accumulation mechanisms and genetic and environmental factors, e.g. as light and temperature in greenhouse conditions (Antoniadis et al. 2006).

Factors regulating metal and metalloid availability may affect plant uptake, but their concentration in plant tissues also depends on total and available concentrations in soils where plants are growing. Amongst different metals/metalloids, it is known that Zn, Cd, Ni, As, Se and Cu are examples of mobile elements that are readily bioavailable, whereas Co, Mn and Fe are moderately bioavailable and the ones more strongly sorbed to humic substances, i.e. Cr and Pb, are less available (Ali et al. 2013; Prasad 2003).

Phytoextraction can be natural (with no soil amendments) or induced/assisted (Ali et al. 2013) in order to increase metal solubility. One option is the addition of chelating agents, for instance, synthetic aminopolycarboxylic acids, e.g. ethylenediamine tetra-acetic acid (EDTA), and the natural ones such as ethylenediamine disuccinate (EDDS) and nitrilotriacetic acid but also natural low-molecular-weight organic acids such as tartaric and citric acid, as reviewed by Evangelou et al. (2007). By applying a chelating agent, contamination levels may start to decrease as plant uptake is enhanced due to the dissolution of precipitated or adsorbed heavy metals. Chelating agents enhance metal solubility (Ali et al. 2013; Saifullah et al. 2009; Evangelou et al. 2007; Leštan et al. 2008; González et al. 2011; Gheju and Stelescu 2013; Kos and Lestan 2004; Lambrechts et al. 2011; Liu et al. 2008; Neugschwandtner et al. 2008) as they form water-soluble complexes with metals present in soil.

The performance of chelating agents depends both on metals to remediate and used plant species (Evangelou et al. 2007). Indian mustard and ryegrass were tested together with the application of biodegradable amendments, citric acid, $NH_4$-citrate/citric acid, oxalic acid, S,S-ethylenediamine disuccinic acid (EDDS) or nitrilotriacetic acid, with the increased uptake of Cd, Cr, Cu, U, Zn and Pb (Duquène et al. 2009). As pointed by Evangelou et al. (2007), when dealing with increased metal mobility, the rate of degradation becomes of special importance as it encloses a direct impact on leaching probability, this has been considered as one of the potential hazards intrinsically related with this technology (Saifullah et al. 2009). It can involve the risk of drinking water quality deterioration and groundwater contamination due to its possible migration. In consequence, the addition of these agents should take into consideration subsurface geochemical and hydrologic conditions (Cameselle et al. 2013).

Additionally, environmental persistence and environmental pollution as a consequence of using some chelating agents, e.g. EDTA, need a search for more degradable alternatives in assisted phytoextraction practices (Saifullah et al. 2009).

The chemistry of metals and/or metalloids is, normally, pH dependent—with higher pH, the metals bound more strongly to inorganic phases but became more available when in acidic pH. Low pH can also increase metal bioavailability as their salts are soluble in acidic conditions (Ali et al. 2013). To increase metal solubilisation, plant roots can also exudate phytosiderophores (Lone et al. 2008), substances that will increase metal mobilisation. Secretion of $H^+$ ions by plant roots will lower rhizosphere pH and thus increase metal dissolution displacing metal cations adsorbed to soil particles (Ali et al. 2013; Alford et al. 2010).

### 17.2.2 Phytoremediation of Arsenic and Antimony in Mining Areas/Soil

Mining areas can be rehabilitated by plants with potential to accumulate metals and/or metalloids. Plant species spontaneously growing in mining areas contaminated with Sb and As naturally accumulate these metalloids, mainly when in an available form (Levresse et al. 2012). The presence of plants that specifically accumulate one or both metalloids has also been studied for biogeochemical prospecting and mine stabilisation potential (Pratas et al. 2005). The mechanism of metal tolerance and detoxification enabled some plant species to survive, grow and reproduce in contaminated sites (Pratas et al. 2005). Vaculik et al. (2013) reported shoot concentrations range between 1 and 519 mg $kg^{-1}$ for As and 10 and 920 mg $kg^{-1}$ for Sb in medicinal plants (*Fragaria vesca*, *Taraxacum officinale*, *Tussilago farfara*, *Plantago major*, *Veronica officinalis*, *Plantago media* and *Primula elatior*) naturally growing in old mining sites in Slovakia, Central Europe. Other plant species such as *Dittrichia viscosa* (Pérez-Sirvent et al. 2012), *Pinus sylvestris* (pines), *Betula pendula* (birches), *Juncus effusus* (bulrush), *Cytisus scoparius* and the herbaceous *Plantago major* and *Deschampsia flexuosa* (Jana et al. 2012) are some examples of effective Sb and As accumulators. When in the presence of a plant that specifically accumulates one of the metalloids, the performance of As or Sb uptake can be variable. *Pteris cretica* L. (Cretan brake fern), an As hyperaccumulator, simultaneously accumulate As and Sb under hydroponic conditions, but when Sb is present in a medium level, As uptake is slightly enhanced, whereas it can be suppressed in the presence of high levels of Sb (Feng et al. 2011). Muller et al. (2013) also reported that when *Pteris vittata* (Chinese brake fern), another specific As hyperaccumulator, is in the presence of both metalloids, an increase of As concentration enhances Sb uptake, with metalloids going to different organs inside the plant.

The level of accumulation depends on the plant tissue (shoot or root) (Qi et al. 2011) and can be season dependent (Murciego et al. 2007). The accumulation levels are also related with the soil type, bioavailability and plant species, as observed for Sb (Qi et al. 2011). When in small quantities, some heavy metals such as Fe, Zn, Cu, Mn and Ni can be considered essential to organisms, whereas Cd, As, Pb, Cr and Hg are examples of non-essential metals/metalloids that can interfere with the functioning of living systems ((Ali et al. 2013) and references therein). Similarly with As, Sb is a non-essential element for plant living, and when present in elevated concentrations in plant tissues, they are phytotoxic for plant cells (Vaculík et al. 2013).

A study about Sb distribution and accumulation in plants from Xikuangshan Sb deposit area, a superlarge ore deposit, located in Hunan province, China, was carried out by Qi et al. (2011). The average Sb concentration was ca. 6,000 mg $kg^{-1}$, and the area presented 21 families and 34 plant species (amongst them, 23 were perennial herbs and annual forbs) with concentrations between ca. 4 and 144 mg $kg^{-1}$. The team also reported that bioavailability was dependent from different soil sites and plant species.

## 17.3 Electrokinetic Process for Remediations of Inorganic Contaminants

### 17.3.1 The Concept

Electrokinetic (EK) process relies on the application of a low-level direct current density in a partially saturated or even saturated soil, in the order of a few mA $cm^{-2}$ and/or low potential gradient, in the order of some V $cm^{-1}$, between electrodes. With this set-up, electric current will promote physicochemical changes in the contaminated matrix leading to the transport of contaminants (metals and/or organics) by mechanisms as electromigration, electro-osmosis (direct or reverse) and electrophoresis. The transport processes include (Mateus et al. 2010; Ribeiro et al. 2000, 2005):

1. Electro-osmosis, the mass flux of a pore fluid (water), i.e. the movement of soil moisture or groundwater, generally takes place from the anode to the cathode.
2. Electromigration, a directional movement, in which charged species, ions and ion complexes move towards the electrode with an opposite charge.
3. Electrophoresis, the movement of charged particles or colloids under the influence of an electric field; contaminants bound to mobile particulate matter are also transported.

Electro-osmosis and electromigration are non-selective processes implying that besides target contaminants, other elements (i.e. Mg, Ca and Fe associated with acid mobilisation (Ottosen et al. 2001)) are also transported. This can have implications in the soil nutritional status, but it can also decrease energy efficiency associated with EK process.

For these reasons, the electric field acts as a "cleaning agent", the contaminants being moved out of the contaminated matrix towards one of the electrode compartments, where they concentrate and may be removed (Ribeiro et al. 2005).

EK remediation process has been applied at laboratory and/or field scales, in different (1) matrices, soil, fly ash from straw combustion (Ottosen et al. 2007) or from municipal solid waste incinerators, impregnated wood waste (Mateus et al. 2010; Ribeiro et al. 2000), mine waste and sediments, and (2) contaminants, heavy metals (Lima et al. 2008; Wang et al. 2007), petroleum hydrocarbons, phenols and PAH (Alcántara et al. 2010), chlorinated solvents and pesticides/herbicides (Ribeiro et al. 2005, 2011).

Comparing to traditional remediation processes, EK presents advantages: (1) effective results for low permeability matrices (e.g. clay soils where conventional methods do not work), (2) effectiveness in small periods of time (Mateus et al. 2010; Ribeiro et al. 2000, 2005; Alcántara et al. 2010, 2012), (3) separation of different contaminants towards one of the electrode compartments (Ribeiro et al. 2005) and (4) remediation of toxic/persistent organic compounds (e.g. PAH (Alcántara et al. 2010)).

The transport processes and geochemical evolution of the contaminated matrix are complex during EK treatment, thus depending on soil and contaminant characteristics, treatment time, applied potential and/or electrolytic dissolutions (Reddy and Saichek 2004).

Electrokinetic soil treatment relies on several interacting mechanisms, but the dominant is the electron transfer reactions that occur at electrodes during the electrokinetic process which is the electrolysis of water (Eq. 17.1):

$$H_2O \rightarrow 2H^+ + \tfrac{1}{2}O_2(g) + 2e^-(\text{anode}) \tag{17.1}$$

$$2H_2O + 2e^- \rightarrow 2OH^- + H_2(g)(\text{cathode}) \tag{17.2}$$

The hydrolysis of water produces $H^+$ and $OH^-$ ions that are able to move across the matrix. Hydrogen ions will be responsible for the pH decrease near the anode and acid front that will be carried towards the cathode by electrical migration, diffusion and advection. At the same time, there is a pH increase near the cathode due to the formation of hydroxide ions (Virkutyte et al. 2002).

Matrix acidification and the continuous removal of its constituents dramatically change its physico-chemical characteristics with acid front helping to mobilise metals.

### 17.3.2 EK Process in the Remediation of Soil with Metals and Metalloids

Parameters that affect the efficiency of EK (current density, potential difference between electrodes, etc.) as well as the type of contaminant/nutrient, matrix and interactions matrix-contaminants have been studied in different matrices. The position of electrodes has also been explored with studies with electrodes horizontally or vertically installed in a contaminated soil.

The use of assisting agents allow a selective desorption of heavy metals and can help in the contaminant removal using EK process. For example, pH can be adjusted (Zhou et al. 2005) or chelating agents can be added (Yuan and Chiang 2008). The use of such agents is adequate, but not limited to, in the following situations, a particular combination of metals/metalloids implying a complex remediation process, when metals/metalloids are in insoluble form, in the presence of matrices with high CEC and intrinsic slow development of an acid front, implying a slow metal removal.

Due to water electrolysis (Eqs. 17.1 and 17.2), the soil pH can decrease between 2 and 3 values near the anode section and increase to 8–12 near the cathode, in soils with low buffer capacity (Zhou et al. 2005). A consequence of increased soil pH is the precipitation of metal hydroxides near the cathode, thus reducing removal efficiencies (Zhou et al. 2005). The alkaline front tends to precipitate the heavy metals, an effect that should be attenuated by suitably adjusting the pH in the cathode compartment (Zhou et al. 2004a, b, 2005; Lee and Yang 2000a).

Electrolyte pH adjustment can be an option to equilibrate soil pH and thus promote a higher metal removal. As pH decreases in the anode end, the heavy metal species should become more available. pH adjustment with lactic acid and calcium chloride in the catholyte caused an efficient removal of Cu, 63 %, and Zn, 65 %, from a low pH soil after ca. 9 days of experiment (Zhou et al. 2005). But without controlling pH of catholyte, pH near cathode was higher than 6 resulting in the accumulation of Cu and Zn in soil sections (Zhou et al. 2005).

EDTA has been used in EK treatments (Gidarakos and Giannis 2006), due to its strong chelant capacity and high complex stability with elements such as Zn, Cd, Pb and As (Sun et al. 2001).

#### 17.3.2.1 EK Process in the Remediation of Arsenic

All soluble arsenic species are anionic above pH 9, and As (V) is more strongly sorbed than As (III) (Virkutyte et al. 2002). Therefore, a careful management of the pH and other electrolyte conditions within the electrode reservoir to enhance desorption and increase the electro-osmotic flow rate is one of the critical factors in controlling EK system performance (Zhou et al. 2004b; Lee and Yang 2000b; Saichek and Reddy 2003). Both acids and bases can be used to mobilise As, depending on the dominant As species (Lee et al. 2007; Yang et al. 2009). As (V) dissolves under alkaline conditions but not under acidic conditions, whereas As (III) behaves in the opposite way (Alam and Tokunaga 2006). Catholyte conditioning with 0.1 M nitric acid showed removal efficiency for As of 62 % after 28 days, at 4 mA $cm^{-2}$ of current density (Baek et al. 2009). Remediation of As (V) with EK process and assisting agents, like EDTA, was reported by Yuan and Chiang (2008), and as potential gradient increased from 2.0 to 3.0 V $cm^{-1}$, the removal efficiency of As(V) was increased from approximately 35 to 45 % in the system. Oxalate and phosphate solutions might also serve as mobility-enhancing agents, and EK experiments conducted with mine tailings showed that in the section near the anode, As removal was 30 % with oxalate and 48 % with phosphate, after 20 days (Isosaari and Sillanpää 2012). But, due to the accumulation of As in the middle section, the respective overall removals in the entire tailing material were only 6 and 12 %. Potassium phosphate was also the most efficient in extracting arsenic from kaolinite, probably due to anion exchange of arsenic species by phosphate, whereas sodium hydroxide seemed to be the most efficient in removing arsenic from the tailing soil (Kim et al. 2005). This result may be explained by the fact that the sodium hydroxide increased the soil pH and accelerated ionic migration of the species through the desorption of arsenic species as well as the dissolution of arsenic-bearing minerals (Kim et al. 2005).

## 17.4 Electrokinetic Process Coupled with Phytoremediation

### 17.4.1 The Concept

One of the key aspects of innovation in the remediation field refers to the development of combined treatments and operational methods that contribute to improve the mobilisation and the transport of the contaminants out of the matrix. Due to its mobilisation potential, EK process can be considered an integrated tool for contaminant removal, alone and coupled with phytoremediation. The coupling of EK process with phytoremediation (also known as EK-assisted phytoremediation) is an innovative technique that deserves a deeper knowledge to enlarge the scope of EK application.

This technique has been developed since the 2000 decade (O'Connor et al. 2003) and has been optimised and successfully tested (Cang et al. 2011; Zhou et al. 2007).

As pointed in the review performed by Cameselle et al. 2013, the rehabilitation of a contaminated site by phytoremediation requires a long treatment time as it depends on the growth rate, biomass production and even adaptation of the plant to environmental conditions (including contaminant toxicity). Additionally, the action of roots is mainly in the range between 20 cm and 2 m and its effectiveness linked with the bioavailability of contaminants together with soil properties. Joining electrokinetics to the process may be an attempt to deal with limitations of phytoremediation (Cameselle et al. 2013). EK field may enhance the removal of the contaminants by increasing its bioavailability (enhancement of solubilisation and/or selective mobilisation) by desorption and transport of metals or metalloids, thus facilitating phytoextraction process. The removal of contaminant from soil is performed by the plant with a synergistic effect of the DC field that enhances plant activity by increasing bioavailability of contaminant, while the plant simultaneously rehabilitates soil properties changed by the presence of electrokinetics. The presence of plants brings most of the benefits of a "regular" phytoremediation scheme, recovery of soil properties and improvement of its structure.

### 17.4.2 Applications with Metals and Metalloids

In 2003, O'Connor and his team (O'Connor et al. 2003) coupled phytoremediation with EK process by applying a horizontal DC field of 30 V to a contaminated soil in order to evaluate the effect on Cu/Cd/As uptake by ryegrass. For that, two contaminated soils were used in lab scale reactors. The first soil was heavily contaminated with Cu and was treated for 98 days, whereas the second was contaminated with Cd and As and was treated for 80 days. With the coupled technique, a redistribution of soil metals was observed from anode to cathode. It also reported an increase of Cu uptake in the cathode region for the Cu soil. Due to the DC field, pH changed in the anode compartment, where the soil was acidified, but only a slight change in the other cell compartments was observed.

Cang et al. (2011) exposed Indian mustard (grown for 35 days) to different voltage gradients (0, 1, 2, 4 V $cm^{-1}$) of DC current applied in cycles of 8 h $day^{-1}$ for 16 days, aiming Pb, Cd, Cu and Zn removal. Heavy metals presented a redistribution from anode to cathode, and the uptake of Cd, Pb and Zn was increased by the action of DC field, namely, in the presence of a voltage gradient of 2 V $cm^{-1}$. Shoot concentration of Pb, Cd and Cu in the anode compartment was higher than in other sections due to soil acidification and thus activation of soil heavy metals in the anode region (Cang et al. 2011). The intensity of applied voltage was determinant in enhancing the uptake of a plant species as it reported an enhancement with 2 V $cm^{-1}$ but little or none with 1 V $cm^{-1}$, 4 V $cm^{-1}$ or no applied voltage (Cang et al. 2011).

The effect of DC and alternate field (AC) was tested together with potato tubers in Zn-, Pb-, Cu- and Cd-contaminated soil (Aboughalma et al. 2008). The AC field did not promote a significant metal redistribution or pH variation between anode and cathode, an opposite pattern than with DC field. Overall metal uptake was enhanced by an electric current with AC field presenting higher accumulation of heavy metals in plant shoots than DC field or no electric field. In general, AC current was more effective in phytoextraction as it did not change metal redistribution nor pH (Cameselle et al. 2013).

The effect of AC and/or DC field was also studied by Bi et al. (2011) with rapeseed and tobacco plants in soils spiked with Cd and multi-contaminated with Cd, Pb, Cu and Zn. As DC field induces pH alterations in soil, the team inverted the polarity of the field in intervals of 3 h allowing the comparison of AC and DC field without the "induced" variable of pH change. The presence of AC electric field showed a positive effect on rapeseed biomass enhancing the total metal uptake. Tobacco plants showed an opposite pattern, DC field has a negative effect on biomass, and AC field did not enhance biomass but slightly increased metal uptake. Multi-contaminated soil presented lower metal extraction efficiency probably related with contaminant bioavailability. In a Pb-contaminated sandy soil, Putra et al. (2013) used a continuous DC field during 15 days to enhance Pb uptake of *Poa pratensis* L. (Kentucky bluegrass) when compared with the results of phytoremediation alone for 30 days.

EK process can affect root and shoot development at different extents. O'Connor (O'Connor et al. 2003) reported a slight inhibition of plant growth in the anode compartment due to soil acidification and movement of nutrient cations (e.g. Ca). Cang et al. (2011) reported a positive effect in plant growth for lower voltage (1 V $cm^{-1}$) but also a decrease for 2 and 4 V $cm^{-1}$ comparing with control. The biomass of potato tubers was higher with AC current than control and this one higher than DC treatment, this last case explained by the acidic conditions and increased amount of metals in the soil which lead to an adverse effect on plant growth as a symptom of phytotoxicity (Aboughalma et al. 2008).

EK-assisted phytoremediation was also used with chelating agents promoting metal/metalloid dissolution but avoiding the risk of contaminant leaching. EK can deliver chelators into the soil facilitating contaminant solubilisation and transport of metal complexes in the direction of root plants (Cameselle et al. 2013). Zhou et al. (2007) tested ryegrass uptake of Cu/Zn with a vertical direct current electrical field with the presence of EDTA/EDDS. The use of vertical DC field, 1 V $cm^{-1}$, controlled the migration of metal complexes in soil columns, favouring transport towards the soil surface where the plant was growing. The use of EDTA/EDDS increased uptake of Cu/Zn when compared with no application of chelating agents at all tested depths, 15, 30 and 50 cm. Electrodes were inserted in the extremities of soil column with anode 5 cm below soil surface and cathode at bottom causing a redistribution of Cu/Zn concentration. Shoot Cu concentration with EDTA/EDDS together with DC field presented better results than without DC field. This work provides an alternative to a concerning situation already raised by scientists (please see Sect. 2.1.)—the possibility of metals leaching due to increased solubility provided by chelating agents. Phytoremediation, by itself, could be a viable option to take advantage of metal solubility and thus remove them from the contaminated soil. But its removal rate could not be sufficient to avoid metal leaching. By using the EK process, an efficient control of leaching was reported by Zhou et al. (2007) with low metal concentrations in pore water.

Lim et al. (2004, 2012) reported the positive effect of a chelator (EDTA) together with electrokinetics in the enhanced Pb uptake of Indian mustard. Indeed, simultaneous addition of EDTA with electric field increased the accumulation of Pb in the shoots by 2–4 times compared with the use of EDTA only (Lim et al. 2004).

On one hand, EK process affects soil chemistry, most commonly the acidification of the soil (when a DC field is applied), and possible disappearance of most of the natural microflora due to the toxic effect of the acidic pH, but, on the other hand, plant growth favours increased enzymatic and microbial activity (Cameselle et al. 2013). Cang et al. (2012) carried out a study with the purpose of understanding the effect of electric current on physico-chemical soil properties and enzymatic and microbial activity and reported differences between the three soil compartments (anode, central compartment and cathode). $NO_3^-$, $NH_4^+$, available K and P increased compared with their initial soil concentration. Basal soil respiration and microbial biomass carbon significantly increased near the anode and cathode. All tested enzymatic parameters (urease, invertase and phosphatase) were inhibited by DC field. DC field was the main factor affecting the soil properties, but plant growth counteracted to the same extent its impact on soil properties.

## 17.5 Case Study from a Mine Soil from China

### 17.5.1 The Problem

The present work (Couto 2015) is a consequence of the works carried out by Zhou et al. (2007) and Cang et al. (2011). The aim was to assess the potential and applicability of EK-assisted phytoremediation to remediate soil contaminated with Sb and As. Soil from a mining area located in southern Hunan province (China) was used. Arsenic concentration was $65.8 \pm 0.8$ mg $kg^{-1}$ and Sb $546.5 \pm 0.8$ mg $kg^{-1}$. The soil pH, CEC and soil organic carbon (SOC) were 6.58, 14.5 cmol $kg^{-1}$ and 45.1 g $kg^{-1}$, respectively.

### 17.5.2 Materials and Methods

The effect of Indian mustard and ryegrass plant species in the enhanced removal of Sb and As from the soil was evaluated in the presence of an electrical field. Each experimental box was divided in three different parts using a vertical nylon mesh—anode to central compartment to cathode (Fig. 17.1)

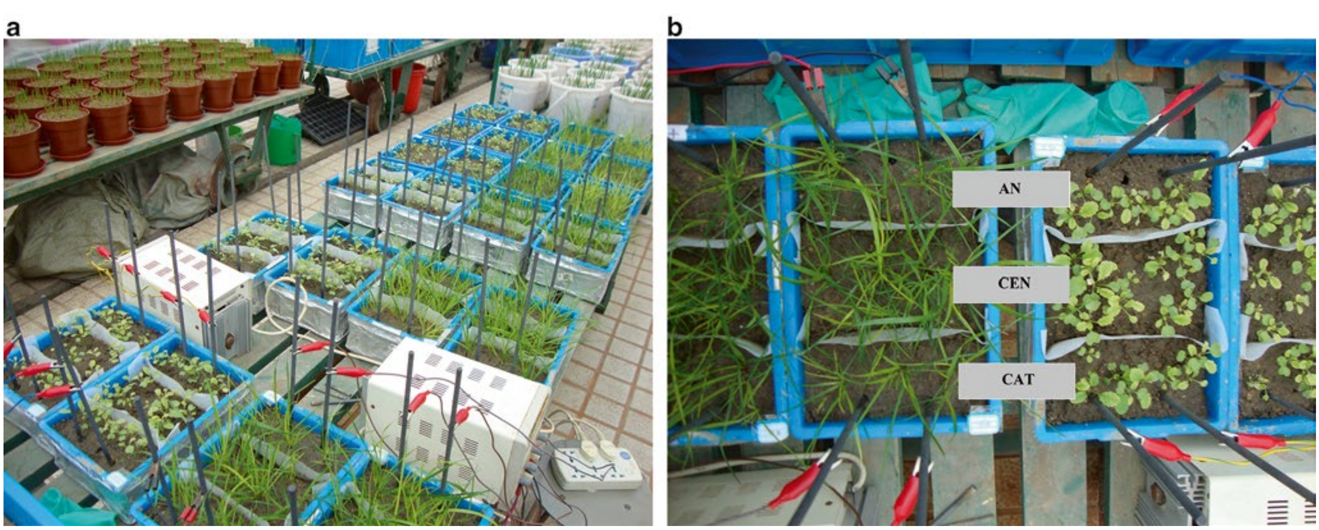


**Fig. 17.1** *Boxes* with contaminated soil and EK treatment: experiment overview (**a**) and details of experimental boxes with ryegrass at left and Indian mustard at right (**b**)

with a soil proportion of 900:1,200:900 g in each box. Following soil fertilisation (solution of 0.99 g urea and 0.96 g of $KH_2PO_4$), pre-germinated seedlings were grown in each experimental pot during 35 days, with a proportion of 12:16:12 plants per pot. Graphite rods (length 15 cm, diameter 6 mm) were used as working electrodes as they are low cost, inert and widely available. Four rods (2+2) were vertically inserted into the soil in opposite sides of the box. A voltage gradient was applied, 20 V, for 15 days and during 8 h per day. The experiment was carried out in a glass greenhouse, and the soil was kept at 70 % of its holding water capacity. Results discussed in this chapter are those from (1) plant effect and (2) plant with simultaneous presence of DC field.

### 17.5.3 Results and Discussion

At the end of the experiment, soil pH values changed slightly when comparing to control experiment pH (6.58) (data not shown). In the anode compartment pH decreased (ca. 0.5), whereas it increased in the cathode compartment (ca. 0.8) being pH variations more expressive between Indian mustard compartments with statistical significance at 95 %. The application of the electric field did not present a negative effect on plant biomass, and inclusive, a tendency for higher biomass production was found when comparing with plant biomass achieved without electric field, namely, for Indian mustard (data not shown).

Ryegrass and Indian mustard accumulated As and Sb (Figs. 17.2 and 17.3), and overall Indian mustard showed higher metal accumulation than ryegrass. For example, Indian mustard accumulated significantly more Sb than ryegrass in its shoot and As and Sb in the root. Additionally, after applying EK, As and Sb accumulations in the shoot and root of Indian mustard were improved. The enhanced remediation efficiency of the coupled technology was more expressive in the cathode compartment of Indian mustard shoot with, for example, more 45 % Sb uptake than in the central compartment. However, no significant improvement of As and Sb uptake was observed for ryegrass in the presence of EK treatment.

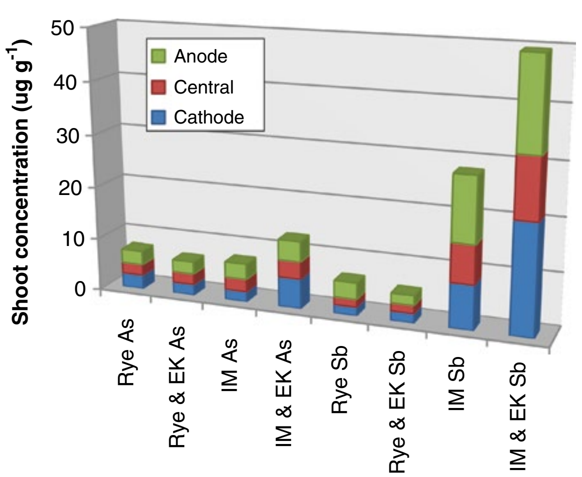


**Fig. 17.2** Concentration of As and Sb in the shoot of Indian mustard (IM) and ryegrass (rye) with and without EK treatment

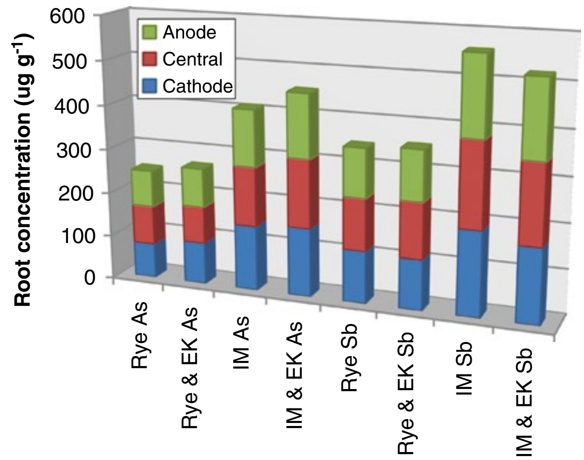


**Fig. 17.3** Concentration of As and Sb in the root of Indian mustard (IM) and ryegrass (rye) with and without EK treatment

## 17.6 Conclusions

Soil contamination with metals/metalloids is a worldwide problem that needs to be addressed. There are several approaches that can be applied aiming soil remediation/rehabilitation, and both EK and phytoremediation seem to be a viable option.

Results demonstrate that both plant species can accumulate metalloids, and for that, they are considered valuable for phytoremediation schemes of mining areas co-contaminated with As and Sb. In general, Indian mustard accumulated more than doubled As and Sb concentrations in their tissues compared to ryegrass. EK-assisted phytoremediation seems a suitable combination for the upgrade of mine-contaminated areas.

Gathering fundamentals and research on applied EK-assisted phytoremediation will allow deeper knowledge on the application of this hybrid technology, which assemble advantages from both processes, in the design of a remediation scheme.

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## References

Aboughalma H, Bi R, Schlaak M (2008) Electrokinetic enhancement on phytoremediation in Zn, Pb, Cu and Cd contaminated soil using potato plants. J Environ Sci Health A Tox Hazard Subst Environ Eng 43(8):926–933

Alam MGM, Tokunaga S (2006) Chemical extraction of arsenic from contaminated soil. J Environ Sci Health A Tox Hazard Subst Environ Eng 41(4):631–643

Alcántara MT, Gómez J, Pazos M, Sanromán MA (2010) Electrokinetic remediation of PAH mixtures from kaolin. J Hazard Mater 179(1–3):1156–1160

Alcántara MT, Gómez J, Pazos M, Sanromán MA (2012) Electrokinetic remediation of lead and phenanthrene polluted soils. Geoderma 173–174:128–133

Alford ER, Pilon-Smits EAH, Paschke MW (2010) Metallophytes—a view from the rhizosphere. Plant Soil 33:733–750

Ali HH, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881

Alloway BJ (1995) Heavy metals in soils. Blackie Academic & Professional, Glasgow

Antoniadis V, Tsadilas C, Samaras V, Sgouras J (2006) Availability of heavy metals applied to soil through sewage sludge. In: Prasad MNV, Sajwan KS, Naidu R (eds) Trace elements in the environment, biogeochemistry, biotechnology, and bioremediation. Taylor and Francis, FL

Azcue JM, Nriagu JO (1994) Arsenic: historical perspectives, In: Nriagu JO (ed). Arsenic in the environment part 1: cycling and characterisation. John Wiley and Sons, Toronto

Baek K, Kim DH, Park SW, Ryu BG, Bajargal T, Yang JS (2009) Electrolyte conditioning-enhanced electrokinetic remediation of arsenic-contaminated mine tailing. J Hazard Mater 161(1):457–462

Bech J, Corrales I, Tume P, Barceló J, Duran P, Roca N, Poschenrieder C (2012) Accumulation of antimony and other potentially toxic elements in plants around a former antimony mine located in the Ribes Valley (Eastern Pyrenees). J Geochem Explor 113:100–105

Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120

Bi R, Schlaak M, Siefert E, Lord R, Connolly H (2011) Influence of electrical fields (AC and DC) on phytoremediation of metal polluted soils with rapeseed (Brassica napus) and tobacco (Nicotiana tabacum). Chemosphere 83(3):318–326

Bradshaw AD (1993) Understanding the fundamentals of succession. In: Miles J, Dalton DH (eds) Primary succession on land. Blackwell, Oxford

Cameselle C, Chirakkara RA, Reddy KR (2013) Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosphere 93:626–636

Cang L, Wang QY, Zhou DM, Xu H (2011) Effects of electrokinetic-assisted phytoremediation of a multiple-metal contaminated soil on soil metal bioavailability and uptake by Indian mustard. Separat Purific Technol 79(2):246–253

Cang L, Zhou D-M, Wang Q-Y, Fan G-P (2012) Impact of electrokinetic-assisted phytoremediation of heavy metal contaminated soil on its physicochemical properties, enzymatic and microbial activities. Electrochim Acta 86:41–48

Casiot C, Ujevic M, Munoz M, Seidel JL, Elbaz-Poulichet F (2007) Antimony and arsenic mobility in a creek draining an antimony mine abandoned 85 years ago (upper Orb basin, France). Appl Geochem 22:788–798

Couto N, Guedes P, Zhou DM, Ribeiro A (2015) Integrated perspectives of a greenhouse study to upgrade an antimony and arsenic mine soil—potential of enhanced phytotechnologies. Chem Eng J 262:563–570

Duquène L, Vandenhove H, Tack F, Meers E, Baeten J, Wannijn J (2009) Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments. Sci Tot Environ 407(5):1496–1505

Ellwood MJ, Maher WA (2002) Arsenic and antimony species in surface transects and depth profiles across a frontal zone: The Chatham Rise, New Zealand. Deep Sea Research Part I. Oceanogr Res Papers 49(11):1971–1981

Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68(6):989–1003

Feng R, Wei C, Tu S, Tang S, Wu F (2011) Simultaneous hyperaccumulation of arsenic and antimony in Cretan brake fern: evidence of plant uptake and subcellular distributions. Microchem J 97(1):38–43

Filella M, Belzile N, Chen YW (2002) Antimony in the environment: a review focused on natural waters. I. Occurrence. Earth Sci Rev 57(1–2):125–176

Filella M, Williams PA, Belzile N (2009) Antimony in the environment: knowns and unknowns. Environ Chem 6:95–105

Flynn HC, Meharg AA, Bowyer PK, Paton GI (2003) Antimony bioavailability in mine soils. Environ Pollut 124(1):93–100

Gheju M, Stelescu I (2013) Chelant-assisted phytoextraction and accumulation of Zn by *Zea mays*. J Environ Manage 128:631–636

Gidarakos E, Giannis A (2006) Chelate agents enhanced electrokinetic remediation for removal cadmium and zinc by conditioning catholyte pH. Water Air Soil Poll 172:295–312

González I, Cortes A, Neaman A, Rubio P (2011) Biodegradable chelate enhances the phytoextraction of copper by Oenothera picensis grown in copper-contaminated acid soils. Chemosphere 84(4):490–496

He M, Wang X, Wu F, Fu Z (2012) Antimony pollution in China. Sci Tot Env 421–422:41–50

Hiller E, Lalinská B, Chovan M, Jurkovi A, Klimko T, Jankulár M, Hovori R, Šottník P, Fl'aková R, Ženišová Z, Ondrejková L (2012) Arsenic and antimony contamination of waters, stream sediments and soils in the vicinity of abandoned antimony mines in the Western Carpathians, Slovakia. Appl Geochem 27(3):598–614

http://www.indexmundi.com/en/commodities/minerals/antimony/antimony_t9.html. Accessed 24 July 2013

Isosaari P, Sillanpää M (2012) Effects of oxalate and phosphate on electrokinetic removal of arsenic from mine tailings. Sep Purif Technol 86:26–34

Jana U, Chassany V, Bertrand G, Castrec-Rouelle M, Aubry E, Boudsocq S, Laffray D, Repellin A (2012) Analysis of arsenic and antimony distribution within plants growing at an old mine site in Ouche (Cantal, France) and identification of species suitable for site revegetation. J Environ Manage 110:188–193

Kim SO, Kim WS, Kim KW (2005) Evaluation of electrokinetic remediation of arsenic-contaminated soils. Environ Geochem Health 27(5–6):443–453

Kos B, Lestan D (2004) Chelator induced phytoextraction and in situ soil washing of Cu. Environ Pollut 134:333–339

Lambrechts T, Gustot Q, Couder E, Houben D, Iserentant A, Lutts S (2011) Comparison of EDTA-enhanced phytoextraction and phytostabilisation strategies with Lolium perenne on a heavy metal contaminated soil. Chemosphere 85(8):1290–1298

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31(1):109–120

Lee H, Yang J (2000) A new method to control electrolytes pH by circulation system in electrokinetic soil remediation. J Hazard Mater 77:227–240

Lee M, Paik I, Do W, Kim I, Lee Y, Lee S (2007) Soil washing of As-contaminated stream sediments in the vicinity of an abandoned mine in Korea. Environ Geochem Health 29(4):319–329

Leštan D, Luo C-L, Li X-D (2008) The use of chelating agents in the remediation of metal-contaminated soils: a review. Environ Pollut 153(1):3–13

Levresse G, Lopez G, Tritlla J, López EC, Chavez AC, Salvador EM, Soler A, Corbella M, Sandoval LGH, Corona-Esquivel R (2012) Phytoavailability of antimony and heavy metals in arid regions: the case of the Wadley Sb district (San Luis, Potosí, Mexico). Sci Tot Environ 427–428:115–125

Lim JM, Salido ALJ, Butcher D (2004) Phytoremediation of lead using Indian mustard (Brassica juncea) with EDTA and electrodics. Microchem J 76(1–2):3–9

Lim JM, Jin B, Butche DJ (2012) A comparison of electrical stimulation for electrodic and EDTA-ENHANCED phytoremediation of lead using Indian mustard (Brassica juncea). Bull Korean Chem Soci 33(8):2737–2740

Lima AT, Ottosen LM, Pedersen AJ, Ribeiro AB (2008) Characterization of fly ash from bio and municipal waste. Biomass Bioenerg 32(3):277–282

Liu D, Islam E, Li T, Yang X, Jin X, Mahmood Q (2008) Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of Sedum alfredii Hance. J Hazard Mater 153(1–2):114–122

Lone MI, He Z, Stoffella PJ, Yang X (2008) Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. J Zhejiang Univ-Sci B 9:210–220

Mateus E, Zrostlíková J, Gomes da Silva M, Ribeiro A, Marriott P (2010) Electrokinetic removal of creosote from treated timber waste: a comprehensive gas chromatographic view. J Appl Electrochem 40(6):1183–1193

Müller K, Daus B, Mattusch J, Vetterlein D, Merbach I, Wennrich R (2013) Impact of arsenic on uptake and bio-accumulation of antimony by arsenic hyperaccumulator Pteris vittata. Environ Poll 174:128–133

Murciego AM, Sánchez AG, González MAR, Gil EP, Gordillo CT, Fernández JC, Triguero TB (2007) Antimony distribution and mobility in topsoils and plants (Cytisus striatus, Cistus ladanifer and Dittrichia viscosa) from polluted Sb-mining areas in Extremadura (Spain). Environ Poll 145(1):15–21

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216

Neugschwandtner RW, Tlustoš P, Komárek M, Száková J (2008) Phytoextraction of Pb and Cd from a contaminated agricultural soil using different EDTA application regimes: laboratory versus field scale measures of efficiency. Geoderma 144(3–4):446–454

O'Connor CS, Lepp NW, Edwards R, Sunderland G (2003) The combined use of electrokinetic remediation and phytoremediation to decontaminate metal-polluted soils: a laboratory-scale feasibility study. Environ Monit Assess 84(1–2):141–158

Okkenhaug G, Zhu YG, Luo L, Lei M, Li X, Mulder J (2011) Distribution, speciation and availability of antimony (Sb) in soils and terrestrial plants from an active Sb mining area. Environ Pollut 159(10):2427–2434

Ottosen LM, Hansen HK, Ribeiro AB, Villumsen A (2001) Removal of Cu, Pb and Zn in an applied electric field in calcareous and non-calcareous soils. J Hazard Mater 85:291–299

Ottosen LM, Pedersen AJ, Hansen HK, Ribeiro AB (2007) Screening the possibility for removing cadmium and other heavy metals from wastewater sludge and bio-ashes by an electrodialytic method. Electrochem Acta 52(10):3420–3426

Pérez-Sirvent C, Martínez-Sánchez MJ, Martínez-López S, Bech J, Bolan N (2012) Distribution and bioaccumulation of arsenic and antimony in Dittrichia viscosa growing in mining-affected semiarid soils in southeast Spain. J Geochem Explor 123:128–135

Prasad MNV (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–700

Pratas J, Prasad MNV, Freitas H, Conde L (2005) Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. J Geochem Explor 85(3):99–107

Putra RS, Ohkawa Y, Tanaka S (2013) Application of EAPR system on the removal of lead from sandy soil and uptake by Kentucky bluegrass (Poa pratensis L.). Separat Purific Technol 102:34–42

Qi C, Wu F, Deng Q, Liu G, Mo C, Liu B, Zhu J (2011) Distribution and accumulation of antimony in plants in the super-large Sb deposit areas, China. Microchem J 97(1):44–51

Reddy KR, Saichek RE (2004) Enhanced electrokinetic removal of phenanthrene from clay soil by periodic electric potential application. J Environ Sci Health A Tox Hazard Subst Environ Eng 39:1189–1212

Ribeiro AB, Mateus EP, Ottosen LM, Bech-Nielsen G (2000) Electrodialytic removal of Cu, Cr, and As from chromated copper arsenate-treated timber waste. Environ Sci Technol 34(5):784–788

Ribeiro AB, Rodriguez-Maroto JM, Mateus EP, Gomes H (2005) Removal of organic contaminants from soils by an electrokinetic process: the case of atrazine: experimental and modeling. Chemosphere 59:1229–1239

Ribeiro AB, Mateus EP, Rodríguez-Maroto JM (2011) Removal of organic contaminants from soils by an electrokinetic process: the case of molinate and bentazone. Experimental and modeling. Separat Purific Technol 79(2):193–203

Sáez R, Pascual E, Toscano M, Almodóvar GR (1999) The Iberian type of volcano-sedimentary massive sulphide deposits. Miner Deposita 34(5–6):549–570

Saichek RE, Reddy KR (2003) Effect of pH control at the anode for the electrokinetic removal of phenanthrene from kaolin soil. Chemosphere 51(4):273–287

Saifullah ME, Qadir M, De-Caritat P, Tack FMG, Du-Laing G, Zia MH (2009) EDTA-assisted Pb phytoextraction. Chemosphere 74(10): 1279–1291

Sarkar D, Datta R (2006) Arsenic concentration and bioavailability in soils as a function of soil properties: a Florida case study. In: Prasad MNV, Sajwan KS, Naidu R (eds) Trace elements in the environment: biogeochemistry, Biotechnology, and bioremediation. Taylor and Francis, FL

Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41(2):168–214

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC (2000) Laboratory to field validation in an integrative assessment of an acid mine drainage–impacted watershed. Environ Toxicol Chem 19(4): 1036–1043

Sun B, Zhao FJ, Lombi E, McGrath SP (2001) Leaching of heavy metals from contaminated soils using EDTA. Environ Poll 113: 111–120

Vaculík M, Jurkovi A, Matejkovi P, Molnárová M, Lux A (2013) Potential risk of arsenic and antimony accumulation by medicinal plants naturally growing on old mining sites. Water Air Soil Pollut 224(5):1–16

Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8(1):1–17

Virkutyte J, Sillanpää M, Latostenmaa P (2002) Electrokinetic soil remediation—critical overview. Sci Tot Environ 289(1–3):97–121

Wang JY, Huang XJ, Kao JCM, Stabnikova O (2007) Simultaneous removal of organic contaminants and heavy metals from kaolin using an upward electrokinetic soil remediation process. J Hazard Mater 144(1–2):292–299

WHO (1989) Evaluation of certain food additives and contaminants. Thirty third report of the joint FAO/WHO expert committee on food additives (JECFA). World Health Organization, Geneva

WHO (2003) Antimony in drinking water. Background document for development of WHO guidelines for drinking water quality World Health Organization. WHO, Geneva

Wilson SC, Lockwood PV, Ashley PM, Tighe M (2010) The chemistry and behaviour of antimony in the soil environment with comparisons to arsenic: a critical review. Environ Pollut 158(5):1169–1181

Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50(6): 775–780

Yamauchi H, Fowler BA (1994) Toxicity and metabolism of inorganic and methylated arsenicals. In: Nriagu JO (ed) Arsenic in the environment. Part II; human health and ecosystem effects, Wiley, New York

Yang JS, Lee JY, Baek K, Kwon TS, Choi J (2009) Extraction behavior of As, Pb, and Zn from mine tailings with acid and base solutions. J Hazard Mater 171(1–3):443–451

Yuan C, Chiang TS (2008) Enhancement of electrokinetic remediation of arsenic spiked soil by chemical reagents. J Hazard Mater 152(1):309–315

Zhou DM, Alshawabkeh AN, Deng CF, Cang L, Si YB (2004a) Electrokinetic removal of chromium and copper from contaminated soils by adding lactic acid in cathode chamber as an enhancing reagent. J Environ Sci 16:529–532

Zhou DM, Deng CF, Cang L (2004b) Electrokinetic remediation of a Cu contaminated red soil by conditioning catholyte pH with different enhancing chemical reagents. Chemosphere 56:265–273

Zhou DM, Deng CF, Cang L, Alshawabkeh AN (2005) Electrokinetic remediation of a Cu–Zn contaminated red soil by controlling the voltage and conditioning catholyte pH. Chemosphere 61(4):519–527

Zhou DM, Chen HF, Cang L, Wang YJ (2007) Ryegrass uptake of soil Cu/Zn induced by EDTA/EDDS together with a vertical direct-current electrical field. Chemosphere 67(8):1671–1676

# Chromium Phyto-transformation in Salt Marshes: The Role of Halophytes

# 18

Isabel Caçador and Bernardo Duarte

## 18.1 Salt Marshes, Halophytes and Contaminants

Several studies (Morris et al. 1986; Bewers and Yeats 1989; Vale 1990) describe estuaries as efficient filters of suspended particulate matter (SPM) and heavy metals, majorly through particle-solute interactions, flocculation processes and settling down of metal-charged particles. Since the industrial revolution, large amounts of contaminants have been released to the atmosphere, soils and watercourses. Most of these emissions will be transferred in ultimate analysis into a water matrix, either by atmospheric deposition or by soil erosion, where they will stay dissolved or associated to sediment particles (Nriagu 1988; Viers et al. 2009). This is a very dynamic mechanism greatly influenced by the river hydrological conditions.

Salt marshes are natural deposits of heavy metals in the estuarine system (Doyle and Otte 1997; Williams et al. 1994). When located near polluted areas, these ecosystems receive large amounts of pollutants from industrial and urban wastes that either drifts downstream within the river flow or of waste dumping from the near industrial and urban areas (Reboreda and Caçador 2007). When metals enter salt marshes, they spread along with the tides and periodic floods and interact with the sediment and the biotic community (Suntornvongsagul et al. 2007). Most salt marsh plants accumulate large amounts of metals in their aerial and belowground organs (Caçador et al. 1996). Their ability to phytostabilize those contaminants in the rhizosediment is an important aspect in the ecosystem self-remediative processes and biogeochemistry (Weis and Weis 2004).

In the last decades, phytoremediation has become a promising biotechnology for cleaning up contaminants, namely, metals (Cunningham et al. 1995; Cunningham and Ow 1996). Several works concerning a large variety of plant species have been published in the last decades identifying possible hyperaccumulator species (Salt et al. 1997; Brown et al. 1994). Besides accumulation, some other abilities have arisen as potential metal detoxification mechanisms to less harmful forms, either by chelation (Duarte et al. 2007) or redox reactions, changing the metal's oxidation states. However two important aspects must be considered when choosing the best phytoremediator species for a specific location and level of contamination: the biomass production ability and the ecology of the species (Redondo-Gómez et al. 2011). When considering contaminated wetland phytoremediation, the number of potential species becomes reduced to a few due to the specificities of these environments, like tidal saltwater flooding and waterlogging. Even though a species is considered to fit in this description and with a phytoremediative potential, it is also important to consider its ecophysiological response (like oxidative feedback) to this metal accumulation. This oxidative feedback will be important to determine whether this species can tolerate high metal concentrations and with this maintain its ecophysiological health and carry out the phytoremediative process.

Chromium is used in many industrial processes, and its unregulated use and dumping have led to water, sediment and biota contamination (Vale et al. 2008; Duarte et al. 2008, 2009). Industrial activities such as plating, tanning, corrosion inhibition, glassware-cleaning solutions, wood preservation, metal finishing or chromite ore processing (COP), are the main sources of trivalent and hexavalent toxic chromium compounds (Barceloux 1999; Losi et al. 1994). Cr-elevated contents (up to 600 ppm) in some phosphate fertilizers may be also a significant source of this metal in soils, although the most hazardous addition of Cr to a soil is related to tannery sludge, which can contain up to 2.8 % of this metal (Kabata-Pendias and Pendias 2001). Estuarine areas are often affected by both these industrial and agricultural activities, unbalancing their critical environmental equilibrium (Pazos-Capeáns et al. 2010).

I. Caçador, Ph.D. • B. Duarte (✉)
MARE – Marine and Environmental Sciences Centre,
Faculty of Sciences of the University of Lisbon, Campo Grande
1749-016 Lisbon, Portugal
e-mail: baduarte@fc.ul.pt

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## 18.2 Chromium Sediment Storage

As stated before, one of the major sources of Cr supply to wetlands, and particularly in estuarine salt marshes, is the tidal flooding. When dissolved and particulate Cr reaches the salt marshes, it will be deposited in the sediments establishing chemical bounds with it. This can be easily observed by its speciation (how Cr is bound to each of the sediment components) as shown in Fig. 18.1.

The sediments become this way the major storage pool of Cr in the estuarine system. As seen in Fig. 18.1, Cr has high affinity for the organic matter, establishing relatively stable chemical bounds (Duarte et al. 2008, 2009). In fact, Cr is well described in the literature as being a strong oxidizer of the organic matter, being even used as reagent for the determination of some of the organic components of the sediments and water due to this property. This way, salt marsh sediments tend to store Cr in a rather stable form but which is accessible throughout oxidizable conditions or throughout organic matter hydrolysis, for example, by the microbial community (Duarte et al. 2008, 2009). This leads to shifts and fluctuations in the bioavailability of Cr, making it accessible to the biota. In fact, if the Cr concentrations in the estuarine environment are considered, the second largest pool of this metal appears in the roots of the halophytes.

## 18.3 Chromium Natural Phytoremediation

Considering the case of polluted salt marshes, the metal cycling throughout the sediment and the vegetation becomes of great interest. According to several works and as stated before (Duarte et al. 2010; Caçador et al. 2009), it is possible to observe that the two major pools of heavy metals in the salt marshes are the sediment and the root system of resident species (Fig. 18.2). The higher metal budgets in roots corroborate their increased ability for heavy metal accumulation. The calculated root decomposition rates suggest that these metal pools are quite mobile particularly in *S. maritima*. This mobility is very important since it creates a cycle of metals between the sediment and the root system. Although a higher fraction of biomass losses in the aboveground organs was found, their low metal concentration makes the detritus generated by the aboveground less contributing for the metal budgets. Conversely, the comparatively low losses of biomass in the root system generate less necromass, but with very high concentrations of metals. This necromass becomes important to the metal budget of the sediment, not only due to its input of heavy metals but also due to the increase of organic matter content of this matrix. The bioaccumulation of these elements in the belowground organs is rather mobile, being able to return to the sediment matrix due to necromass generation and mineralization processes subdue. The return of metals due to these decaying processes and consequent input of metals into the sediments, although in rather lower concentrations comparatively to the existent in this matrix, is very important to be considered not only due to the amount of metal released through this process but also by the metal forms it introduces into the sediment.

These organically bound metals were already reported as being one of the most important fractions of metals present in these sediments, being subjected to microbial degradation processes (Duarte et al. 2008), that can lead to more bioavailable metal forms and contamination of the pore waters. All this process is the equivalent to a phytoremediation process,

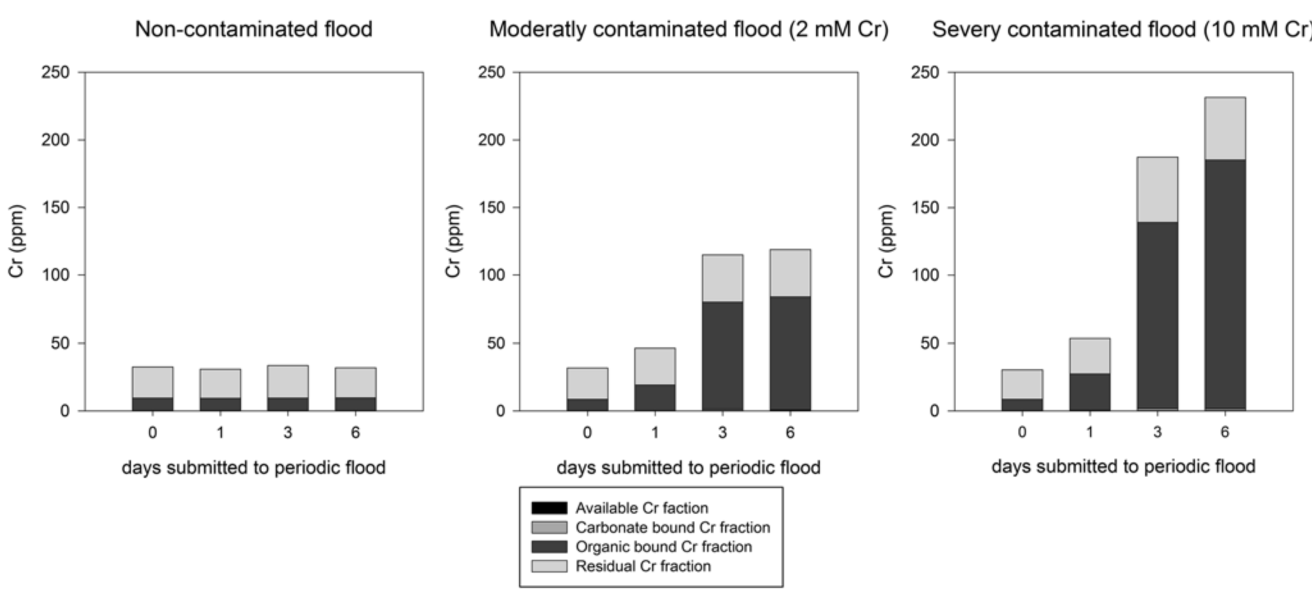



**Fig. 18.1** Chromium speciation and total concentrations in sediment exposed to different levels of contamination during 6 days of repeated periodic flooding with Cr-contaminated water

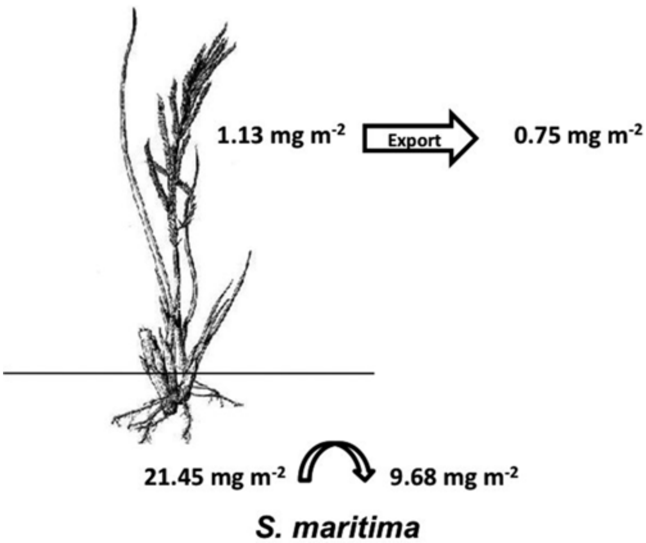



**Fig. 18.2** Chromium accumulation and exportations (due to senescence) per square metre by *Spartina maritima* in a low polluted salt marsh, during a year life cycle (adapted from Couto et al. 2013)

occurring everyday in natural ecosystem such as salt marsh, indicating the suitability of these halophytes as potential applications in promoted phytoremediation processes. This natural process is a well-documented ecological role of these ecosystems, contributing naturally for the estuarine depuration and promoting the maintenance of the ecosystem ecological status (Caçador et al. 2013).

## 18.4 Chromium-Assisted Phytoremediation

Recently, a new approach has also been taken based on this natural phytoremediation processes. This approach is based on the enhancement of this mechanism throughout the addition of chemical compounds (organic or inorganic) that would intervene in the phytoremediative process (Duarte et al. 2007, 2010). Plants can modify metal speciation throughout several processes, like radial oxygen loss (ROL) and exudation of organic acids (Sundby et al. 1998; Jacob and Otte 2004; Duarte et al. 2007). This last process is well documented for plants with agricultural interest (Jones 1998 and paper herein referred), but there are much less papers investigating wetland plants. One of the groups of substances exudated by salt marsh plants are low-molecular-weight organic acids (LMWOA), such as malic, citric and acetic acids (Mucha et al. 2005). These LMWOA are able to bind to metals establishing complexes and changing their bioavailability (Parker et al. 2001). These exudates have been related to nutrient uptake (Marschner 1995), metal detoxification (Ma et al. 2001) and microbial communication (Jones 1998) in agricultural ecosystems. The use of these LMWOA can be a useful tool in the so-called assisted phytoremediation processes improving the metal uptake by plants. As observed in Fig. 18.3, the application of LMWOA improved not only the phyto-extractability of Cr but also in some cases its allocation within the plant (Duarte et al. 2010). Although some of the changes result directly from the application of the LMWOA in study, they are often products of reactions in which these molecules are involved at the root surface. Cr exhibited higher uptake upon application of organic acids. All of the applications showed important increases in the Cr uptake probably due to the interaction of Cr(III) with these organic ligands resulting in the formation of very mobile organic-bound Cr(III) complexes (Srivastava et al. 1999). Although the presence of manganese oxides could lead to the oxidation of Cr(III) to Cr(VI) and formation of Cr(VI) organic-bound complexes that are less taken up by plants, there are several side reactions that slow down this oxidation. These maintain the Cr(III) organic-bound complexes as the more abundant species of Cr(III) (Bartlett 1991). This is in agreement with the present data, which shows a high increase of Cr uptake upon organic acids application, due to the formation of this highly mobile organic-bound Cr(III) complexes. Previous work showed that most of the Cr found in the roots was present in the form of Cr acetate (Bluskov et al. 2005). This form is mostly stored in the cortex of the roots and lately translocated to the aboveground parts by conversion into Cr oxalate. In the plants treated with acetic acid, there was a very high increase in Cr content not only in the roots but also in the shoots. This should probably be attributed to this mechanism of acetate-oxalate conversion, in contrast to what was found in the other LMWOA treatments where only the root Cr content increased.

The tenfold increase in Cr uptake observed when acetic acid is applied points out to a very promising technique for bioremediation of Cr-contaminated sediments. The potential environmental risk must be recognized at the early stage of LMWOA application. With this application, the labile complexes-associated metals could be absorbed and taken up directly by plants. New techniques could facilitate the decomposition of these organo-metal complexes in the short term, increasing the proportion of free ions and enhancing the uptake by plants, thus minimizing the environmental risk. Metals can therefore be removed of the system by harvesting of the aboveground biomass. This points out to a research need to make the use of these environmental-friendly phytoextraction enhancers feasible for commercial phytoextraction. In addition, the use of natural compounds in contraposition to synthetic chelates sounds better for the public acceptance of phytoextraction as a technology to clean up metal-polluted soils.

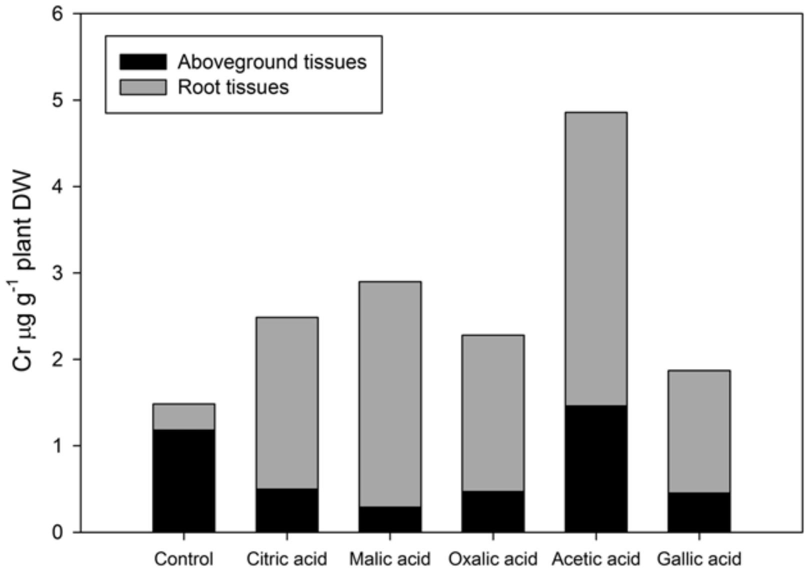



**Fig. 18.3** Chromium concentrations in above- and belowground tissues of *Spartina maritima* upon the application of LMWOA (10 mM) to its rhizosediments. Controls were supplemented with ultra-pure water only

## 18.5 The Phyto-transformation of Chromium (VI) to (III)

Chromium has two states of oxidation, Cr (III) and Cr (VI). The latter has been classified as a primary contaminant (Lytle et al. 1998) due to its mobility and reported harmful effects in animals and humans (Kortenkamp et al. 1996). Although this toxic effect is associated to Cr (VI) form, Cr is also an essential element in the nutrition of several organisms in its stable Cr (III) form (Katz and Salem 1994). This reduction of a toxic form to a stable nontoxic and beneficial form has gathered the attention of several investigative teams. Abiotically, the reduction of Cr (VI) can occur by the reactions with other ions, metallic or mineral surfaces and organic molecules (Wittbrodt and Palmer 1996). More important for phytoremediation proposes, the reduction of Cr (VI) to Cr (III) can be biologically mediated (Mikalsen et al. 1991; Stearns et al. 1995). Bacteria-mediated Cr (VI) to Cr (III) reduction pathway by a specific Cr reductase is well described (Shen and Wang 1993; Nies 1992; Romheld and Marschner 1983), although this mechanism has not been identified in non-engineered plants. Some recent studies (Duarte et al. 2012) point out some halophyte species as potential phyto-converters and phyto-accumulators of Cr. This has implications both at ecological and cellular levels. Ecologically it was found that, for example, *H. portulacoides* could accumulate very high Cr concentrations, in particular in the root system (Fig. 18.4).

Earlier reports pointed out in the same direction using non-halophyte-rooted aquatic plants (Gupta et al. 1999; Sinha et al. 2003; Suseela et al. 2002) and registered significantly higher values in the roots than their upper parts. High metal accumulation in the fine roots is also in agreement with earlier reports (Sinicrope et al. 1992). Qian et al. (1999) also reported the highest concentration of Cr in the plant roots and lower level in shoots similar to other ten elements studied in twelve aquatic plants. Also field-monitoring studies directed to this species showed that the main biological metal sink is the halophyte root system (Caçador et al. 2009; Duarte et al. 2010). This can be due to metal binding to organic ligands, thus reducing its mobility from roots to aerial parts. Another important aspect focused in this study (Duarte et al. 2012) was the *H. portulacoides*-mediated Cr (VI) to Cr (III) reduction (Fig. 18.5). It was found that this species can convert large Cr (VI) amounts to its less toxic form. This was already pointed out as a defence mechanism in sediments colonized by *H. portulacoides*, where a reduction of Cr (VI) to Cr (III) would have as consequence the retention, of this element, in Fe oxyhydroxide fraction, decreasing its bioavailability (Tanackovic et al. 2008). Lytle et al. (1998) found similar data concerning soluble Cr (VI) reduction by water hyacinth, suggesting that wetland plants uptake Cr in its less toxic form, throughout external reduction of Cr (VI) by their lateral fine roots, probably due to oxalate exudation.

This points out for two potential phytoremediative applications. Not only this species can accumulate large amounts of Cr withdrawn from its surrounding medium, but it can also convert high percentages of the remaining Cr (VI) into a less toxic form. This toxicity reduction is important not only for this plant species but also for the remaining surrounding biota, with a potentially essential role for environmental detoxification. This phytoremediation potential must be allied to a healthy and tolerant metabolism.

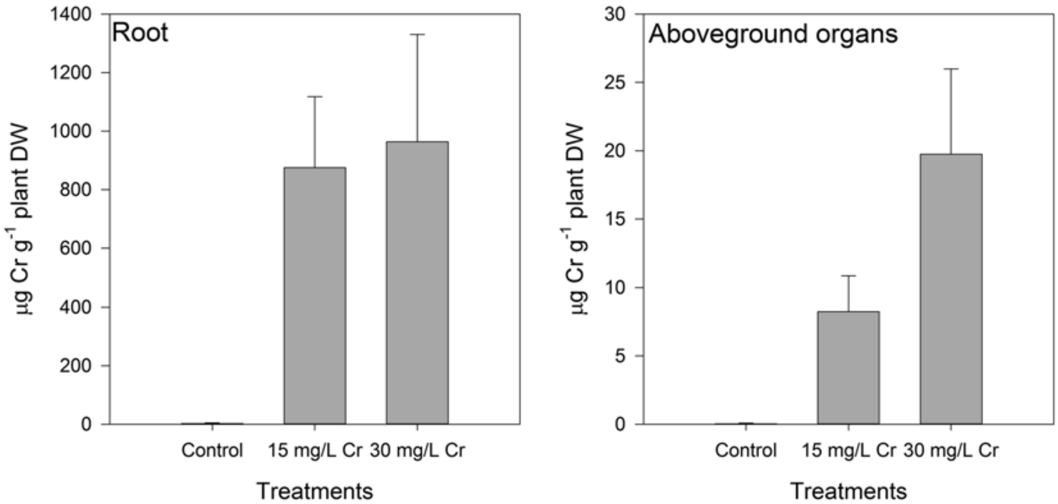
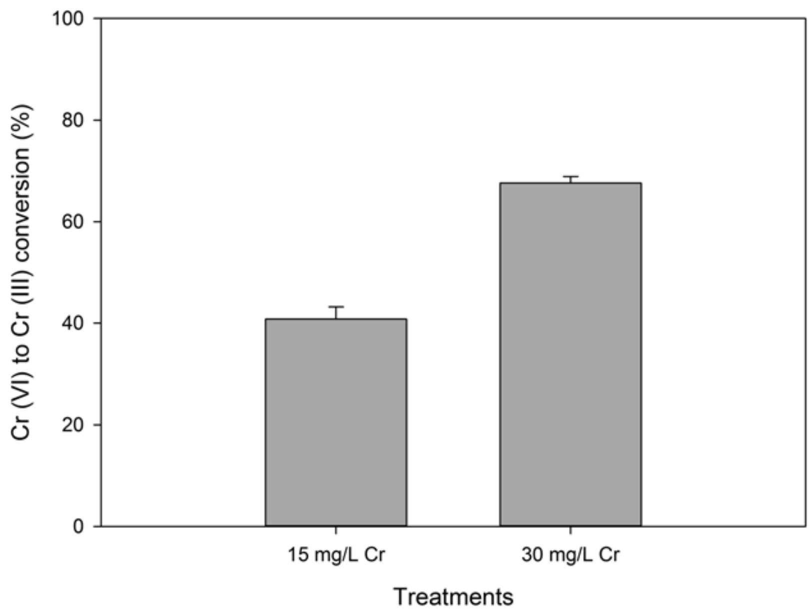



**Fig. 18.4** Chromium concentration in *H. portulacoides* aerial organs and root tissues subjected to different Cr (VI) concentrations

**Fig. 18.5** Cr (VI) to Cr (III) conversion percentage in both the considered treatments




## 18.6 Final Remarks

Salt marshes are very interesting field laboratories to study metal biogeochemistry, namely, Cr. Salt marsh localization in estuarine systems, where large concentrations of industrial activities are gathered, makes them target systems to store metals. Being preferentially bound to the organic matter present in the sediment, the removal of Cr throughout natural or enhanced processes occurs throughout plant-mediated processes. Naturally, plants acquire during their life cycle nutrients from their sediments but also some non-nutritional elements, like Cr, and store them in their tissues. This natural ability can also be used and enhanced by the application of transporter molecules, like LMWOA, in order to increase the sediment-plant Cr transport. At this interface, it is also interesting to analyse the important root-mediated process of phyto-conversion of Cr (VI) toxic form to the less toxic Cr (III). Again, halophytes acquire an important role with high conversion efficiencies. All these passive and enhanced processes point out to a promising biotechnology using halophytes as potential cleaners of Cr-contaminated sediments, using environmental-friendly and low-cost technologies.

## References


Barceloux D (1999) Chromium. J Tox Clin Tox 37:173–194

Bartlett RJ (1991) Cr cycling in soils and water: links, gaps and methods. Environ Health Persp 92:17–24

Bewers J, Yeats P (1989) Transport of river-derived trace metals through the coastal zone. Nether J Sea Res 23:359–368

Bluskov S, Arocena J, Omotoso O, Young J (2005) Uptake, distribution, and speciation of chromium in *Brassica Juncea*. Int J Phytoremediation 7:153–165

Brown S, Chaney R, Angle J, Baker A (1994) Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc- and cadmium-contaminated soil. J Environ Qual 23:1151–1157

Caçador I, Caetano M, Duarte B, Vale C (2009) Stock and losses of trace metals from salt marsh plants. Mar Environ Res 67:75–82

Caçador I, Neto JM, Duarte B, Barroso DV, Pinto M, Marques JC (2013) Development of an Angiosperm Quality Assessment Index (AQuA – Index) for ecological quality evaluation of Portuguese water bodies – a multi-metric approach. Ecol Ind 25:141–148

Caçador I, Vale C, Catarino F (1996) The influence of plants on concentration and fractionation of Zn, Pb, and Cu in salt marsh sediments (Tagus Estuary, Portugal). J Aquat Ecosyst Health 5:193–198

Couto T, Duarte B, Barroso D, Caçador I, Marques JC (2013) Halophytes as sources of metals in estuarine systems with low levels of contamination. Funct Plant Biol 40:931–939

Cunningham S, Ow D (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719

Cunningham S, Berti W, Huang J (1995) Phytoremediation of contaminated soils. Trends Biotechnol 13:393–397

Doyle M, Otte M (1997) Organism-induced accumulation of Fe, Zn and AS in wetland soils. Environ Pollut 96:1–11

Duarte B, Caetano M, Almeida P, Vale C, Caçador I (2010) Accumulation and biological cycling of heavy metal in the root-sediment system of four salt marsh species, from Tagus estuary (Portugal). Environ Pollut 158:1661–1668

Duarte B, Delgado M, Caçador I (2007) The role of citric acid in cadmium and nickel uptake and translocation, in *Halimione portulacoides*. Chemosphere 69:836–840

Duarte B, Raposo P, Caçador I (2009) *Spartina maritima* (cordgrass) rhizosediment extracellular enzymatic activity and its role on organic matter decomposition and metal speciation processes. Mar Ecol 30:65–73

Duarte B, Reboreda R, Caçador I (2008) Seasonal variation of extracellular enzymatic activity (EEA) and its influence on metal speciation in a polluted salt marsh. Chemosphere 73:1056–1063

Duarte B, Silva V, Caçador I (2012) Hexavalent chromium reduction, uptake and oxidative biomarkers in *Halimione portulacoides*. Ecotoxicol Environ Saf 83:1–7

Gupta M, Cuypers A, Vangronsveld J, Clijsters H (1999) Copper affects the enzymes of the ascorbate-glutathione cycle and its related metabolites in the roots of Phaseolus vulgaris. Physiol Plant 106:262–267

Jacob D, Otte M (2004) Influence of *Typha latifolia* and fertilization on metal mobility in two different Pb Zn mine tailings types. Sci Total Environ 333:9–24

Jones DL (1998) Organic acids in the rhizosphere a critical review. Plant Soil 205:25–44

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants. CRC Press, Boca Ratón, FL

Katz S, Salem H (1994) The biological and environmental chemistry of chromium. VCH, New York

Kortenkamp A, Casadevall M, Faux SP, Jenner A, Shayer R, Woodbridge N, O'Brien P (1996) A role for molecular oxygen in the formation of DNA damage during the reduction of the carcinogen chromium(VI) by glutathione. Arch Biochem Biophys 329:199–207

Losi M, Amrhein C, Frankenberger W (1994) Environmental biochemistry of chromium. Rev Environ Contam Toxicol 136:91–121

Lytle C, Lytle F, Yang N, Qian J, Hanen D, Zayed A, Terry N (1998) Reduction of Cr (VI) to Cr (III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32:3087–3093

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273–278

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press Limited, London

Mikalsen A, Alexander J, Wallin J, Ingelman-Sundberg M, Andersen R (1991) Reductive metabolism and protein binding of chromium(VI) by P450 protein enzymes. Carcinogenesis 12:825–831

Morris A, Bale A, Howland R, Millward G, Ackroyd D, Loring D, Rantala T (1986) Sediment mobility and its contribution to trace metal cycling and retention in a macrotidal estuary. Water Sci Technol 18:111–119

Mucha AP, Almeida CM, Bordalo AA, Vasconcelos MT (2005) Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. Estuarine Coastal Shelf Sci 65:191–198

Nies D (1992) Resistance to cadmium, cobalt, zinc and nickel in microbes. Plasmid 27:17–28

Nriagu J (1988) A silent epidemic of environmental metal poisoning. Environ Pollut 50:139–161

Parker D, Pedler J, Ahnstrom Z, Resketo M (2001) Re-evaluating the free-ion activity model of trace metal toxicity toward higher plants: experimental evidence with copper and zinc. Environ Toxicol Chem 20:899–906

Pazos-Capeáns P, Barciela-Alonso M, Herbello-Hermelo P, Bermejo-Barra P (2010) Estuarine increase of chromium surface sediments: distribution, transport and time evolution. Microchem J 96: 362–370

Qian J-H, Zayed A, Zhu Y-L, Yu M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants. III. Uptake and accumulation of trace elements by 12 plant species. J Environ Qual 28: 1448–1455

Reboreda R, Caçador I (2007) Copper, zinc and lead speciation in salt marsh sediments colonised by *Halimione portulacoides* and *Spartina maritima*. Chemosphere 69:1655–1661

Redondo-Gómez S, Mateos-Naranjo E, Vecino-Bueno I, Feldman S (2011) Accumulation and tolerance characteristics of chromium in a cordgrass Cr-hyper-accumulator, *Spartina argentinensis*. J Hazard Mater 185:862–869

Romheld V, Marschner H (1983) Mechanism of iron uptake by peanut plants: I. FeIII reduction, chelate splitting, and release of phenolics. Plant Physiol 71:949–954

Salt D, Pickering I, Prince R, Gleba D, Dushenkov S, Smith R, Raskin I (1997) Metal accumulation by aquacultured seedlings of Indian mustard. Environ Sci Technol 31:1636–1644

Shen H, Wang Y (1993) Characterization of enzymatic reduction of hexavalent chromium by Escherichia coli ATCC 33456. Appl Environ Microbiol 59:3771–3777

Sinha S, Bhatt K, Pandey K, Singh S, Saxena R (2003) Interactive metal accumulation and its toxic effects under repeated exposure in submerged plant Najas indica Cham. Bull Environ Contam Toxicol 70:696–704

Sinicrope T, Langis R, Gergberg R, Busnardo M, Zedler J (1992) Removal of metals by wetland mesocosms subjected to different hydroperiods. Ecol Eng 1:309–322

Srivastava S, Prakash S, Srivastava M (1999) Chromium mobilization and plant availability – the impact of organic complexing ligands. Plant Soil 212:203–208

Stearns D, Kennedy L, Courtney K, Giangrande P, Phieffer L, Wetterhahn K (1995) Reduction of chromium(VI) by ascorbate leads to chromium-DNA binding and DNA strand breaks in vitro. Biochemistry 34:910–919

Sundby B, Vale C, Caçador I, Catarino F, Madureira MJ, Caetano M (1998) Metal-rich concretions on the roots of salt marsh plants: mechanism and rate of formation. Limnol Oceanogr 43:245–252

Suntornvongsagul K, Burke D, Hamerlynck E, Hahn D (2007) Fate and effects of heavy metals in salt marsh sediments. Environ Pollut 149:79–91

Suseela MR, Sinha S, Singh S, Saxena R (2002) Scanning electron microscopic studies of Scirpus lacustris L. treated with Cr and tannery effluent: accumulation of metal. Bull Environ Contam Toxicol 68:540–548

Tanackovic S, Caetano M, Vale C (2008) Effect of salt-marsh plants on the mobility of Cr in sediments. Ciencias Marinas 34:363–372

Vale C (1990) Temporal variations of particulate metals in the Tagus estuary. Sci Total Environ 97:137–154

Vale C, Canário J, Caetano M, Lavrado J, Brito P (2008) Estimation of the anthropogenic fraction of elements in surface sediments of the Tagus Estuary (Portugal). Mar Pollut Bull 56:1353–1376

Viers J, Dupré B, Gaillardet J (2009) Chemical composition of suspended sediments in World Rivers: new insights from a new database. Sci Total Environ 407:853–868

Weis J, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ Int 30:685–700

Williams TP, Bubb JM, Lester JN (1994) Metal accumulation within salt marsh environments: a review. Mar Pollut Bull 28:277–290

Wittbrodt P, Palmer C (1996) Effect of temperature, ionic strength, background electrolytes, and Fe(III) on the reduction of hexavalent chromium by soil humic substances. Environ Sci Technol 30:2470–2477

# Molecular Mechanisms in the Phytoremediation of Heavy Metals from Coastal Waters

19

Subrata Trivedi and Abid Ali Ansari

## 19.1 Heavy Metal Pollution in Coastal Areas: An Introduction

The rapid pace of industrialization and urbanization of coastal areas has created several pollution-related problems in which heavy metal pollution is a major area of concern. Coastal areas are one of the most important places for human inhabitation (McKinley et al. 2011). Major world cities like Los Angeles, San Francisco, New York, Tokyo, Osaka, Beijing, Singapore, Hong Kong, Sidney, Mumbai, Dubai, etc. are located along the coastal regions around the world. With rapid urbanization and industrialization, heavy metals are continuously carried to the estuarine and coastal areas from upstream of tributaries and from the industrial and sewage discharge. Major part of the anthropogenic metal load in coastal areas has a terrestrial source, mainly from mining and industrial activities along major rivers and estuaries (Mitra et al. 1995; Ridgway et al. 2003; Caeiro et al. 2005; Usero et al. 2005; Sundaray et al. 2011).

Water pollution by heavy metals is a global issue that needs to be addressed properly. The coastal areas where the terrestrial and marine ecosystems converge are of great significance because a large number of plants and animals, both marine and estuarine, thrive in this dynamic and fragile habitat. Heavy metal contamination could affect the water quality and bioaccumulation of metals in aquatic organisms, resulting in potential long-term implication on the health of humans and also the ecosystem (Fernandes et al. 2007; Abdel-Baki et al. 2011; Trivedi et al. 1995; Mitra et al. 1996). Pollution by heavy metals is a very serious problem due to their toxicity and ability to accumulate in the biota (Islam and Tanaka 2004). In many countries drinking water is produced and then supplied to different locations by desalination of seawater. The presence of high level of metals in seawater, especially coastal waters, poses severe problem for such activities.

Some heavy metals which are essential components in metabolism may become toxic when present in high concentration. Some of these heavy metals, like Hg, Cd, Pb, As, and Se, are not essential for most of the plants, since they do not perform any known physiological function. Other heavy metals like Zn, Co, Cu, Fe, Mn, Mo, and Ni are considered as essential elements because they are required for normal growth and metabolism of plants. These latter elements can easily lead to poisoning at higher concentrations. Heavy metal toxicity may result from alterations of numerous physiological processes caused at cellular or molecular level by inactivating enzymes. They may block functional groups of metabolically important molecules, displace or substitute essential elements, and also disrupt membrane integrity. Heavy metal poisoning commonly results in the enhanced production of reactive oxygen species (ROS) due to interference with electron transport activities, particularly in the chloroplast membranes. Increase in ROS has several negative consequences like exposure of cells to oxidative stress leading to lipid peroxidation, biological macromolecule deterioration, ion leakage, membrane dismantling, and DNA-strand cleavage.

### 19.1.1 Phytoremediation and Types of Phytoremediation (Definitions)

Bioremediation is one of the most applicable methods for the management of environmental contaminants by biological mechanisms (including microorganisms) in soil and water. Plant-based bioremediation technologies are collectively termed as phytoremediation. Thus, phytoremediation refers

S. Trivedi, M.Sc., M.Phil., Ph.D. (✉) • A.A. Ansari, Ph.D.
Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia

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to the use of the plants to clean up contaminated soil and water. Phytoremediation is also called green remediation, agro-remediation, botano-remediation, or vegetative remediation. Different techniques used in phytoremediation are:

#### 19.1.1.1 Phytoextraction

Phytoextraction refers to the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants. It is one of the best methods to remove contaminants from soil, sediment, and sludge.

#### 19.1.1.2 Rhizofiltration

In rhizofiltration, plants, both terrestrial and aquatic, are used to absorb and concentrate contaminants including heavy metals from polluted aqueous sources in their roots.

#### 19.1.1.3 Phytostabilization

Phytostabilization refers to the use of plants to reduce the mobility or bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into food chains.

#### 19.1.1.4 Phytovolatilization

In this method plants are used in the uptake of contaminants from soil and waste water, transforming them into volatilized compound and then transpiring into the atmosphere.

## 19.2 Phytoremediation of Heavy Metals from Coastal Waters (General)

It is reported that a common water hyacinth *Eichhornia crassipes* may serve as a phytoremediation tool for cleaning up of several metals from coastal areas. In a study at the coastal area of Ondo State in Nigeria, enrichment factor (EF) and translocation factor (TF) were evaluated for ten metals, namely, As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, V, and Zn. Heavy metal accumulation was observed in water as well as in the roots and shoots of *Eichhornia crassipes*. The results indicated that *Eichhornia crassipes* was able to accumulate high levels of Cr, Cd, Pb, and As both in the roots and shoots (Agunbiade et al. 2009). Phytoremediation using halophytes could be a possible bioremediation technique to control heavy metal pollution in coastal environments. Phytoremediation using halophytes is being applied to recover polluted coastal lagoons (Madejón et al. 2006; Lone et al. 2008).

### 19.2.1 Phytoremediation of Cadmium

Cadmium is not essential for plant growth and is very toxic to many organisms. In humans, cadmium causes several health problems such as damage to the kidneys and lung tissues, emphysema, and carcinogenesis. It is also an endocrine disruptor and interferes with calcium regulation in biological systems (Degraeve (1981), Salem et al. (2000), and Awofolu (2005)). Cadmium is used in industrial operations to prevent corrosion of machinery. Plants show Cd toxicity like chlorosis, reddish veins and petioles, curled leaves, severe reduction in growth of roots and tops, and a number of tillers. *Echinochloa polystachya*, a fast-growing perennial grass, is able to accumulate high levels of cadmium and is a good candidate for Cd phytoextraction. A study conducted in Saudi Arabia showed that the translocation factor (TF) was high for Cd in *Calotropis procera* (Badr et al. 2012). *Azolla pinnata* accumulates 740 mg $kg^{-1}$ Cd (Rai 2008) and *Thlaspi caerulescens* and *Solanum photeinocarpum* accumulate 263 and 158 mg $kg^{-1}$ Cd, respectively (Lombi et al. 2001; Zhang et al. 2011). Hyperaccumulation of cadmium in *Arabidopsis halleri* (Cosio and Keller 2004; Kupper et al. 2000) and *Brassica juncea* (Salt et al. 1997) has also been reported.

### 19.2.2 Phytoremediation of Arsenic

It is reported that over 137 million people in more than 70 countries are affected by arsenic poisoning from drinking water (Arsenic in drinking water seen as threat, "USAToday.com," August 30, 2007). Arsenic poisoning or arsenicosis is caused by the ingestion, absorption, or inhalation of high levels of arsenic. Initial symptoms of arsenic poisoning include headaches, confusion, diarrhea, and drowsiness. With increased poisoning, convulsions and changes in fingernail pigmentation (leukonychia striata) occur. In acute arsenic poisoning severe diarrhea, vomiting, blood in the urine, hair loss, cramping muscles, stomach pain, and more convulsions take place. Arsenic poisoning also affects the skin, lungs, kidneys, and liver (Test ID: ASU. Arsenic, 24 h, Urine, Clinical Information, *Mayo Medical Laboratories Catalog*, Mayo Clinic. Retrieved 2012-09-25).

Arsenic is related to heart disease and cancer (Smith et al. 1992; Chiou et al. 1997). Cancers related to As in drinking water are reported in Taiwan, Argentina, Chile, Bangladesh, and India (WHO 2001). Arsenic pollution is also linked to chronic lower respiratory diseases and diabetes (Navas-Acien et al. 2008; Kile and Christiani 2008). Chronic exposure to arsenic may result in deficiency of vitamin A and night blindness (Hsueh et al. 1998). Arsenic poisoning ultimately results in coma and death. As (as arsenate) is an analogue of phosphate and interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis (Tripathi et al. 2007). Hence, removal of arsenic from water is of prime importance.

Some marine algae, which are constantly exposed to arsenate in seawater, have the biochemical capability to convert arsenate to harmless organo-arsenic compounds intercellularly (Edmonds and Francesconi 1987). *Pteris vittata*

(Chinese brake fern) can accumulate up to 95 % of the arsenic from soil in its fronds (Ma et al. 2001; Zhang et al. 2011). This hyperaccumulator fern species can be used in phytoremediation of arsenic (Wilkins and Salter 2003). Some properties like high biomass, fast growth, versatility and hardiness, extensive root system, high As accumulation in fronds, perennial, resistance to disease and pests, diverse ecological niches, and mycorrhizal associations of *P. vittata* make it an ideal candidate for phytoremediation of arsenic. The plant is reported to accumulate approximately 1,000 mg $kg^{-1}$ As (Baldwin and Butcher 2007).

Another study suggested that the level of As in *P. vittata* is 8,331 mg $kg^{-1}$ (Kalve et al. 2011). Besides *P. vittata* four other species belonging to the genus *Pteris*, namely, *P. biaurita, P. cretica, P.* quadriaurita, and *P. ryukyuensis*, also accumulate high levels of arsenic, i.e., ~2,000, ~1,800, ~2,900, and 3,647 mg $kg^{-1}$, respectively (Srivastava et al. 2006). In recent years much progress has been made in understanding As tolerance and its hyperaccumulation by *P. vittata.* However, more research on *P. vittata* is needed and certain technical barriers are to be overcome. *Corrigiola telephiifolia*, which accumulates 2,110 mg $kg^{-1}$ As, is also a known hyperaccumulator species for arsenic (Garcia-Salgado et al. 2012). In seawater, marine algae can transform arsenate into nonvolatile methylated arsenic compounds (methanearsonic and dimethylarsinic acids). It is a beneficial step to the primary producers and also to the higher trophic levels, since nonvolatile methylated arsenic is much less toxic to marine invertebrates.

### 19.2.3 Phytoremediation of Lead

Lead is an extremely toxic heavy metal, which can cause severe problems in children such as impaired development, reduced intelligence, short-term memory loss, learning disabilities, and coordination problems. High level of lead causes renal failure and increased risk for development of cardiovascular disease (Salem et al. 2000; Padmavathiamma and Li 2007; Wuana and Okieimen 2011; Iqbal 2012). Several plant species have been reported as important hyperaccumulators of lead. A leguminous shrub *Sesbania drummondii* and several *Brassica* species can accumulate significant amounts of lead in their roots (Blaylock et al. 1997; Sahi et al. 2002; Wong et al. 2001). *Piptatherum miliaceum* accumulate lead directly correlating to soil concentrations and do not show any symptoms of toxicity for 3 weeks. *Sesbania drummondii* can tolerate lead levels up to 1,500 mg $L^{-1}$ and accumulate 40 g $kg^{-1}$ shoot dry weight (Sahi et al. 2002). Lead is relatively insoluble because most of the lead is accumulated in the stems and not in the leaves (Kumar et al. 1995). The main problem for the phytoremediation of lead is its extremely low solubility (Huang et al. 1997). The aquatic weed, *Eichhornia crassipes*, has phytoremediation potential for removal of lead from effluent (Sukumaran 2013).

### 19.2.4 Phytoremediation of Copper

Copper is an essential element and enzyme cofactor for oxidases like cytochrome oxidase, superoxide dismutase, and tyrosinases but some plants and animals can accumulate even toxic levels of copper. High levels of copper can cause brain and kidney damage, liver cirrhosis and chronic anemia, and stomach and intestinal irritation (Salem et al. 2000; Wuana and Okieimen 2011). *Salix nigra* can accumulate more cadmium and copper but more studies are necessary to determine the feasibility of this species for phytoremediation (Kuzovkina et al. 2004). *Eichhornia crassipes* is estimated to accumulate high levels of copper and could be potentially used for phytoremediation (Liao and Chang 2004). Aquatic weed, *Typha latifolia*, can prominently remove copper, cadmium, and arsenic from effluent (Sukumaran 2013). *Eleocharis acicularis* which hyperaccumulates Cu at 20,200 mg $kg^{-1}$ may be considered as a potential phytoremediation species for removal of copper (Sakakibara et al. 2011).

### 19.2.5 Phytoremediation of Chromium

High level of chromium causes cancer and extensive hair loss (Salem et al. 2000). *Crotalaria juncea* and *Crotalaria dactylon* can remediate Cr and Cu (Saraswat and Rai 2009). Tumbleweeds *Salsola kali* (Gardea-Torresday et al. 2005) and *Gynura pseudochina* are known to be Cr hyperaccumulators (Mongkhonsin et al. 2011). *Pteris vittata* is a potential chromium phytoremediation species which accumulates 20,675 mg $kg^{-1}$ Cr (Kalve et al. 2011). The species is also considered as phytoremediation tool for arsenic management.

### 19.2.6 Phytoremediation of Manganese

Manganese has been reported for its negative effects on the respiratory and nervous system. Symptoms of manganese poisoning are hallucinations, forgetfulness, and nerve damage. Higher levels of manganese can also cause Parkinson disease, lung embolism, bronchitis, and impotency. Manganese can cause both toxicity and deficiency symptoms in plants. *Chengiopanax sciadophylloides* is a Mn hyperaccumulator species and ZIP family transporter genes have been isolated from this species (Mizuno et al. 2008). *Austromyrtus bidwillii* (Bidwell et al. 2002), *Phytolacca* *americana* (Pollard et al. 2009), and *Maytenus founieri* (Fernando et al. 2008) are capable of Mn hyperaccumulation. *Schima superba* is capable of hyperaccumulating Mn to a level of 62,412.3 mg $kg^{-1}$ (Yang et al. 2008).

### 19.2.7 Phytoremediation of Nickel

Nickel can cause allergic dermatitis known as nickel itch. Inhalation of Ni can cause cancer of the lungs, nose, throat, and stomach. Ni is hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, and hepatotoxic metal. Furthermore it causes hair loss (Salem et al. 2000; Khan et al. 2007; Das et al. 2008). Ni-hyperaccumulating plants are *Berkheya coddii* (Robinson et al. 1997; Moradi et al. 2010), *Sebertia acuminate* (Jaffre et al. 1976; Perrier 2004), *Phidiasia lindavii* (Reeves et al. 1999), and *Bornmuellera kiyakii* (Reeves et al. 2009). *Isatis pinnatiloba* can accumulate Ni to a level of 1,441 mg $kg^{-1}$ (Altinozlu et al. 2012). Five species belonging to the genus *Alyssum*, namely, *Alyssum bertolonii*, *A. caricum, A. pterocarpum, A. murale*, and *A. corsicum*, hyperaccumulate Ni with levels of 10,900, 12,500, 13,500, 15,000, and 18,100 mg $kg^{-1}$, respectively (Li et al. 2003).

A different study showed that *Alyssum murale* hyperaccumulates 4,730–20,100 mg $kg^{-1}$ and *Alyssum markgrafii* accumulates 19,100 mg $kg^{-1}$ Ni, respectively (Bani et al. 2010). *Alyssum serpyllifolium* accumulates 10,000 mg $kg^{-1}$ Ni (Prasad 2005). Hence it is evident that all the seven different *Alyssum* species are capable of hyperaccumulating Ni and can be considered as a potential candidate for phytoremediation of nickel. After phytoremediation, plant biomass containing accumulated heavy metals can be combusted to get energy and the remaining ash is called bio-ore which can be processed for the recovery or extraction of the heavy metals. Phytomining is commercially used for Ni and it is less expensive compared to conventional extraction methods. Using *Alyssum murale* and *Alyssum corsicum*, we may grow biomass containing 400 kg Ni $ha^{-1}$ with production costs of \$250–500 $ha^{-1}$. Considering Ni price of \$40 $kg^{-1}$ (in 2006, Ni metal was trading on the London Metal Exchange at more than \$40 $kg^{-1}$), Ni phytomining is considered a highly profitable agricultural technology (crop value = \$16,000 $ha^{-1}$) for Ni-contaminated soils (Chaney et al. 2007). This bio-based mining would be more attractive since it would be helpful in limiting environmental pollution (Siddiqui et al. 2009).

### 19.2.8 Phytoremediation of Vanadium

Vanadium is used mainly to produce certain alloys, and $V_2O_5$ is used as a catalyst in manufacturing sulfuric acid and maleic anhydride and in making ceramics. The blood cells of some marine animals like ascidians hyperaccumulate vanadium which is $10^7$ times higher than the vanadium found in seawater (Trivedi et al. 2003). In humans the acute effects of vanadium are irritation of the lungs, throat, eyes, and nasal cavities. Very little information is available on the phytoremediation of this metal. In seawater, many marine algae accumulate vanadium which is utilized in the functioning of vanadium-dependent haloperoxidases. The levels of vanadium in sediments, roots, stems, and leaves of a mangrove species *Avicennia marina* have been reported from Mtoni, Msimbazi, and Mbweni mangrove ecosystems (Mremi and Machiwa 2003). Among mushrooms, *Amanita muscaria* concentrates vanadium to levels of 100 times (2 mmol $kg^{-1}$ dry weight) than those found in other mushrooms and higher plants.

### 19.2.9 Phytoremediation of Zinc

Overdosage of zinc can cause dizziness and fatigue (Hess and Schmid 2002). The unicellular green alga, *Dunaliella salina*, showed high tendency for zinc accumulation and is a candidate for phytoremediation of zinc (Magda 2008). Studies conducted in China have identified *Sedum alfredii* as hyperaccumulator for Zn and Cd, and it has been intensively investigated by various researchers in their studies conducted in hydroponics and/or the uncontaminated and contaminated soils. *Thlaspi caerulescens* (Kupper and Kochian 2010), *Arabis gemmifera, A. paniculata* (Kubota and Takenaka 2003; Tang et al. 2009), *Arabidopsis halleri* (Zhao et al. 2000), and *Picris divaricata* (Du et al. 2011) also have the capability to hyperaccumulate zinc. *Eleocharis acicularis* which accumulates 11,200 mg $kg^{-1}$ Zn is a potential candidate for zinc phytoremediation (Sakakibara et al. 2011).

### 19.2.10 Phytoremediation of Metals by Mangroves

Located between marine and terrestrial environments, mangroves are transitional coastal ecosystems which are found mostly in the tropical and subtropical regions. In these regions, about 75 % of the coastline and nearly 18 million hectares are occupied by mangrove forests (Kathiresan and Qasim 2005). There are more than 14.5 million hectares of mangrove forests in the Indo-Pacific region (6.9 million), Africa (3.5 million), and the Americas (4.1 million) Sahoo and Dhal (2009). The mangrove ecosystems are of great ecological and economic significance. They serve as buffer zone and provide primary protection against storm surges and coastal erosion. Besides this, they are also an important nursery for various marine and estuarine faunas including fish, crustaceans, etc. The global economic value (USD) of mangrove habitat is estimated as 181 billion (Alongi 2002).

One excellent example of mangrove ecosystem is Sunderbans which is located in India and Bangladesh. The Sunderbans is the single largest block of tidal halophytic mangrove forest listed in the UNESCO world heritage list (http://whc.unesco.org/en/list) which is regarded as a biodiversity hot spot. This region is also regarded as the world's largest natural nursery where a large number of marine and estuarine species come to breed and the juveniles stay back to exploit its rich natural resources (Trivedi et al. 2007). Several researches on heavy metal accumulation by different floras and faunas in this region have been reported (Mitra et al. 1994a, b, 1995, 1996; Trivedi et al. 1995). A comparative study on the heavy metal accumulation by different mangrove plants in this area was conducted (Mitra et al. 1994c).

Mangroves act as sink or buffer and remove/immobilize metals before reaching the nearby aquatic ecosystems like the estuaries and creeks. Due to high proportion of fine clays, organic matter and low pH, mangrove mud effectively sequester metals, often immobilized as sulfides in anaerobic sediments. Mangroves are considered to be tolerant and significantly adaptive to the presence of heavy metals (Chiu and Chou 1991; Walsh et al. 1979). A concentration of trace metals is reported for at least 33 mangrove species (Lewis et al. 2011).

The widely distributed fast-growing mangrove plant *Rhizophora mucronata* has the potential for metal phytoremediation (Pahalawattaarachchi et al. 2009). *Sonneratia caseolaris*, a mangrove species belonging to family Sonneratiaceae, is found near the banks of tidal rivers in brackish water and provides essential congregating place for fireflies. The fermented juices of this mangrove species have the ability to arrest hemorrhage and the half-ripe fruit is used to treat coughs (Perry 1980). Recent studies showed that *Sonneratia caseolaris* possess the capacity to take up selected heavy metals through its roots and store certain heavy metals in its leaves without any sign of injury, thereby suggesting the potential of *Sonneratia caseolaris* as a phytoremediation species (Nazli and Hashim 2010). Bioaccumulation of heavy metals by certain mangrove species reveals that these plants can act as bio-purifier or biofilter (Zaman et al. 2013). The concentration of heavy metals in different parts of mangrove plants may be efficiently used for water quality monitoring program (Mitra et al. 2004). Different mangroves can be used for phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization. Table 19.1 shows examples of trace metal bioaccumulation in mangrove tissue.

### 19.2.11 Molecular Mechanisms of Metal Phytoremediation

Heavy metal ions that are incorporated to the tissues of living organisms can bind to macromolecules like protein. In order to understand the mechanism of accumulation of the metal, it is important to search for the metal-binding proteins. Since these proteins are in turn encoded by specific genes, the searching and subsequent analysis of those genes provides important clues for the hyperaccumulation of the metals. The analysis of the expressed sequence tags (ESTs) plays an important role in elucidating the metal hyperaccumulation sites. It is noted that the frequency of ESTs for a gene encoding metal-binding protein in each developmental stage and in the adult tissue roughly reflects the level of mRNA expression.

If the cDNA encoding the metal-binding protein is available, then the recombinant metal-binding protein can be produced in the laboratory. In this case, the cDNA encoding the metal-binding protein is ligated to a cloning vector by the process of genetic engineering. The recombinant DNA is then transformed using suitable competent cells. These bacterial cells when grown in proper condition in appropriate culture medium produce the metal-binding protein of interest. During the process of isolation of metal-binding protein, sometimes it is difficult to purify the protein from the mixture of several proteins. This situation often arises with the newly discovered metal-binding proteins or their genes which are not well characterized. In such a situation, it is a good idea to prepare fusion proteins. For example, vanadium-binding protein (CiVanabin5) was ligated to maltose-binding protein (MBP) to produce fusion protein CiVanabin5-MBP. This fusion protein was then subjected to amylose resin column chromatography. The fusion protein CiVanabin5-MBP was eluted from the column using a buffer containing maltose. Subsequently, the junction between the CiVanabin5 and MBP was cut, and metal-binding assay was conducted using immobilized metal ion affinity chromatography or IMAC (Trivedi et al. 2003).

There has been significant progress in determining the molecular basis for metal accumulation, which provides a strong scientific basis to outline several strategies for phytoremediation of metals. Biotechnological approaches are now used to produce improved plant varieties for enhancing natural hyperaccumulation of heavy metals. After mobilization, metals first bind to the cell wall, which is an ion exchanger of comparatively low selectivity. Subsequently, transport systems and intracellular high-affinity binding sites then mediate and help the uptake across the plasma membrane through secondary transporters such as channel proteins and/or $H^+$-coupled carrier proteins (Chaney et al. 2007). In recent years several membrane transporter gene families have been identified and characterized by heterologous complementation screens and sequencing of ESTs and plant genome studies.

Many cation transporters have been identified in recent years, most of which are Zn-regulated transporter (ZRT), Fe-regulated transporter (IRT), natural resistance-associated macrophage proteins (NRAMP), Al-activated malate transporter (ALMT), cation diffusion facilitator (CDF), P-type ATPase (heavy metal associated), yellow stripe-like (YSL),

**Table 19.1** Example of trace metal bioaccumulation (μg/g dry wt) in mangrove tissue

| Species | Location | | Tissue | Cd | Cu | Cr | Mn | Ni | Pb | Zn | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Laguncularia racemosa* | Panama | R | Leaves | | 2.3–5.0 | | | | 2.7–9.3 | 33.4–41.4 | Defew et al. (2005) |
| *Avicennia marina* | Australia | RM | Leaves | | 2.9–24.8 | | | | 0.1–3.9 | 8.8–57.7 | MacFarlane. (2002) |
| *Rhizophora mangle* | Brazil | MC | Fruits | | | | | | | | Silva et al. (1990) |
| 14 species including<br>*Rhizophora conjugata*<br>*Acanthus ilicifolius*<br>*Bruguiera caryophylloides*<br>*Carapa moluccensis*<br>*Scyphiphora hydrophyllacea*<br>*Excoecaria agallocha*<br>*Avicennia alba*<br>*Avicennia marina* | West Malaysia | R | Leaves | | BD-12 | | | | | BD-42 | Peterson et al. (1979) |
| *Avicennia marina* | China | R | Leaf | 0.01–0.30 | 1.8–13.8 | 0.28–0.73 | 25–1,552 | 0.43–7.7 | 0.4–3.5 | 3.4–69.5 | Peng et al. (1997) |
| *Avicennia marina* | Australia | MC | Roots | | 101 | | | | 164 | 295 | MacFarlane et al. (2003) |
| | | | Leaf | | 9 | | | | 5 | 25 | |
| *Avicennia marina* | Australia | R | Young leaves | BD | 18.3 | | | | 2.4–4.3 | 16.4–43.7 | Saenger and McConchie (2004) |
| *Aegiceras corniculatum* | | | Old leaves | | 3.6–12.2 | | | | 2.7–4.2 | 13.7–34.3 | |
| *Hibiscus tiliaceus* | | | Wood | | 4.3–20.5 | | | | 2.6–15.1 | 9.9–20.8 | |
| *Excoecaria agallocha* | | | Bark | | 6.6–21.4 | | | | 7.5–15.7 | 12.6–54.9 | |
| *Bruguiera gymnorrhiza* | | | Fruit | | 6.1–4.8 | | | | 2.1–4.8 | 8.8–31.1 | |
| Not reported | Puerto Rico | SV | Leaves | <6.0 | 5.2 | <1.0 | 561 | 2.6 | | 4.5 | Ragsdale and Thorhaug (1980) |
| *Rhizophora mangle* (mg/g) | Brazil | MC | Leaves | <0.02 | <0.05 | <0.2 | 101 | | <0.06 | 7.2 | Lacerda (1998) |
| *Rhizophora mangle* | China | M | Standing crop | 0.11 | 0.98 | 0.5 | 14.6 | 1.6 | 0.86 | 4.91 | Weng-jiao et al. (1997) |
| Many species (review) | | R | Leaves | | 1.2–102 | | | | 0.2–168 | 0.5–120 | MacFarlane et al. (2007) |
| | | | Roots | | 1.1–101 | | | | 0.2–164 | 0.3–640 | |
| *Avicennia marina* | India | SV | Leaves | 3–1 | 7–24 | | | | 26–5 | 78–28 | Chakrabarti et al. (1993) |
| *Acanthus ilicifolius* | | | Stems | | | | | | | | |
| *Ceriops decandra* | | | Roots | | | | | | | | |
| *Avicennia officinalis*<br>*Rhizophora apiculata*<br>*Rhizophora mucronata*<br>*Excoecaria agallocha*<br>*Bruguiera cylindrica*<br>*Ceriops decandra*<br>*Aegiceras corniculatum*<br>*Acanthus ilicifolius* | India | R | Leaves | BD | 8.1–95.1 | | 51.1–391 | | 11.8–27.4 | 9.3–116.9 | Agoramoorthy et al. (2008) |
| *Avicennia marina* | Australia | R | Aerial roots | | 2–12 | BD-4 | | | | 29–60 | Preda and Cox (2002) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Avicennia officinalis*<br>*Acanthus ilicifolius*<br>*Bruguiera gymnorrhiza*<br>*Sonneratia caseolaris*<br>*Barringtonia racemosa*<br>*Acanthus ilicifolius*<br>*Aegiceras corniculatum* | India | R | Twigs | | 6.1–190.2 | | 46–2,472 | | 25–225 | 18–248 | Thomas and Fernandez (1997) |
| *Kandelia candel* | Hong Kong | RMC | Roots | | | | | 14.3–18.3 | 40–30 | 83–207 | OngChe (1999) |
| *Avicennia marina* | Saudi Arabia | R | Leaves | <0.4–3.1 | 0.5–23.3 | <0.15–9.2 | 4.5–66.6 | <0.2–7.5 | <0.3–22.8 | 3.8–31.7 | Sadiq and Zaidi (1994) |
| *Rhizophora mangle* | French Guiana (nmolg-$^{1}$) | R | Leaves | | 13–193 | 203–347 | | 16–108 | | 212–668 | Marchand et al. (2006) |
| *Avicennia germinans*<br>*Crenea maritima*<br>*Laguncularia racemosa* | | | Stems | | BD-207 | 151–346 | | 8e83 | | 67–1,988 | |
| *Rhizophora mangle*<br>*Bruguiera gymnorrhiza*<br>*Ceriops tagal*<br>*Avicennia officinalis*<br>*Lumnitzera racemosa*<br>*Aegiceras corniculatum*<br>*Acanthus ilicifolius*<br>*Aeluropus lagopoides*<br>*Clerodendron inerme* | India | R | Leaves | | 4.1–10.8 | | 25.8–158.7 | | | 8.3–107.1 | Bhosale (1979) |
| *Kandelia candel* | Taiwan | R | Root | 0.2–1.8 | 9.8–165.2 | | | 4–50.2 | 5–77 | 24.9–340 | Chiu and Chou (1991) |
| | | | Branch | <0.05–0.6 | 39–39.2 | | | 0.4–1.8 | 4–24 | 8.3–36.5 | |
| | | | Leaf | <0.05–0.9 | 1.8–10.3 | | | <0.4–3.7 | <1–7 | 9.2–21.8 | |
| | | | Seedling | 0.1–0.9 | 3.2–5.0 | | | 0.7–2.8 | <1–8 | 7.9–11.4 | |
| *Ceriops tagal*<br>*Aegiceras corniculatum*<br>*Bruguiera sexangula*<br>*Sonneratia caseolaris*<br>*Rhizophora stylosa*<br>*Bruguiera gymnorrhiza*<br>*Kandelia candel*<br>*Avicennia marina*<br>*Sonneratia ovata*<br>*Acanthus ilicifolius* | China | RMC | Propagules | 0.02–0.06 | 2.1–7.8 | | 5.1–28 | | 0.02–0.04 | 5.7–60 | Lian et al. (1999) |
| *Rhizophora mucronata*<br>*Avicennia officinalis*<br>*Xylocarpus granatum*<br>*Ceriops decandra*<br>*Bruguiera cylindrica* | India | RMC | Leaves | | 0.8–3.7 | | 5.7–7.2 | | | 0.7–1.6 | Sarangi et al. (2002) |

*R* range, *RMC* range of mean concentrations, *MC* mean concentrations, *SV* single value, *BD* below direction (Lewis et al. 2011)

**Table 19.2** Important metal transporter genes in different plant species involved in heavy metal tolerance and accumulation (Bhargava et al. 2012)

| Family | Gene | Plant | Metal transported | References |
|---|---|---|---|---|
| Zn-regulated transporter (ZRT) | *zip1-12* | *Arabidopsis thaliana* | Zn | Weber et al. (2004); Roosens et al. (2008a, b) |
| | *zip4* | *Oryza sativa* | Zn | Ishimaru et al. (2005) |
| | *zip* | *Medicago truncatula* | Zn | Lopez-Millan et al. (2004) |
| | *znt1-2* | *T. caerulescens* | Zn | van de Mortel et al. (2006) |
| Fe-regulated transporter (IRT) | *irt1* | *Arabidopsis thaliana* | Fe | Kerkeb et al. (2008) |
| | *irt1-2* | *Lycopersicon esculentum* | Fe | Bereczky et al. (2003) |
| | *irt1-2* | *T. caerulescens* | Fe | Schikora et al. (2006); Plaza et al. (2007) |
| Natural resistance-associated macrophage proteins (NRAMP) | *nramp1-3* | *Lycopersicon esculentum* | Fe | Bereczky et al. (2003) |
| | *nramp4* | *Thlaspi japonicum* | Fe | Mizuno et al. (2005) |
| | *nramp1* | *Malus baccata* | Fe | Xiao et al. (2008) |
| Cation diffusion facilitator (CDF) | *mtp1* | *Arabidopsis thaliana* | Zn | Kawachi et al. (2008) |
| | *mtp1* | *Arabidopsis halleri* | Zn | Willems et al. (2007) |
| | *mtp1* | *Thlaspi goesingense* | Zn, Ni | Kim et al. (2004) |
| | *mtp1* | *Nicotiana tabacum* | Zn, Co | Shingu et al. (2005) |
| Al-activated malate transporter (ALMT) | *almt1* | *Triticum* sp. | Al | Sasaki et al. (2004) |
| | *almt1* | *Secale cereale* | Al | Collins et al. (2008) |
| P-type ATPase (heavy metal associated) | *hma8* | *Glycine max* | Cu | Bernal et al. (2007) |
| | *hma9* | *Oryza sativa* | Cu, Zn, Cd | Lee et al. (2007) |
| | *hma4* | *Arabidopsis halleri* | Cd | Courbot et al. (2007) |
| | *hma3* | *Arabidopsis thaliana* | Co, Zn, Cd, Pb | Morel et al. (2008) |
| Nicotianamine synthase (NAS) | *nas2, nas3* | *Arabidopsis halleri* | Zn | Talke et al. (2006) |
| Copper transporter | *copt1* | *Arabidopsis thaliana* | Cu | Sancenon et al. (2004) Andres-Colas et al. (2010) |
| Yellow stripe-like (YSL) | *ysl2* | *Arabidopsis thaliana* | Fe, Cu | DiDonato et al. (2004) |
| | *ysl3* | *T. caerulescens* | Fe, Ni | Gendre et al. (2006) |

copper transporter, and nicotianamine synthase (NAS) (Guerinot 2000; Williams et al. 2000; Talke et al. 2006; van de Mortel et al. 2006; Kramer et al. 2007; Memon and Schroder 2009; Maestri et al. 2010) (Table 19.2). After its entry into the plant, further movement of metal-containing sap from roots to the aerial parts is controlled by root pressure and also transpiration pull (Robinson et al. 2003).

Thereafter, transport of the metal to the shoot primarily takes place through the xylem. Because metals are extremely toxic at high intracellular concentrations, plants generally catalyze redox reactions and alter the chemistry of these metal ions in order to allow their accumulation in nontoxic forms. Two such examples are reduction of $Cr^{6+}$ to $Cr^{3+}$ in *Eichhornia crassipes* (Lytle et al. 1998) and reduction of $As^{5+}$ to $As^{3+}$ in *B. juncea* (Pickering et al. 2000). Some heavy metals like Zn, Cd, and Pb do not occur in different oxidation states. Alternatively, some intracellular metals are detoxified by binding to low molecular mass organic compounds, by localization in the vacuoles as a metal-organic acid complex, or by binding to histidine (Persans et al. 1999; Kramer et al. 2000). In the case of metals like Zn, there are various mechanisms for regulation of cytoplasmic metal concentration which include sequestration in a subcellular organelle to low molecular mass organic ligands, low uptake across the plasma membrane, and precipitation as insoluble salts and active extrusion across the plasma membrane into the apoplast (Brune et al. 1994).

The application of molecular and genetic engineering technologies led to the well understanding of mechanisms of heavy metal tolerance and/or accumulation in plants. As a consequence, several transgenic plants with increased resistance and uptake of heavy metals were developed for the purpose of heavy metal phytoremediation. Once the rate-limiting steps for uptake, translocation, and detoxification of metals in hyperaccumulating plants are identified, precise and proper construction of transgenic plants can be achieved with improved applicability of the phytoremediation technology (Yang et al. 2005).

An excellent biotechnological approach for enhancing the potential for metal phytoremediation, by the method of phytoextraction, may be to improve the growth rate of hyperaccumulator plants through selective breeding or by the transfer of metal hyperaccumulation genes to high biomass species. Recently, somatic hybrids have been generated between *Thlapsi caerulescens* and *Brassica napus*. High biomass hybrids selected for Zn tolerance are capable of accumulating Zn level that would have been toxic to *B. napus* (Brewer et al. 1999). This result indicates that the transfer of the metal hyperaccumulating phenotype is quite feasible. It was also noticed that somatic hybrids from *T. caerulescens* and

*B. juncea* were also able to remove significant amounts of Pb (Gleba et al. 1999).

There are several reports on the bioengineered plants that are tolerant to the presence of toxic levels of As (Lee 2003), Cd (Kawashima et al. 2004), Se (Berken et al. 2002), and Zn, Cr, Cu, and Pb (Bennet et al. 2003). A combination of transporter genes has also been used in rapidly growing plant species leading to promising results. Transgenic *B. juncea* showed higher uptake of Se and enhanced Se tolerance than the wild species (Pilon-Smits et al. 1999; Van Huysen et al. 2004). In order to enhance Se tolerance, the selenocysteine methyltransferase (SMT) gene has been transferred from the Se hyperaccumulator *A. bisulcatus* to Se-non-tolerant *B. juncea*. SMT transgenic plants of *B. juncea* grown in a contaminated soil were able to accumulate 60 % more Se than the wild-type plant (Zhao and McGrath 2009).

Although transgenic plant approach is promising, only very few studies have been performed till now under field conditions (Zhao and McGrath 2009). It is also noticed that accumulation and tolerance of heavy metals and thus phytoremediation potential of a given plant are controlled by many genes, so that genetic manipulations to improve these traits in fast-growing plants will require to change the expression levels in a number of genes, rather than a single gene and to cross them to determine the number of genes involved and their characteristics. Functional assay, expression, and regulations of genes involved in metal hyperaccumulation, uptake, root-to-shoot translocation, detoxification, and/or sequestration mechanisms need to be fully elucidated in order to make the transgenic metal phytoremediation technique successful.

## References


Abdel-Baki AS, Dkhil MA, Al-Quraishy S (2011) Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. Afr J Biotech 10:2541–2547

Agoramoorthy G, Chen FA, Hou MJ (2008) Threat of heavy metal pollution in halophytic and mangrove plants of Tamil Nadu, India. Environ Pollut 155:320–326

Agunbiade FO, Olu-Owolabi BI, Adebowale KO (2009) Phytoremediation potential of *Eichornia crassipes* in metal-contaminated coastal water. Bioresour Technol 100(19):4521–4526

Alongi DM (2002) Present state and future of the world's mangrove forests. Environ Conserv 29:331–349

Altinozlu H, Karagoz A, Polat T, Ünver I (2012) Nickel hyperaccumulation by natural plants in Turkish serpentine soils. Turk J Bot 36:269–280

Andres-Colas N, Perea-Garcia A, Puig S, Penarrubia L (2010) Deregulated copper transport affects *Arabidopsis* development especially in the absence of environmental cycles. Plant Physiol 153:170–184

Awofolu O (2005) A survey of trace metals in vegetation, soil and lower animal along some selected major roads in metropolitan city of Lagos. Environ Monit Assess 105:431–447

Badr N, Fawzy M, Al-Qahtani MK (2012) Phytoremediation: an ecological solution to heavy-metal-polluted soil and evaluation of plant removal ability. World Appl Sci J 16(9):1292–1301

Baldwin PR, Butcher DJ (2007) Phytoremediation of arsenic by two hyperaccumulators in a hydroponic environment. Microchem J 85:297–300

Bani A, Pavlova D, Echevarria G, Mullaj A, Reeves RD, Morel JL, Sulçe S (2010) Nickel hyperaccumulation by the species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. Bot Serb 34:3–14

Bennet LE et al (2003) Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. J Environ Qual 32:432–440

Bereczky Z, Wang HY, Schubert V, Ganal M, Bauer P (2003) Differential regulation of Nramp and IRT metal transporter genes in wild type and iron uptake mutants of tomato. J Bio Chem 278:24697–24704

Berken A, Mulholland MM, LeDuc DL, Terry N (2002) Genetic engineering of plants to enhance Selenium phytoremediation. Crit Rev Plant Sci 21:567–582

Bernal M, Testillano PS, Alfonso M, Del Carmen RM, Picorel R, Yruela I (2007) Identification and subcellular localization of the soybean copper P1B-ATPase GmHMA8 transporter. J Struct Biol 158:146–158

Bhargava A, Carmona FF, Bhargava M et al (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120

Bhosale LJ (1979) Distribution of trace elements in the leaves of mangroves. Ind J Marine Sci 8:58–59

Bidwell SD, Woodrow IE, Batianoff GN, Sommer-Knudsen J (2002) Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwillii* (Myrtaceae) from Queensland, Australia. Funct Plant Bio 29:899–905

Blaylock M, Salt D, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley B, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865

Brewer EP, Saunders JA, Angle JS et al (1999) Somatic hybridization between the zinc accumulator *Thlaspi caerulescens* and *Brassica napus*. Theor Appl Genet 99:761–771

Brune A, Urbach W, Dietz KJ (1994) Compartmentation and transport of Zn in barley primary leaves as basic mechanisms involved in Zn tolerance. Plant Cell Environ 17:153–162

Caeiro S, Costa MH, Fernandes F, Silveira N, Coimbra A et al (2005) Assessing heavy metal contamination in Sado Estuary sediment: an index analysis approach. Ecolog Indicat 5:151–169

Chakrabarti C, Kundu SK, Ghosh PB, Choudhury A (1993) A preliminary study on certain trace metals in some plant and animal organisms from mangroves of Sundarbans, India. Mahasagar 26:17–20

Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual 36:1429–1443

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH, Chen CJ (1997) Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28(9):1717–1723, doi: 10.1161/01.STR.28.9.1717, 10.1161 %2F01.STR.28.9.1717. PMID 9303014

Chiu CY, Chou CH (1991) The distribution and influence of heavy metals in mangrove forests of the Tamshui estuary in Taiwan. Soil Sci Plant Nutr 37:559–669

Collins NC, Shirley NJ, Saeed M, Pallotta M, Gustafson JP (2008) An ALMT1 gene cluster controlling aluminum tolerance at the Alt4 locus of rye (*Secale cereale L.*). Genetics 179:669–682

Cosio C, Keller C (2004) Hyper accumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. Plant Physiol 134:716–725

Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, Saumitou-Laprade P, Verbruggen N (2007) A major quantitative trait locus for

Cd tolerance in *Arabidopsis halleri* colocalizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiol 144:1052–1065
Das K, Das S, Dhundasi S (2008) Nickel, its adverse health effects and oxidative stress. Indian J Med Res 128:412–425
Defew LH, Mair JM, Guzman HM (2005) An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Panama. Marine Pollution Bulletin 50:547–552
Degraeve N (1981) Carcinogenic, teratogenic and mutagenic effects of cadmium. Mut Res 86:115–135
DiDonato RJ, Roberts LA, Sanderson T, Eisley RB, Walker EL (2004) *Arabidopsis* Yellow Stripe-Like2 (YSL2): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine metal complexes. Plant J 39:403–414
Du RJ, He EK, Tang YT, Hu PJ, Ying RR, Morel JL, Qiu RL (2011) How phytohormone IAA and chelator EDTA affect lead uptake by Zn/Cd hyperaccumulator *Picris divaricata*. Int J Phytoremed 13:1024–1036
Edmonds JS, Francesconi KA (1987) Transformations of arsenic in the marine environment. Experientia 43:553–557
Fernandes C, Fontainhas-Fernandes A, Peixoto F, Salgado MA (2007) Bioaccumulation of heavy metals in Liza saliens from the Esmoriz-Paramos coastal lagoon, Portugal. Ecotoxicol Environ Safe 66:426–431
Fernando DR, Woodrow IE, Jaffre T, Dumontet V, Marshall AT, Baker AJM (2008) Foliar manganese accumulation by *Maytenus founieri* (Celastraceae) in its native New Caledonian habitats: populational variation and localization by X-ray microanalysis. New Phytol 177:178–185
Garcia G, Faz A, Cunha M (2004) Performance of *Piptatherum miliaceum* (Smilo grass) in edaphic Pb and Zn phytoremediation over a short growth period. Int Bio Dete Bio Deg 54:245–250
Garcia-Salgado S, Garcia-Casillas D, Quijano-Nieto MA, Bonilla-Simon MM (2012) Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. Water Air Soil Pollut 223:559–572
Gardea-Torresday JL, De la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jimenez G, Cano-Aguilera I (2005) Differential uptake and transport of trivalent and hexavalent chromium by tumbleweed (*Salsola kali*). Arch Environ Contam Toxicol 48:225–232
Gendre D, Czernic P, Conéjéro G, Pianelli K, Briat JF, Lebrun M, Mari S (2006) TcYSL3, a member of the YSL gene family from the hyperaccumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. Plant J 49:1–15
Gleba D et al (1999) Use of plant roots for phytoremediation and molecular farming. Proc Natl Acad Sci U S A 96:5973–5977
Guerinot ML (2000) The ZIP family of metal transporters. Biochim Biophys Acta 1465:190–198
Hess R, Schmid B (2002) Zinc supplement overdose can have toxic effects. J Paediat Haematol Oncol 24:582–584
Hsueh YM, Wu WL, Huang YL, Chiou HY, Tseng CH, Chen CJ (1998) Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. Atherosclerosis 141(2):249–257, doi: 10.1016/S0021-9150(98)00178-6, 10.1016%2FS0021-9150 %2898 %2900178-6. PMID 9862173
Huang J, Chen J, Berti W, Cunningham S (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environ Sci Technol 31:800–805
Iqbal MP (2012) Lead pollution – a risk factor for cardiovascular disease in Asian developing countries. Pak J Pharm Sci 25:289–294
Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J Exp Bot 56:3207–3214
Islam MD, Tanaka M (2004) Impact of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Marine Pollut Bull 48:624–649
Jaffre T, Brooks RR, Lee J, Reeves RD (1976) *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. Science 193:579–580
Kalve S, Sarangi BK, Pandey RA, Chakrabarti T (2011) Arsenic and chromium hyperaccumulation by an ecotype of *Pteris vittata-prospective* for phytoextraction from contaminated water and soil. Curr Sci 100:888–894
Kathiresan K, Qasim SZ (2005) Biodiversity of mangrove ecosystems. Hindustan Publishing Corporation, New Delhi, p 251
Kawachi M, Kobae Y, Mimura T, Maeshima M (2008) Deletion of a histidine-rich loop of AtMTP1, a vacuolar Zn2þ/Hþ antiporter of *Arabidopsis thaliana*, stimulates the transport activity. J Biol Chem 283:8374–8383
Kawashima CG et al (2004) Heavy metal tolerance of transgenic plants over-expressing cysteine synthase. Biotech Lett 26:153–157
Kerkeb L, Mukherjee I, Chatterjee I, Lahner B, Salt DE, Connolly EL (2008) Iron-induced turnover of the *Arabidopsis* Iron-Regulated Transporter1 metal transporter requires lysine residues. Plant Physiol 146:1964–1973
Khan MA, Ahmad I, Rahman I (2007) Effect of environmental pollution on heavy metals content of *Withania somnifera*. J Chin Chem Soc 54:339–343
Kile ML, Christiani DC (2008) Environmental arsenic exposure and diabetes. JAMA 300(7):845–846, doi: 10.1001/jama.300.7.845, 10.1001%2Fjama.300.7.845. PMID 18714068
Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun DJ, Salt DE (2004) The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. Plant J 39:237–251
Kramer U, Talke I, Hanikenne M (2007) Transition metal transport. FEBS Lett 581:2263–2272
Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of Ni in hyperaccumulator and non-accumulator *Thlaspi* species. Plant Physiol 122:1343–1353
Kubota H, Takenaka C (2003) *Arabis gemmifera* is a hyperaccumulator of Cd and Zn. Int J Phytoremed 5:197–201
Kumar PB, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238
Kupper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator, *Thlaspi caerulescens* (Ganges population). New Phytol 185:114–129
Kupper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyper accumulator *Arabidopsis halleri*. Planta 212:75–84
Kuzovkina YA, Knee M, Quigley MF (2004) Cadmium and copper uptake and translocation in five willow (*Salix* L.) species. Int J Phytorem 6:269–287
Lacerda LD (1998) Biogeochemistry of trace metals and diffuse pollution in mangrove ecosystems. International Society for Mangrove Ecosystems, Okinawa
Lee S, Kim YY, Lee Y, An G (2007) Rice P1B-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. Plant Physiol 145:831–842
Lee J (2003) Functional expression of a bacterial heavy metal transporter in Arabidopsis enhances resistance to and decreases uptake of heavy metals. Plant Physiol 133:589–596
Lewis M, Pryor R, Wilking L (2011) Fate and effects of anthropogenic chemicals in mangrove ecosystems: a review. Environ Pollut 159:2328–2346
Li YM, Chaney R, Brewer E, Roseberg R, Angle JS, Baker A, Reeves R, Nelkin J (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant Soil 249:107–115
Liao S, Chang N (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquatic Plant Manag 42:60–68
Lian Y, Xu J, Lin P, Meguro S, Kawachi S (1999) Five heavy metals in propagules of ten mangrove species of China. J Wood Sci 45:343–347

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemical enhanced phytoextraction. J Environ Qual 30:1919–1926
Lone MI, He Z, Stoffella PJ, Yang X (2008) Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. J Zhejiang Univ Sci 9(3):210–220
Lopez-Millan AF, Ellis DR, Grusak MA (2004) Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. Plant Mol Biol 54:583–596
Lytle CM, Lytle FW, Yang N, Qian JH, Hansen D, Zayed A, Terry N (1998) Reduction of Cr (VI) to Cr (III) by wetland plants: potential for in situ heavy metal detoxification. Environ Sci Technol 32:3087–3093
Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579
MacFarlane GR, Koller CE, Blomberg SP (2007) Accumulation and partitioning of heavy metals in mangroves: a synthesis of field studies. Chemosphere 69:1454–1464
MacFarlane GR, Pulkownik A, Burchett MD (2003) Accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina* (Forsk). Vierh: biological indication potential. Environ Pollut 123:139–151
MacFarlane GR (2002) Leaf biochemical parameters in *Avicennia marina* (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. Marine Pollut Bull 44:244–256
Madejón P, Murillo JM, Marañón T, Espinar JL, Cabrera F (2006) Accumulation of As, Cd and selected trace elements in tubers of *Scirpus maritimus* L. from Doñana marshes (South Spain). Chemosphere 64:742–748
Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between trade and environment. Environ Exp Bot 68:1–13
Magda AS (2008) Phytoremediation of some heavy metals by *Dunaliella salina*. Glob J Environ Res 2(1):1–11
Marchand C, Lallier-Verges E, Baltzer F, Alberic P, Cossa D, Baillif P (2006) Heavy metals distribution in mangrove sediments along the coastline of French Guiana. Mar Chem 98:1–17
McKinley AC, Dafforn KA, Taylor MD, Johnston EL (2011) High levels of sediment contamination have little influence on estuarine beach fish communities. PLoS One 6(10):e26353. doi:10.1371/journal.pone.0026353
Memon AR, Schroder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Environ Sci Poll Res 16:162–175
Mitra A, Banerjee K, Bhattacharyya DP (2004) The other face of mangroves. Department of Environment, Government of West Bengal (India) Press, Kolkata, p 185
Mitra A, Trivedi S, Choudhury A (1994a) Inter relationship between gross primary production and metal accumulation by *Crassostrea cucullata*. Poll Res 13(4):391–394
Mitra A, Trivedi S, Chaudhuri A et al (1994b) Inter-relationship between trace metal pollution and physico-chemical variables in the frame work of Hooghly estuary. Indian Ports 2:27–35
Mitra A, Trivedi S, Choudhuri A, Gupta A, Chakraborty K, Sanyal T, Choudhury A (1994c) Comparative study of heavy metal accumulation by different mangrove plants, technical papers of National convention on environment of India-challenges for the 21st Century, November 1994, Institution of Public Health Engineers, Calcutta, pp 9–14
Mitra A, Trivedi S, Gupta A et al (1996) Distribution of heavy metals in the sediments from Hooghly estuary, India. Pollut Res 15(2):137–141
Mitra A, Trivedi S, Gupta A et al (1995) *Balanas balanoides* as indicator of heavy metals. Indian J Environ Health 37(1):42–45
Mizuno T, Hirano K, Kato S, Obata H (2008) Cloning of ZIP family transporter genes from the manganese hyperaccumulator plant *Chengiopanax sciadophylloides* and its metal transport and resistance abilities in yeast. Soil Sci Plant Nutr 54:86–94
Mizuno T, Usui K, Horie K, Nosaka S, Mizuno N, Obata H (2005) Cloning of three ZIP/NRAMP transporter genes from a Ni hyperaccumulator plant *Thlaspi japonicum* and their Ni 2þ-transport abilities. Plant Physiol Biochem 43:793–801
Mongkhonsin B, Nakbanpote W, Nakai I, Hokura A, Jearanaikoon N (2011) Distribution and speciation of chromium accumulated in *Gynura pseudochina* (L.) DC. Environ Exp Bot 74:56–64
Moradi AB, Swoboda S, Robinson B, Prohaska T, Kaestner A, Oswald SE, Wenzel WW, Schulin R (2010) Mapping of nickel in root cross-sections of the hyperaccumulator plant *Berkheya coddii* using laser ablation ICP-MS. Environ Exp Bot 69:24–31
Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P (2008) AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. Plant Physiol 149:894–904
Mremi SD, Machiwa JF (2003) Heavy metal contamination of mangrove sediments and the associated biota in the Dar es Salam, Tanzania. Tanz J Sci 29(1):60–76
Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, Guallar E (2008) Arsenic exposure and prevalence of type 2 diabetes in US adults. JAMA 300(7):814–822, doi:10.1001/jama.300.7.814, 10.1001%2Fjama.300.7.814. PMID 18714061
Nazli FM, Hashim RN (2010) Heavy metal concentrations in an important mangrove species, *Sonneratia caseolaris*, in Peninsular Malaysia. Environ Asia 3(Special issue):50–55
Ong Che RG (1999) Concentration of seven heavy metals in sediments and mangrove root samples from Mai Po, Hong Kong. Marine Pollut Bull 39:269–279
Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyperaccumulation metals in plants. Water Air Soil Pollut 184:105–126
Pahalawattaarachchi V, Purushothaman CS, Vennila A (2009) Metal phytoremediation potential of *Rhizophora mucronata* (Lam). Ind J Marine Sci 38(2):178–183
Peng L, Zheng W, Li Z (1997) Distribution and accumulation of heavy metals in *Avicennia marina* community in Shenzhen, China. J Environ Sci 4:472–479
Perrier N (2004) Nickel speciation in *Sebertia acuminata*, a plant growing on a lateritic soil of New Caledonia. Geoscience 336: 567–577
Perry LM (1980) Medicinal plants of East and Southeast Asia. MIT Press, Cambridge, UK
Persans MW, Yan X, Patnoe J-MML, Kramer U, Salt DE (1999) Molecular dissection of the role of histidine in Ni hyperaccumulation in *Thlaspi goesingense* (Halacsy). Plant Physiol 121:1117–1126
Peterson PJ, Burton MAS, Gregson M, Nye SM, Porter EK (1979) Accumulation of tin by mangrove species in West Malaysia. Sci Tot Environ 11:213–221
Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122:1171–1177
Pilon-Smits E et al (1999) Over-expression of ATP sulfurylase in *Brassica juncea* leads to increased selenate uptake, reduction and tolerance. Plant Physiol 119:123–132
Plaza S, Tearall KL, Zhao FJ, Buchner P, McGrath SP, Hawkesford MJ (2007) Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 58:1717–1728
Pollard AJ, Stewart HL, Roberson CB (2009) Manganese hyperaccumulation in *Phytolacca americana* L. from the southeastern united states. Northeastern Natur 16:155–162
Prasad MNV (2005) Nickelophilous plants and their significance in phytotechnologies. Braz J Plant Physiol 17:113–128
Preda M, Cox ME (2002) Trace metal occurrence and distribution in sediments and mangroves, Pumicestone region, Southeast Queensland, Australia. Environ Int 28:433–449
Ragsdale HL, Thorhaug A (1980) Trace metal cycling in the U.S. coastal zone: a synthesis. Americ J Bot 67:1102–1112

Rai PK (2008) Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. Int J Phytorem 10:430–439
Reeves RD, Adiguzel N, Baker AJM (2009) Nickel hyperaccumulation in *Bornmuellera kiyakii* and associated plants of the Brassicaceae from Kızıldaäÿ Derebucak (Konya), Turkey. Turk J Bot 33:33–40
Reeves RD, Baker AJM, Borhidi A, Berazain R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. Ann Bot 83:29–38
Ridgway J, Breward N, Langston WJ, Lister R, Rees JG et al (2003) Distinguishing between natural and anthropogenic sources of metals entering the Irish Sea. Appl Geochem 18:283–309
Robinson BH, Brooks RR, Howes AW (1997) The potential of the high-biomass nicke1 hyperaccumulator Berkheya coddii for phytoremediation and phytomining. J Geochem Explor 60:115–126
Roosens NH, Willems G, Saumitou-Laprade P (2008a) Using Arabidopsis to explore zinc tolerance and hyperaccumulation. Trends Plant Sci 13:208–215
Roosens NHCJ, Glenda W, Cécile G, Adeline C, Pierre SL (2008b) The use of comparative genome analysis and synthetic relationships allows extrapolating the position of Zn tolerance QTL regions from *Arabidopsis halleri* into *Arabidopsis thaliana*. Plant Soil 306:105–116
Sadiq M, Zaidi TH (1994) Sediment composition and metal concentrations in mangrove leaves from the Saudi coast of the Arabian Gulf. Sci Total Environ 155:1–8
Saenger P, McConchie D (2004) Heavy metals in mangroves: methodology, monitoring and management. ENVIS Forestry Bullet 4:52–62
Sahi SV, Bryant NL, Sharma NC, Singh SR (2002) Characterization of a lead hyper accumulator shrub, *Sesbania drummondii*. Environ Sci Technol 36:4676–4680
Sahoo KD, Dhal NK (2009) Potential microbial diversity in mangrove ecosystems: a review. Ind J Marine Sci 38:249–256
Sakakibara M, Ohmori Y, Ha NTH, Sano S, Sera K (2011) Phytoremediation of heavy metal contaminated water and sediment by *Eleocharis acicularis*. Clean Soil Air Water 39:735–741
Salem HM, Eweida EA, Farag A (2000) Heavy metals in drinking water and their environmental impact on human health. ICEHM2000. Cairo University, Egypt, pp 542–556
Salt DE, Pickering IJ, Prince RC, Gleba D, Dushenkov S, Smith RD, Raskin I (1997) Metal accumulation by aquacultured seedlings of Indian mustard. Environ Sci Technol 31:1635–1644
Sancenon V, Puig S, Mateu-Andres I, Dorcey E, Thiele DJ, Penarrubia L (2004) The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. J Bio Chem 279:15348–15355
Saraswat S, Rai JPN (2009) Phytoextraction potential of six plant species grown in multimetal contaminated soil. Chem Ecol 25:1–11
Sarangi RK, Kathiresan K, Subramanian AN (2002) Metal concentrations in five mangrove species of the Bhitarkanika, Orissa, east coast of India. Ind J Marine Sci 31:251–253
Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, Ryan PR, Delhaize E, Matsumoto H (2004) A wheat gene encoding an aluminum-activated malate transporter. Plant J 37:645–653
Schikora A, Thimm O, Linke B, Buckhout TJ, Müller M, Schmidt W (2006) Expression, localization, and regulation of the iron transporter LeIRT1 in tomato roots. Plant Soil 284:101–108
Shingu Y, Kudo T, Ohsato S, Kimura M, Ono Y, Yamaguchi I, Hamamoto H (2005) Characterization of genes encoding metal tolerance proteins isolated from *Nicotiana glauca* and *Nicotiana tabacum*. Biochem Biophys Res Commun 331:675–680
Siddiqui MH, Kumar A, Kesari KK, Arif JM (2009) Biomining – a useful approach toward metal extraction. Am-Eurasian J Agron 2:84–88
Silva CAR, Lacerda LD, Rezende CE (1990) Metals reservoir in a red mangrove forest. Biotropica 22:339–345
Smith AH, Hopenhayn-Rich C, Bates MN et al (1992) Cancer risks from arsenic in drinking water. Environ Health Persp 97:259–267, doi: 10.2307/3431362, 10.2307%2F3431362. PMC 1519547, PMID 1396465
Srivastava M, Ma LQ, Santos JAG (2006) Three new arsenic hyperaccumulating ferns. Sci Total Environ 364:24–31
Sukumaran D (2013) Phytoremediation of heavy metals from industrial effluent using constructed wetland technology. Appl Ecol Environ Sci 1(5):92–97
Sundaray SK, Nayak BB, Lin S, Bhatta D (2011) Geochemical speciation and risk assessment of heavy metals in the river estuarine sediments – a case study: Mahanadi basin, India. J Hazard Mater 186:1837–1846
Talke I, Hanikenne M, Krämer U (2006) Zn dependent global transcriptional control, transcriptional de-regulation and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 142:148–167
Tang YT, Qiu RL, Zeng XW, Ying RR, Yu FM, Zhou XY (2009) Lead, zinc cadmium accumulation and growth simulation in *Arabis paniculata* (Franch). Environ Exp Bot 66:126–134
Thomas G, Fernandez TV (1997) Incidence of heavy metals in the mangrove flora and sediments in Kerala, India. Hydrobiologia 352:77–87
Tripathi RD, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis FJM (2007) Arsenic hazards: strategies for tolerance and remediation by plants. Trend Biotech 25:158–165
Trivedi S, Banerjee K, Mukhopadhyay N, and Mitra A (2007) Threat associated with shrimp culture practice in the ichthyoplankton community of coastal West Bengal, international conference on biodiversity: issues and concerns, 21–23 Nov 2007, Indian Statistical Institute, Kolkata, India
Trivedi S, Mitra A, Bag M et al (1995) Heavy metal concentration in mud-skipper *Boleophthalmus boddaerti* of Nayachara Island, India. Indian J Environ Health 37(2):120–122
Trivedi S, Ueki T, Yamaguchi N et al (2003) Novel Vanadium-binding proteins (vanabins) identified in cDNA libraries and the genome of the ascidian *Ciona intestinalis*. Biochem Biophys Acta 1630:64–70
Usero J, Morillo J, Gracia I (2005) Heavy metal concentrations in mollusks from the Atlantic coast of southern Spain. Chemosphere 59:1175–1181
van de Mortel JE, Villanueva LA, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Loren V, van Themaat E, Koornneef M, Aarts MGM (2006) Large expression differences in genes for iron and Zn homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 142:1127–1147
Van Huysen T, Terry N, Pilon-Smits E (2004) Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine-synthase. Int J Phytoremed 6:111–118
Walsh GE, Ainsworth KA, Rigby R (1979) Resistance of red mangrove (*Rhizophora mangle* L.) seedlings to lead, cadmium and mercury. Biotropica 11:22–27
Weber M, Harada E, Vess C, von Roepenack-Lahaye E, Clemens S (2004) Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J 37:269–281
Weng-jiao Z, Xiao-yong C, Peng L (1997) Accumulation and biological cycling of heavy metal elements in *Rhizophora stylosa* mangroves in Yingluo Bay, China. Mar Ecol Progr Ser 159:293–301
WHO (2001) Arsenic in drinking water. http://www.who.int/int-fs/en/fact210.html. Fact sheet No. 210. 30 May 2000
Wilkins C, Salter L (2003) Arsenic hyperaccumulation in ferns: a review. Environmental Chemistry Group Bulletin of the Royal Society of Chemistry, July 2003 edition
Willems G, Drager DB, Courbot M, Gode C, Verbruggen N, Saumitou-Laprade P (2007) The genetic basis of Zn tolerance in the metallophyte *Arabidopsis halleri ssp. halleri* (Brassicaceae): an analysis of quantitative trait loci. Genetics 176:659–674

Williams LE, Pittman JK, Hall JL (2000) Emerging mechanisms for heavy metal transport in plants. Biochim Biophys Acta 1465:104–126

Wong JWC, Lai KM, Su DS, Fang M (2001) Availability of heavy metals for *Brassica chinensis* grown in an acidic loamy soil amended with domestic and industrial sewage sludge. Water Air Soil Pollut 128:339–353

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2001:1–20

Xiao H, Yin L, Xu X, Li T, Han Z (2008) The Iron-regulated transporter, MbNRAMP1, isolated from *Malus baccata* is involved in Fe, Mn and Cd trafficking. Ann Bot 102:881–889

Yang SX, Deng H, Li MS (2008) Manganese uptake and accumulation in a woody hyperaccumulator, *Schima superba*. Plant Soil Environ 54:441–446

Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Bio 18(4):339–353

Zaman S et al (2013) Rising water salinity: a threat to mangroves of Indian Sundarbans. In water insecurity: a social dilemma. Community Environ Disast Risk Manag 13:167–183

Zhang X, Xia H, Li Z, Zhuang P, Gao B (2011) Identification of a new potential Cd-hyperaccumulator Solanum photeinocarpum by soil seed bank-metal concentration gradient method. J Hazard Mater 189:414–419

Zhao FJ, Lombi E, Breedon T, McGrath SP (2000) Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. Plant Cell Environ 23:507–514

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380

# 20 Lichens as an Alternative Biosorbent: A Review

Demet Cansaran-Duman and Sümer Aras

## 20.1 Introduction

Rapid industrialization and urbanization have resulted in the generation of large quantities of aqueous effluents, many of which contain high levels of toxic pollutants (Krishnani and Ayyappan 2006; Vijayaraghavan and Yun 2008). The deleterious effects of organic and inorganic pollutants on ecosystems and on human health are well known, and much expenditure is devoted to industrial treatment methods to prevent or limit discharges. Other than physical and chemical methods of treatment, biological methods have been in place for many years such as standard sewage and water purification treatments as well as auxiliary reed bed and wetlands approaches (Gadd 2009a). Various physicochemical and biological processes are usually employed to remove pollutants from industrial wastewaters before discharge into the environment (Hai et al. 2007). In the case of treatment of adsorptive pollutants like heavy metals and ionic dyes, however, most of the conventional treatment processes, especially chemical precipitation or coagulation, become less effective and more expensive when the adsorbates are in a low concentration range (Crini 2006; Vieira and Volesky 2000; Volesky 2007a).

The use of biosorbents for the removal of toxic pollutants or for the recovery of valuable resources from aqueous wastewaters is one of the most recent developments in environmental or bioresource technology (Vijayaraghavan and Yun 2008; Volesky 2007a; Aksu 2005; Sağ and Kutsal 2001). These technologies offer many advantages compared to the conventional ones such as low cost, high efficiency, the minimization of chemical or biological sludges, the ability to regenerate biosorbents, and the possibility of metal recovery following adsorption (Volesky 2007a). Although biosorption of heavy metals or dyes has become very popular, their high cost and low efficiency have limited their commercial use in actual industrial scenarios (Vijayaraghavan and Yun 2008).

Several treatment technologies have been developed to remove heavy metal ions from industrial wastewaters and other effluents. These include membrane processing, evaporation, chemical precipitation, coagulation, ion exchange, electrolysis, and adsorption (Gadd 1990; Feng et al. 2004). However all these methods involve high operating cost and may produce large volume of sludge which creates further disposal problem. The major advantages of adsorption over conventional treatment methods include low cost, high efficiency, minimization of chemical sludge, regeneration of biosorbent, and possibility of metal recovery (Sud et al. 2008a; Kaur et al. 2012).

*Sorption* is a term used for both absorption and adsorption; these terms are often confused. *Absorption* is the incorporation of a substance in one state into another different state (i.e., liquids being absorbed by a solid or gases being absorbed by water). *Adsorption* is the physical adherence or bonding of ions and molecules onto the surface of the solid material (Gadd 2009b). *Biosorption* may be simply defined as *the removal of substances from solution by biological material*. Such substances can be organic and inorganic and in soluble or insoluble forms. Biosorption is a physicochemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface complexation, and precipitation. It is a property of living and dead biomass (as well as excreted and derived products): metabolic processes in living organisms may affect physicochemical biosorption mechanisms, as well as pollutant bioavailability, chemical speciation, and accumulation or transformation by metabolism-dependent properties (Gadd 2009a). *Sorbates*: a wide range of target sorbates have been removed from aqueous solutions using biosorbents including metals, dyes, fluoride, phthalates, pharmaceuticals, etc. (Michalak et al. 2013).

D. Cansaran-Duman (✉)
Biotechnology Institute, Ankara University, Tandogan, Ankara, Turkey
e-mail: dcansaran@yahoo.com

S. Aras
Biotechnology Section, Department of Biology, Faculty of Science, University of Ankara, Tandogan, Ankara, Turkey

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*Biosorbents*: a wide range of biomaterials available in nature has been employed as biosorbent for the desired pollutant removal. All kinds of microbial, plant, and animal biomass and their derivative products have received great interest in a variety of ways and in relation to a variety of substances (Volesky 1990a; Volesky 2003a; Al-Masri et al. 2010). However, in recent years attention has been driven toward the agricultural waste materials, polysaccharides, and industrial waste biomaterials (Witek-Krowiak and Reddy 2013; Witek-Krowiak et al. 2011; Reddy et al. 2012; Blázquez et al. 2011). Since Hecker first reported a quantitative study on the copper uptake by fungal spores of *Tilletia tritici* and *Ustilago crameri* in 1902 (McCallan and Miller 1956; Muraleedharan et al. 1991), over 3,000 research articles on biosorption have been published in different journals from many different countries. In addition, about 70 review papers and some books have appeared about biosorption phenomena, equilibrium and kinetic modeling, reactor operation, and application to real industries (John Wase and Forster 1997; Volesky 1990b; Volesky 2004). However, for general readers, access to these special books or perusal of the large number of papers available on this topic is challenging. In many review papers, biosorptive capacities of various biomass types have been quantitatively compared (Vijayaraghavan and Yun 2008; Aksu 2005; Volesky and Holan 1995a; Ahluwalia and Goyal 2007a; Bishnoi and Garima 2005; Gupta and Mohapatra 2003; Kaushik and Malik 2009; Lodeiro et al. 2006; Mack et al. 2007; Sağ 2001; Wan Ngah and Hanafiah 2008; Romera et al. 2006). In some cases, the uptake of heavy metals by biomass reached as high as 50 % of its dry weight (Vieira and Volesky 2000; Volesky 1994). Further, a vast array of biological materials, especially bacteria, cyanobacteria, algae (including microalgae, macroalgae, seaweeds), yeasts, fungi, and lichens, have drawn much attention for the removal and recovery of heavy metal ions due to their good performance, low cost, and availability in large quantities. Because of their abundant chelating functional groups, biological materials have greater affinity for metal ions (Volesky 2007b).

It was believed that by using this new method in which biomass is used as a sorbent, the toxic pollutants could be selectively removed from aqueous solutions to desired low levels. The remarkable properties of lichens in the transformation and detoxification of organic and inorganic pollutants are well known, and many processes have received attention in the general area of environmental biotechnology and microbiology.

### 20.1.1 Mechanism: Biosorbent–Sorbate Interactions

The importance of any given group for biosorption of a certain pollutant by a certain biomass depends on various factors, including the number of reactive sites in the biosorbent, accessibility of the sites, chemical state of the sites (i.e., availability), and affinity between the sites and the particular pollutant of interest (i.e., binding strength) (Vieira and Volesky 2000). Biosorption of metals or dyes occurs mainly through interactions such as ion exchange, complexation, adsorption by physical forces, precipitation, and entrapment in inner spaces (Sud et al. 2008b; Park et al. 2010).

The biosorption process involves interaction between the biosorbent (solid phase) and a solvent (liquid phase) containing the dissolved species to be sorbed. This phenomenon can be explained by different mechanisms, complexation, chemisorption, adsorption on surface and pores, ion exchange, chelation, adsorption by physical forces, etc. (Sud et al. 2008a). The biosorption continues till equilibrium is maintained between the amount of sorbed species and its portion remaining in the solution. The extent of biosorbent affinity for the dissolved species determines its distribution between the solid and liquid phase. Among the different species dissolved in the liquid phase, metal biosorption is a two-step process, where the first step involves a stoichiometric interaction between the metal ions and the reactive functional groups forming monolayer on the cell wall and the second step is an inorganic deposition of increased amounts of metals (Kaur et al. 2012).

In general, dead biomass were preferred as biosorbent; most of the biosorbents used were of dead biomass; this exhibits specific advantages in comparison with the use of living microorganisms: dead cells can be easily stored or used for longer time periods, dead biomass is not the subject to metal toxicity limitations and does not require nutrients, and biosorbents can be easily desorbed from the metal ions and reused (Baysal et al. 2009; Selatnia et al. 2004). However, the use of nonliving biomass in powdered form displays some disadvantages such as difficulty in the separation of biomass from the reaction system, mass loss after regeneration, poor mechanical strength, and small particle size which makes it difficult to use in batch and continuous systems (Michalak et al. 2013; Arica et al. 2004). On the other hand, immobilization is a straightforward method to overcome these obstacles.

### 20.1.2 Process Factors Influencing Biosorption

For the industrial application of biosorption technology for pollutant removal, it is very important to investigate the removal efficiency of a given biosorbent for the target pollutant. Pollutant uptake can involve different types of biosorption processes that will be affected by various physical and chemical factors, and these factors will determine the overall biosorption performance of a given biosorbent (i.e., its uptake rate, its specificity for the target, and the quantity of target removed) (Park et al. 2010).

These are many factors that can affect biosorption. Physical and chemical treatments such as boiling, drying, autoclaving, and mechanical disruption will affect binding properties, while chemical treatments such as alkali treatment often improve biosorption capacity (Wang and Chen 2006). Growth and nutrition of the biomass and age can also influence biosorption due to changes in cell size, wall composition, extracellular product formation, etc. The surface area to volume ratio may be important for individual cells or particles, as well as the available surface area of immobilized biofilms. In addition, the biomass concentration may also affect biosorption efficiency with a reduction in sorption per unit weight occurring with increasing biomass concentration (de Rome and Gadd 1987a). Apart from these, physicochemical factors such as pH, the presence of other anions and cations, metal speciation, pollutant solubility and form, and temperature may also have an influence. With living cell systems, the provision of nutrients and optimal growth conditions is an obvious requirement (Gadd 2009a). Among these factors, the pH appears to be the most important regulator of the biosorptive process. In general, as solution pH increases, the biosorptive removal of cationic metals or basic dyes is also increased, while that of anionic metals or acidic dyes is reduced (Park et al. 2010). Metal biosorption has frequently been shown to be strongly pH dependent in almost all systems examined, including bacteria, cyanobacteria, algae, and fungi.

### 20.1.3 Kinetics and Equilibrium Modeling

A variety of models have been used to characterize biosorption (de Rome and Gadd 1987a; Sag et al. 1998; Volesky et al. 2003; Volesky 2003b; Yang and Volesky 2000; Chen et al. 2007; Beolchini et al. 2006; Pagnanelli et al. 2004a; Pagnanelli et al. 2004b; Pagnanelli et al. 2003; Beolchini et al. 2001; Volesky and Holan 1995b). These range from simple single component models, of which the Langmuir and Freundlich models are probably the most widely used, to complex multicomponent models (the bed depth-service time (BDST) model; Thomas model; Yoon and Nelson model; Clark model; Wolborska model; biosorption thermodynamics); some derived from Langmuir–Freundlich models (Pagnanelli et al. 2001; Pagnanelli et al. 2002).

### 20.1.4 Biomass Types

Investigations on the metal-binding capacity of some types of biomass have been accelerated since 1985 (Volesky and Holan 1995c). Indeed, some biomass types are very effective in accumulating heavy metals. Seaweeds, molds, yeasts, bacteria, and crab shells, among other kinds of biomass, have been tested for metal biosorption with very encouraging results (Regine and Volesky 2000).

A wide range of microbial biomass types have been investigated in biosorption studies, including mixed organism/biomass systems (Munoz et al. 2006). These include archaea, bacteria (Pinaki et al. 2004; Bueno et al. 2008; Vijayaraghavan et al. 2007; Tuzen et al. 2008; Ngwenya 2007; Calfa and Torem 2008), cyanobacteria (Pradhan et al. 2007; Kiran et al. 2007; Anjana et al. 2007; Avery et al. 1993; Garnham et al. 1993a; Garnham et al. 1993b; Garnham et al. 1993c), algae (Garnham et al. 1991; Garnham et al. 1992a; Garnham et al. 1992b; Garnham 1997; Mohan et al. 2007; Aksu and Donmez 2006) (including macroalgae, i.e., seaweeds (Davis et al. 2003; Yang and Chen 2008; Vilar et al. 2008; Senthilkumar et al. 2007; Romera et al. 2007; Murphy et al. 2007; Webster and Gadd 1996; Webster et al. 1997)), and fungi, the latter including filamentous forms (Tobin et al. 1994; Gadd and White 1992; Wu; Kapoor and Viraraghavan 1995; Kiran et al. 2006; Bayramoglu and Arica 2007; Zhou 1999), as well as unicellular yeasts (Wang and Chen 2006; de Rome and Gadd 1991; de Rome and Gadd 1987b), fruiting bodies (mushrooms, brackets, etc.), and lichens (Sari et al. 2007; Ekmekyapar et al. 2006a). It is clear that the usage of biomass types that are efficient, cheap, and easy to grow or harvest should be preferred and concentration be given to biomass modifications and/or alteration of bioreactor configuration and physicochemical conditions to enhance biosorption.

Indeed, biomass composition does not show significant differences between species of the same genus or order. For example, cell wall structure and composition (the main site of metal/radionuclide biosorption) is similar throughout all Gram-positive bacteria (Kim and Gadd 2008). Similarly, all Gram-negative bacteria have the same basic cell structure (Kim and Gadd 2008; Dmitriev et al. 2005); main fungal orders are similarly uniform in wall structure and composition, with some known variations due to varying contents of chitin, glucans, etc. (Gow and Gadd 1995). Plant and algal material similarly shows considerable uniformity, albeit with some differences between major genera (Gadd 2009a; Davis et al. 2003).

Many kinds of macroalgae (seaweeds), plant materials (leaves, bark, sawdust), and animal materials (hair, crustaceans) have also been studied (Zhang and Banks 2006; Nasir et al. 2007; Ahluwalia and Goyal 2007b; Niua et al. 2007). A common rationale is that "waste" biomass will provide an economic advantage. A variety of sludges arise from sewage treatment and other waste processing applications, and these have also been investigated for biosorption properties (Barros et al. 2007; Gao and Wang 2007; Pamukoglu and Kargi 2007; Hawari and Mulligan 2006; Hammaini et al. 2007; Nadeema et al. 2008; Veglio et al. 2003), although metal sorbing properties may sometimes be low. Fungal biomass has also received attention as biosorbent materials for metal-contaminated aqueous solutions, because of the ease with which they are grown and the availability of fungal biomass as an industrial waste product, e.g., *A. niger* (citric acid production) and *S. cerevisiae* (brewing) (Gadd 2009a). Fungal cell walls are complex macromolecular structures predominantly consisting of chitins, glucans, mannans, and proteins but also containing other polysaccharides, lipids, and pigments, e.g., melanin (Gadd 1993; Gadd and Griffiths 1980; Gadd and Mowll 1985). This variety of structural components ensures that many different functional groups are able to bind metal ions to varying degrees (Gadd 2009a). Chitin is a very important structural component of fungal cell walls and is an effective biosorbent for metals and radionuclides, as are chitosan and other chitin derivatives (Gadd 2009a). In *Rhizopus arrhizus*, U biosorption involves coordination to the amine N of chitin, adsorption in the cell wall chitin structure, and further precipitation of hydroxylated derivatives (Tsezos and Volesky 1982). Chitosan is of low cost compared with commercial activated carbon (chitosan is derived by deacetylation of chitin, the most abundant amino polysaccharide in nature) and strongly complexes pollutants, especially metals. The most common yeast biomass (*Saccharomyces cerevisiae*) is not usually a waste but a commercial commodity (feedlot uses). Some chemical compounds of yeast cells can also act as ion exchangers with rapid reversible binding of cations. Volesky et al. 1993 working on cadmium biosorption by *Saccharomyces cerevisiae* demonstrated that this yeast is a reasonably potent biosorbent material for cadmium. Niu and Volesky 1999 examined selected bacteria, algae, and the fungus *Penicillium chrysogenum* and found that gold biosorption from cyanide solution is higher at lower pH values, indicating that, in the uptake of anions, biosorbents may act as weak-acid ion exchangers. At pH 2, the gold uptake by *Bacillus* biomass was 8.0 μmol/g, by *Penicillium* 7.2 μmol/g, and by the seaweed *Sargassum* 3.2 μmol/g. The relatively low uptake of the anionic gold complex by *Sargassum* in this work contrasts with excellent uptakes of cationic gold form observed earlier (Kuyucak and Volesky 1989; Volesky and Kuyucak 1988). The results confirmed that waste microbial biomaterials do have some potential for removing and concentrating gold from solutions where it occurs as an anionic gold cyanide complex.

Lichens are usually slow-growing organisms consisting of a fungus and an alga or cyanobacterium which combine in a symbiotic relationship with several unique physiological and morphological characteristics (Ates et al. 2007a). Lichens do not have a complex root system, waxy cuticle, or stoma, and hence they obtain most of their nutrients from the atmosphere through wet and dry deposition (Williams et al. 1996). Lichens have been widely used as air pollution monitors because of their ability to strongly bind and accumulate metals (Akcin et al. 2001; Purvis et al. 2000). The metal ion-binding properties of lichens have been found that nonliving lichen biomass is able to bond metal ions to a greater degree than living lichens (Purvis et al. 2000) because the living plasma membrane excludes metals from entering the cell (Chettri et al. 1998). The mechanism cation uptake by lichen is generally regarded as an abiotic process governed by surface complexation of cations with exposed functional groups on the lichen surface (Pipiska et al. 2007a). Carboxylic, hydroxycarboxylic acids, and chitin have been suggested as metal-binding ligands in lichen (Chettri et al. 1998; Bingöl et al. 2009). The adsorption properties of lichen biomass of *Cladonia rangiformis* Hoffm. for copper(II) were investigated by using batch adsorption techniques. The effects of initial metal ion concentration, initial pH, biosorbent concentration, stirring speed, and contact time on biosorption efficiency were studied. In the experiments, the optimum pH value was determined as 5.0 which was the native pH value of solution. The experimental adsorption data were fitted to the Langmuir adsorption model. The highest metal uptake was calculated from Langmuir isotherm and found to be 7.6923 mg Cu(II)/g inactivated lichen at 15 °C. The results indicated that the biomass of *C. rangiformis* is a suitable biosorbent for removing Cu(II) from aqueous solutions (Ekmekyapar et al. 2006b).

Pipiska et al. removed $Co^{2+}$ by *Hypogymnia physodes* (Pipiska et al. 2007b), Dogan et al. (2006) and Turhan et al. (2005) used *Cetraria islandica* (L.) and *Usnea longissima* for Au(III) and Cu(II) uptake, and Ates et al. removed Ni(II) and Cu(II) by *Pseudevernia furfuracea* (L.) (Ates et al. 2007b) from aqueous solutions.

Equilibrium, thermodynamic, and kinetic studies were carried out for the biosorption of Pb(II) and Ni(II) ions from aqueous solution using the lichen (*Cladonia furcata*) biomass. Langmuir, Freundlich, and Dubinin–Radushkevich (D–R) isotherm models were applied to describe the biosorption of the metal ions onto *C. furcata* biomass. According to the result of these studies, Langmuir model fitted the equilibrium data better than the Freundlich isotherm. The monolayer biosorption capacity of the biomass was found to be 12.3 and 7.9 mg/g for Pb(II) and Ni(II) ions, respectively. From the D–R model, the mean free energy was calculated as 9.1 kJ/mol for Pb(II) biosorption and 9.8 kJ/mol for Ni(II) biosorption, indicating that the biosorption of both metal ions was taken place by chemical ion exchange. Thermodynamic parameters, the change of free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) of the biosorption were also calculated. These parameters showed that the biosorption process of Pb(II) and Ni(II) ions onto *C. furcata* biomass was feasible, spontaneous, and exothermic under studied conditions (Sarı et al. 2007).

On the other studies with lichen species, the biosorption characteristics of Pb(II) and Cr(III) ions from aqueous solution using the lichen (*Parmelina tiliaceae*) biomass were investigated. Langmuir, Freundlich, and Dubinin–Radushkevich (D–R) models were applied to describe the biosorption isotherm of the metal ions by *P. tiliaceae* biomass. Langmuir model fitted the equilibrium data better than the Freundlich isotherm. The monolayer biosorption capacity of *P. tiliaceae* biomass for Pb(II) and Cr(III) ions was found to be 75.8 mg/g and 52.1 mg/g, respectively. From the D–R isotherm model, the mean free energy was calculated as 12.7 kJ/mol for Pb(II) biosorption and 10.5 kJ/mol for Cr(III) biosorption, indicating that the biosorption of both metal ions was taken place by chemical ion exchange. The calculated thermodynamic parameters (DG-, DH-, and DS-) showed that the biosorption of Pb(II) and Cr(III) ions onto *P. tiliaceae* biomass was feasible, spontaneous, and exothermic under examined conditions (Uluozlu et al. 2008).

Bingöl et al. investigated in their study the batch removal of chromate anions ($CrO_4^{2-}$) from wastewater under different experimental conditions using cationic surfactant-modified lichen (*Cladonia rangiformis* (L.)). Cetyltrimethylammonium bromide (CTAB) was used for biomass modification. The results of the experiments showed that biomass modification substantially improved the biosorption efficiency. Effects of pH, biosorption time, initial $CrO_4^{2-}$ concentration, biosorbent dosage, and the existence of the surfactant on the biosorption of $CrO_4^{2-}$ anions were studied. Studies up to date have shown that the biosorption efficiency of chromium increased as the pH of the solution decreased. In this study, the removal of chromate anions from aqueous solutions at high pH values with surfactant-modified lichen was investigated. From the results of the experiments, it was seen that the removal of chromate anions by modified lichen was 61 % at the solution natural pH (pH 5.11), but at the same pH value, the removal of chromate anions by unmodified lichen was 6 %. Also concentrations ranging from 30 to 150 mg/L Cr(IV) were tested, and the biosorptive removal efficiency of the metal ions from aqueous solution at high pH was achieved more than 98 % (Bingöl et al. 2009).

Tüzen et al. evaluated the potential use of the lichen biomass (*Xanthoparmelia conspersa*) to remove mercury(II) ions from aqueous solution by biosorption using the batch method. Effects of pH, contact time, biomass concentration, and temperature on the removal of Hg(II) ions were studied. The Langmuir isotherm model defined the equilibrium data precisely compared to Freundlich model, and the maximum biosorption capacity obtained was 82.8 mg/g. From the D–R isotherm model, the mean free energy was calculated as 9.5 kJ/mol. It shows that the biosorption of Hg(II) ions onto *X. conspersa* biomass was taken place by chemical ion exchange. Experimental data were also performed to the pseudo-first-order and pseudo-second-order kinetic models. The results indicated that the biosorption of Hg(II) on the lichen biomass followed well the second-order kinetics. Thermodynamic parameters _*G*o, _*H*o, and _*S*o indicated the Hg(II) sorption to be exothermic and spontaneous with decreased randomness at the solid–solution interface. Furthermore, the lichen biomass could be regenerated using 1 M HCl, with up to 85 % recovery, which allowed the reuse of the biomass in ten biosorption–desorption cycles without any considerable loss of biosorptive removal capacity (Tüzen et al. 2009).

Uluözlü et al. investigated the biosorption characteristics of antimony(III) from aqueous solution using lichen (*Physcia tribacia*) biomass in terms of equilibrium, thermodynamics, and kinetics. Optimum biosorption conditions were determined with respect to pH, biomass concentration, contact time, and temperature. Langmuir, Freundlich, and Dubinin–Radushkevich (D–R) isotherm models were applied to the equilibrium data. The maximum Sb(III) sorption capacity of *P. tribacia* was found to be 81.1 mg/g at pH 3, biomass concentration 4 g/L, contact time 30 min, and temperature 20 °C. The calculated mean biosorption energy (10.2 kJ/mol) using D–R model indicated that the biosorption of Sb(III) on the biomass has occurred by chemical ion exchange. The highest desorption efficiency (95 %) was achieved using 0.5 M HCI. The biosorption capacity of *P. tribacia* slightly decreased about 10 % after ten times of sorption–desorption process. The calculated thermodynamic parameters showed that the biosorption of Sb(III) onto *P. tribacia* biomass was feasible, spontaneous, and exothermic, respectively. The experimental data was also examined using the Lagergren pseudo-first-order and pseudo-second-order kinetic models. The results revealed that the pseudo-second-order kinetic model provided the best description of the equilibrium data (Uluözlü et al. 2010).

Kiliç et al. investigated the biosorption characteristics of Cu(II) ions from aqueous solution using *Lobaria pulmonaria* (L.) Hoffm. biomass. The biosorption efficiency of Cu(II) onto biomass was significantly influenced by the operating parameters. The maximum biosorption efficiency of *L. pulmonaria* was 65.3 % at 10 mg/L initial metal concentration for 5 g/L lichen biomass dosage. The biosorption of Cu(II) ions onto biomass fits the Langmuir isotherm model and the pseudo-second-order kinetic model well. The thermodynamic parameters indicate the feasibility and exothermic and spontaneous nature of the biosorption. The effective 15 desorption achieved with HCl was 96 %. Information on the nature of possible interactions between the functional groups of the *L. pulmonaria* biomass and Cu(II) ions was obtained via Fourier transform infrared (FTIR) spectroscopy. The results indicated that the carboxyl (–COOH) and hydroxyl (–OH) groups of the biomass were mainly involved in the biosorption of Cu(II) onto *L. pulmonaria* biomass. The *L. pulmonaria* is a promising biosorbent for Cu(II) ions because of its availability, low cost, and high metal biosorption and desorption capacities (Kılıç et al. 2013).

Kiliç et al. determined *Pseudevernia furfuracea* (*L.*) *Zopf.* biosorption efficiency for zinc(II). The biosorption efficiency of Zn(II) onto *P. furfuracea* (*L.*) *Zopf.* significantly affected the parameters, namely, pH, biomass concentration, stirring speed, contact time, and temperature. The maximum biosorption efficiency of *P. furfuracea* (*L.*) *Zopf.* was 92 % at 10 mg/L Zn(II), for 5 g/L lichen biomass dosage. The biosorption of Zn(II) ions onto biomass was better described by the Langmuir model and the pseudo-second-order kinetic. The obtained thermodynamic parameters from biosorption of Zn(II) ions onto biomass were feasible, exothermic, and spontaneous. The different desorbents were used to perform the desorption studies for Zn(II)-loaded *biomass*. The effective desorptions of 96 % was obtained with $HNO_3$. The *P. furfuracea* (*L.*) *Zopf*. is an encouraging biosorbent for Zn(II) ions with the high metal biosorption and desorption capacities, availability, and low cost (Kılıç et al. 2008; Hamutoglu et al. 2012).

## 20.2 Comparison of Biological Materials in Biosorption Studies

All biological materials have greater affinity for metal ions (Michalak et al. 2013). Apart from the abovementioned natural biosorbents, in the literature, few other biomaterials have received much interest, and they are rice husk, coconut shell, plant barks, leaves, sawdust, sugarcane bagasse, and peat moss (Michalak et al. 2013). From the above-discussed biomaterials, special attention was given to the application of lichens (Michalak et al. 2013). Lichens are a strong alkaline material with negatively charged surface at higher pH. Hence, it can be expected that metal ions can be removed from aqueous solutions by precipitation, electrostatic attraction, and ion exchange (Michalak et al. 2013). In an investigation of our lab, it was also reported that the utilization of lichens from biological origin will be a promising alternative to conventional adsorbents used for wastewater treatment. Lichens have also been found to bind metals in a strongly pH-dependent manner. Generally, optimum binding is observed at a pH of around 5.0. Little binding is seen below pH values of 2.0 for most metal ions; the metal ion-binding properties of lichens have been pointed out that nonliving lichen biomass is able to bond metal ions to a greater degree than living lichens. This strong metal-binding ability of lichen biomass from aqueous solutions would seem to make lichen material an ideal biosorbent for the removal of heavy metals (Michalak et al. 2013).

## 20.3 Conclusion

Due to the accumulated knowledge and due to the extremely significant economic margin for application in the metal removal/detoxification process, some new biosorbent materials are currently well poised for commercial exploitation. However, there are no limits to expanding the science of biosorption required to provide deeper understanding of the phenomenon and to support effective application attempts.

Based on all results, it can be also concluded that the lichen biomass can be evaluated as an alternative biosorbent for the treatment of wastewater containing different ions, due to being a low-cost biomass and having a considerable high sorption capacity. In addition, lichen material was chosen as a novel biosorbent in this study due to being naturally abundant, renewable, and thus cost-effective biomass.

## References


Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257

Akcin G, Salyabas O, Yesilcimen F (2001) Biosorption of heavy metal from aqueous solution by dried lichens. Int J Chem 11:141–146

Aksu Z (2005) Application of biosorption for the removal of organic pollutants: a review. Process Biochem 40:997–1026

Aksu Z, Donmez G (2006) Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vulgaris*: co-ion effect on monocomponent isotherm parameters. Process Biochem 41:860–868

Al-Masri MS, Amin Y, Al-Akel B, Al-Naama T (2010) Appl Biochem Biotechnol 160:976–987

Anjana K, Kaushik A, Kiran B, Nisha R (2007) Biosorption of Cr(VI) by immobilized biomass of two indigenous strains of cyanobacteria isolated from metal contaminated soil. J Hazard Mater 148:383–386

Arica MY, Bayramoglu G, Yilmaz M, Genc O, Bektas S (2004) J Hazard Mater B 109:191–199

Ates A, Yildiz A, Yildiz N, Calimli A (2007) Heavy metal removal from aqueous solution by *Pseudevernia furfuracea* (L.) Zopf. Anal Chim 97:385–393

Avery SV, Codd GA, Gadd GM (1993) Biosorption of tributyltin and other organotin compounds by cyanobacteria and microalgae. Appl Microbiol Biotechnol 39:812–817

Barros AJM, Prasad S, Leite VD, Souza AG (2007) Biosorption of heavy metals in upflow sludge columns. Bioresour Technol 98:1418–1425

Bayramoglu G, Arica MY (2007) Biosorption of benzidine based textile dyes “Direct Blue 1 and Direct Red 128” using native and heat-treated biomass of *Trametes versicolor*. J Hazard Mater 143:135–143

Baysal Z, Cinar E, Bulut Y, Alkan H, Dogru M (2009) J Hazard Mater B 161:62–67

Beolchini F, Pagnanelli F, Toro L, Veglio F (2001) Modeling of copper biosorption by *Arthrobacter sp*. in a UF/MF membrane reactor. Environ Sci Technol 35:3048–3054

Beolchini F, Pagnanelli F, Toro L, Veglio F (2006) Ionic strength effect on copper biosorption by *Sphaerotilus natans*: equilibrium study and dynamic modelling in membrane reactor. Water Res 40:144–152

Bingöl A, Aslan A, Çakıcı A (2009) Biosorption of chromate anions from aqueous solution by a cationic surfactant-modified lichen (*Cladonia rangiformis* (L.)). J Hazard Mater 161:747–752

Bishnoi NR, Garima A (2005) Fungus-an alternative for bioremediation of heavy metal containing wastewater: a review. J Sci Ind Res 64:93–100

Blázquez G, Martín-Lara MA, Tenorio G, Calero M (2011) Chem Eng J 168:170–177

Bueno BYM, Torem ML, Molina F, de Mesquita LMS (2008) Biosorption of lead(II), chromium(III) and copper(II) by *R. opacus*: equilibrium and kinetic studies. Min Eng 21:65–75

Calfa BA, Torem ML (2008) On the fundamentals of Cr(III) removal from liquid streams by a bacterial strain. Min Eng 21:48–54

Chen JP, Wang L, Zou S-W (2007) Determination of lead biosorption properties by experimental and modeling simulation study. Chem Eng J 131:209–215

Chettri MK, Cook CM, Vardaka E, Sawidis T, Lanaras T (1998) The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. Environ Exp Bot 39:1–10

Crini G (2006) Non-conventional low-cost adsorbents for dye removal: a review. Bioresour Technol 97:1061–1085

Davis TA, Mucci A, Volesky B (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311–4330 de Rome L, Gadd GM (1987a) Copper adsorption by *Rhizopus arrhizus, Cladosporium resinae* and *Penicillium italicum*. Appl Microbiol Biotechnol 26:84–90

de Rome L, Gadd GM (1987b) Measurement of copper uptake in *Saccharomyces cerevisiae* using a $Cu^{2+}$ selective electrode. FEMS Microbiol Lett 43:283–287

de Rome L, Gadd GM (1991) Use of pelleted and immobilized yeast and fungal biomass for heavy metal and radionuclide recovery. J Ind Microbiol 7:97–104

Dmitriev B, Toukach F, Ehlers S (2005) Towards a comprehensive view of the bacterial cell wall. Trends Biotechnol 13:569–574

Dogan CE, Turhan K, Akcin G, Aslan A (2006) Biosorption of Au(III) and Cu(II) from aqueous solution by a non-living *Cetraria islandica* (L.) Ach. Ann Chim 96:229–236

Ekmekyapar F, Aslan A, Bayhan YK, Cakici A (2006a) Biosorption of copper(II) by nonliving lichen biomass of *Cladonia rangiformis* Hoffm. J Hazard Mater B137:293–298

Ekmekyapar F, Aslan A, Bayhan YK, Cakici A (2006b) Biosorption of copper(II) by nonliving lichen biomass of *Cladonia rangiformis* hoffm. J Hazard Mater 137:293–298

Feng Q, Lin Q, Gong F, Sugita S, Shoya M (2004) Adsorption of lead and mercury by rice husk ash. J Colloid Interface Sci 278:1–8

Gadd GM (1990) Heavy metal accumulation by bacteria and other microorganisms. Cell Mol Life Sci 46:834–840

Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60

Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28

Gadd GM, Griffiths AJ (1980) Effect of copper on morphology of *Aureobasidium pullulans*. Trans Brit Mycol Soc 74:387–392

Gadd GM, Mowll JL (1985) Copper uptake by yeast-like cells, hyphae and chlamydospores of *Aureobasidium pullulans*. Exp Mycol 9:230–240

Gadd GM, White C (1992) Removal of thorium from simulated acid process streams by fungal biomass: potential for thorium desorption and reuse of biomass and desorbent. J Chem Technol Biotechnol 55:39–44

Gao R, Wang J (2007) Effects of pH and temperature on isotherm parameters of chlorophenols biosorption to anaerobic granular sludge. J Hazard Mater 145:398–403

Garnham GW (1997) The use of algae as metal biosorbents. In: Wase J, Forster C (eds) Biosorbents for metal ions. Taylor & Francis, London, pp 11–37

Garnham GW, Codd GA, Gadd GM (1991) Effect of salinity and pH on cobalt biosorption by the estuarine microalgae *Chlorella salina*. Biol Metals 4:151–157

Garnham GW, Codd GA, Gadd GM (1992a) Accumulation of cobalt, zinc and manganese by the estuarine green microalga *Chlorella salina* immobilized in alginate microbeads. Environ Sci Technol 26:1764–1770

Garnham GW, Codd GA, Gadd GM (1992b) Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga, *Chlorella salina*. Appl Microbiol Biotechnol 37:270–276

Garnham GW, Codd GA, Gadd GM (1993a) Accumulation of technetium by cyanobacteria. J Appl Phycol 5:307–315

Garnham GW, Codd GA, Gadd GM (1993b) Uptake of cobalt and caesium by microalgal- and cyanobacterial-clay mixtures. Microb Ecol 25:71–82

Garnham GW, Codd GA, Gadd GM (1993c) Zirconium accumulation by cyanobacteria and microalgae. Appl Microbiol Biotechnol 39:666–672

Gow NAR, Gadd GM (eds) (1995) The growing fungus. Chapman and Hall, London

Gupta R, Mohapatra H (2003) Microbial biomass: an economical alternative for removal of heavy metals from waste water. Indian J Exp Biol 41:945–966

Hai FI, Yamamoto K, Fukushi K (2007) Hybrid treatment systems for dye wastewater. Crit Rev Environ Sci Technol 37:315–377

Hammaini A, Gonzalez F, Ballester A, Blazquez ML, Munoz JA (2007) Biosorption of heavy metals by activated sludge and their desorption characteristics. J Environ Manage 84:419–426

Hamutoglu R, Dinçsoy AB, Cansaran-Duman D, Aras S (2012) Biosorption, adsorption, phytoremediation methods and applications. Turk Hij Den Biyol Derg 69(4):235–253

Hawari AH, Mulligan CN (2006) Biosorption of lead(II), cadmium(II), copper(II) and nickel(II) by anaerobic granular biomass. Bioresour Technol 97:692–700

John Wase DA, Forster CF (1997) Biosorbents for metal ions. CRC, Boca Raton, FL

Kapoor A, Viraraghavan T (1995) Fungal biosorption: an alternative treatment option for heavy metal bearing wastewater—a review. Bioresour Technol 53:195–206

Kaur R, Singh J, Khare R, Ali A (2012) Biosorption the possible alternative to existing conventional technologies for sequestering heavy metal ions from aqueous streams: a review. Univ J Environ Res Technol 2(4):325–335

Kaushik P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Int 35:127–141

Kılıç Z, Atakol O, Aras S, Cansaran-Duman D, Emregul E (2014) Biosorption properties of Zinc (II) from aqueous solutions by *Pseudevernia furfuracea* (L.) Zopf. J Air Waste Manag Assoc 64(10):1112–1121

Kılıç Z, Atakol O, Emregül E, Çelikkol P, Aras S, Cansaran-Duman D (2008) Biosorption characteristics of Zinc(II) from aqueous solutions by *Pseudevernia furfuracea*. 6th International Conference of Chemical Societies of South Eastern European Countries. 10–14 September, Sofia, Bulgaria

Kılıç Z, Atakol O, Aras S, Cansaran-Duman D, Çelikkol P, Emregul E (2013) Evaluation of different isotherm models, kinetic, thermodynamic and copper biosorption efficiency of *Lobaria pulmonaria* (L.) Hoffm. J Air Waste Manag Assoc 64:115–123

Kim BH, Gadd GM (2008) Bacterial physiology and metabolism. Cambridge University Press, Cambridge

Kiran I, Akar T, Ozcan AS, Ozcan A, Tunali S (2006) Biosorption kinetics and isotherm studies of Acid Red 57 by dried *Cephalosporium aphidicola* cells from aqueous solutions. Biochem Eng J 31:197–203

Kiran B, Kaushik A, Kaushik CP (2007) Response surface methodological approach for optimizing removal of Cr(VI) from aqueous solution using immobilized cyanobacterium. Chem Eng J 126:147–153

Krishnani KK, Ayyappan S (2006) Heavy metals remediation of water using plants and lignocellulosic agrowastes. Rev Environ Contam Toxicol 188:59–84

Kuyucak N, Volesky B (1989) Accumulation of gold by algal biosorbent. Biorecovery 1:189–204

Lodeiro P, Herrero R, Sastre de Vicente ME (2006) Thermodynamic and kinetic aspects on the biosorption of cadmium by low cost materials: a review. Environ Chem 3:400–418

Mack C, Wilhelmi B, Duncan JR, Burgess JE (2007) Biosorption of precious metals. Biotechnol Adv 25:264–271

McCallan SEA, Miller LP (1956) Innate toxicity of fungicides. In: Metcalf RL (ed) Advance in pest control research, vol II. Interscience, New York, NY, pp 107–134

Michalak I, Chojnacka K, Witek-Krowiak A (2013) State of the art for the biosorption process: a review. Appl Biochem Biotechnol 170:1389–1416

Mohan SV, Ramanaiah SV, Rajkumar B, Sarma PN (2007) Removal of fluoride from aqueous phase by biosorption onto algal biosorbent

*Spirogyra* sp.-*IO2*: sorption mechanism elucidation. J Hazard Mater 141:465–474

Munoz R, Alvarez MT, Munoz A, Terrazas E, Guieysse B, Mattiasson B (2006) Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. Chemosphere 63: 903–911

Muraleedharan TR, Iyengar L, Venkobachar C (1991) Biosorption: an attractive alternative for metal removal and recovery. Curr Sci 61:379–385

Murphy V, Hughes H, McLoughlin P (2007) Cu(II) binding by dried biomass of red, green and brown macroalgae. Water Res 41:731–740

Nadeema R, Hanif MA, Shaheen F, Perveen S, Zafar MN, Iqbal T (2008) Physical and chemical modification of distillery sludge for Pb(II) biosorption. J Hazard Mater 150:335–342

Nasir MH, Nadeema R, Akhtar K, Hanif MA, Khalid AM (2007) Efficacy of modified distillation sludge of rose (*Rosa centifolia*) petals for lead(II) and zinc(II) removal from aqueous solutions. J Hazard Mater 147:1006–1014

Ngwenya BT (2007) Enhanced adsorption of zinc is associated with aging and lysis of bacterial cells in batch incubations. Chemosphere 67:1982–1992

Niu H, Volesky B (1999) Characteristics of gold biosorption from cyanide solution. J Chem Tech Biotechnol 74:778–784

Niua CH, Volesky B, Cleiman D (2007) Biosorption of arsenic (V) with acid-washed crab shells. Water Res 41:2473–2478

Pagnanelli F, Trifoni M, Beolchini F, Esposito A, Toro L, Veglio F (2001) Equilibrium biosorption studies in single and multi-metal systems. Process Biochem 37:115–124

Pagnanelli F, Esposito A, Veglio F (2002) Multi-metallic modelling for biosorption of binary systems. Water Res 36:4095–4105

Pagnanelli F, Esposito A, Toro L, Veglio F (2003) Metal speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langmuir-type empirical model. Water Res 37:627–633

Pagnanelli F, Veglio F, Toro L (2004a) Modelling of the acid–base properties of natural and synthetic adsorbent materials used for heavy metal removal from aqueous solutions. Chemosphere 54: 905–915

Pagnanelli F, Beolchini F, Di Biase A, Veglio F (2004b) Biosorption of binary heavy metal systems onto *Sphaerotilus natans* cells contained in an UF/MF membrane reactor: dynamic simulations by different Langmuir-type competitive models. Water Res 38: 1055–1061

Pamukoglu Y, Kargi F (2007) Biosorption of copper(II) ions onto powdered waste sludge in a completely mixed fed-batch reactor: estimation of design parameters. Bioresour Technol 98:1155–1162

Park D, Yun YS, Park JM (2010) The past, present, and future trends of biosorption. Biotechnol Bioprocess Eng 15:86–102

Pinaki S, Kazy SK, D'Souza SF (2004) Radionuclide remediation using a bacterial biosorbent. Int Biodeter Biodegrad 54:193–202

Pipiska M, Hornik M, Vrtoch L, Augustin J, Lesny J (2007) Biosorption of $Co^{2+}$ ions by lichen *Hypogymnia physodes* from aqueous solution. Biologia. 62(3):276–282

Pradhan S, Singh S, Rai LC (2007) Characterization of various functional groups present in the capsule of *Microcystis* and study of their role in biosorption of Fe. Ni and Cr Biores Technol 98:595–601

Purvis OW, Williamson BJ, Bartok K, Zoltani N (2000) Bioaccumulation of lead by the lichen *Acarospora smaragdula* from smelter emissions. Res New Phytol 147:591–599

Reddy DHK, Lee S-M, Seshaiah K (2012) Water Air Soil Poll 223:5967–5982

Regine HSF, Volesky VB (2000) Biosorption: a solution to pollution? Int Mıcrobıol 3:17–24

Romera E, González F, Ballester A, Blázquez ML, Muñoz JA (2006) Biosorption with algae: a statistical review. Cri Rev Biotechnol 26:223–235

Romera E, Gonzalez F, Ballester A, Blazquez ML, Munoz JA (2007) Comparative study of biosorption of heavy metals using different types of algae. Bioresour Technol 98:3344–3353

Sağ Y (2001) Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: a review. Separ Purif Method 30:1–48

Sağ Y, Kutsal T (2001) Recent trends in the biosorption of heavy metals: a review. Biotechnol Bioprocess Eng 6:376–385

Sag Y, Kaya A, Kutsal T (1998) The simultaneous biosorption of Cu(II) and Zn on *Rhizopus arrhizus*:application of the adsorption models. Hydrometallurgy 50:297–314

Sari A, Tuzen M, Uluozlu OD, Soylak M (2007) Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass. Biochem Eng J 37:151–158

Sarı A, Tuzen M, Uluozlu OD, Soylak M (2007) Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (Cladonia furcata) biomass. Biochem Eng J 37:151–158

Selatnia A, Madani A, Bakhti MZ, Kertous L, Mansouri Y, Yous R (2004) Minerals Eng 17:903–911

Senthilkumar R, Vijayaraghavan K, Thilakavathi M, Iyer PVR, Velan M (2007) Application of seaweeds for the removal of lead from aqueous solution. Biochem Eng J 33:211–216

Sud D, Mahajan G, Kaur MP (2008) Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions: a review. Bioresour Technol 99:6017–6027

Tobin J, White C, Gadd GM (1994) Fungal accumulation of toxic metals and application to environmental technology. J Ind Microbiol 13:126–130

Tsezos M, Volesky B (1982) The mechanism of uranium biosorption by *R. arrhizus*. Biotechnol Bioeng 24:965–969

Turhan K, Ekinci-Dogan C, Akcin G, Aslan A (2005) Biosorption of Au(III) and Cu(II) from aqueous solution by a non-living *Usnea longissima* biomass. Fresenius Environ Bull 14:1129–1135

Tuzen M, Saygi KO, Usta C, Soylak M (2008) *Pseudomonas aeruginosa* immobilized multiwalled carbon nanotubes as biosorbent for heavy metal ions. Biores Technol 99:1563–1570

Tüzen M, Sarı A, Mendil D, Soylak M (2009) Biosorptive removal of mercury(II) from aqueous solution using lichen (*Xanthoparmelia conspersa*) biomass: Kinetic and equilibrium studies. J Hazard Mater 169:263–270

Uluozlu OD, Sari A, Tuzen M, Soylak M (2008) Biosorption of Pb(II) and Cr(III) from aqueous solution by lichen (Parmelina tiliaceae) biomass. Bioresour Technol 99:2972–3298

Uluözlü OD, Sarı A, Tüzen M (2010) Biosorption of antimony from aqueous solution by lichen (*Physcia tribacia*) biomass. Chem Eng J 163:382–388

Veglio F, Beolchini F, Prisciandaro M (2003) Sorption of copper by olive mill residues. Water Res 37:4895–4903

Vieira RH, Volesky B (2000) Biosorption: a solution to pollution? Int Microbiol 3:17–24

Vijayaraghavan K, Yun Y-S (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291

Vijayaraghavan K, Han MH, Choi SB, Yun Y-S (2007) Biosorption of Reactive black 5 by *Corynebacterium glutamicum* biomass immobilized in alginate and polysulfone matrices. Chemosphere 68:1838–1845

Vilar VJP, Botelho CMS, Boaventura RAR (2008) Copper removal by algae *Gelidium*, agar extraction algal waste and granulated algal waste: kinetics and equilibrium. Bioresour Technol 99:750–762

Volesky B (1990a) Biosorption and biosorbents. In: Volesky B (ed) Biosorption of heavy metals. CRC, Boca Raton, FL, pp 3–5

Volesky B (1990b) Biosorption of heavy metals. CRC, Boca Raton, FL

Volesky B (1994) Advances in biosorption of metals: selection of biomass types. FEMS Microbiol Rev 14:291–302

Volesky B (2003a) Potential of biosorption. In: Volesky B (ed) Sorption and biosorption. BV Sorbex, Inc., Montreal, QC, pp 5–12

Volesky B (2003b) Biosorption process simulation tools. Hydrometallurgy 71:179–190

Volesky B (2004) Sorption and biosorption. BVSorbex Inc., Montreal, QC
Volesky B (2007) Biosorption and me. Water Res 41:4017–4029
Volesky B, Holan ZR (1995a) Biosorption of heavy metals. Biotechnol Progr 11:235–250
Volesky B, Holan ZR (1995b) Biosorption of heavy metals. Biotechnol Prog 11:235–250
Volesky B, Kuyucak N (1988) Biosorbent for gold. US Patent No. 4,769,233
Volesky B, May H, Holan ZR (1993) Cadmium biosorption by *Saccharomyces cerevisiae*. Biotechnol Bioeng 41:826–829
Volesky B, Weber J, Park JM (2003) Continuous flow metal biosorption in a regenerable *Sargassum* column. Water Res 37:297–306
Wan Ngah WS, Hanafiah MA (2008) Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: a review. Bioresour Technol 99:3935–3948
Wang J, Chen C (2006) Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. Biotechnol Adv 24:427–451
Webster EA, Gadd GM (1996) Cadmium replaces calcium in the cell wall of *Ulva lactuca*. Biometals 9:241–244
Webster EA, Murphy AJ, Chudek JA, Gadd GM (1997) Metabolism independent binding of toxic metals by *Ulva lactuca*: cadmium binds to oxygen-containing groups as determined by nuclear magnetic resonance (NMR) spectroscopy. Biometals 10:105–117
Williams RN, Casellas RC, Mangelson NF, Rees LB, Clair LL, Schaalje GB, Swalberg KD (1996) Elemental analysis of lichens from the intermountain Western USA using PIXE. Nucl Instrum Meth B 109(110):336–340
Witek-Krowiak A, Reddy DHK (2013) Bioresour Technol 127:350–357
Witek-Krowiak A, Szafran RG, Modelski S (2011) Removal of microelemental Cr (III) and Cu (II) by using soybean meal waste–unusual isotherms and insights of binding mechanism. Desalination 265: 126–134
Wu J, Yu H-Q. Biosorption of 2,4–dichlorophenol by immobilized white-rot fungus *Phanerochaete chrysosporium* from aqueous solution. Bioresour Technol 98(2):253–259
Yang L, Chen JP (2008) Biosorption of hexavalent chromium onto raw and chemically modified *Sargassum* sp. Bioresour Technol 99:297–307
Yang J, Volesky B (2000) Modeling the uranium–proton ion exchange in biosorption. Environ Sci Technol 33:4049–4058
Zhang Y, Banks C (2006) A comparison of the properties of polyurethane immobilised *Sphagnum* moss, seaweed, sunflower waste and maize for the biosorption of Cu, Pb, Zn and Ni in continuous flow packed columns. Water Res 40:788–798
Zhou JL (1999) Zn biosorption by *Rhizopus arrhizus* and other fungi. Appl Microbiol Biotechnol 51:686–693

# 21 Phytoremediation in Constructed Wetlands

Indika Herath and Meththika Vithanage

## 21.1 Introduction

Contamination of water by toxic pollutants through the discharge of municipal and domestic wastes, industrial wastewater, landfilling sites, etc., has become a worldwide problem, due to its harmful effects on human health and to the fauna and flora of receiving water. The exponential growth of population and civilization, variation in the productivity and consumption habits, increasingly affluent lifestyles and resources use, and rapid development of the industries and technologies have been accompanied by the pollution of water in large extent, during the past several decades. Release of toxic heavy metals, organic pollutants, pesticides, radionuclides, petroleum hydrocarbons, gasoline additives, etc. into the environment has threatened the environment in several aspects causing our planet in great peril. Many industrial processes produce pollutant-rich wastewater that remains as an important environmental issue. Although control technologies have been applied to many industrial and municipal sources, the total quantity of these agents released to the environment remains staggering (Mohan and Pittman Jr 2007). Wastewater treatment is a problem that has plagued man ever since he discovered that discharging wastes into surface waters can lead to many additional environmental problems. Today, a wide range of treatment technologies are available to restore and maintain the chemical, physical, and biological conditions of wastewaters. Conventional technologies used in removal of contaminants from wastewater have been found to be limited, since they often involve high capital and operational cost and may also be associated with the generation of secondary wastes causing treatment problems (Aksu 2002). Membrane filtration is a proven way to remove metal ions, but its high cost limits the use in practice (Amarasinghe and Williams 2007). Because of the drawbacks of conventional technologies in the removal of contaminants from wastewater, a considerable interest has been expressed in the potential use of a variety of natural biological systems to purify water in a controlled manner during the past 20 years. Phytoremediation is a process which uses green plants to remove pollutants from the environment or to render them harmless (Raskin et al. 1994). This is considered as a new and highly promising technology for the remediation of polluted sites due to its competitive performance, cost-effectiveness, and environment friendliness. Phytoremediation technology has been applied to both organic and inorganic pollutants present in soil and water (Salt et al. 1998).

The water purification capability of wetlands is now being recognized as an attractive option in wastewater treatment due to its multi-pollutant treatment capability, low cost, and easy operation. Constructed wetlands (CWs) are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment (Vymazal 2010). Phytoremediation of constructed wetlands has been used to improve the quality of contaminated waters by acting as a sink for various contaminants discharged from sewage, industrial and agricultural wastewaters, landfill leachate, and storm water runoff (Rai 2008; Jomjun et al. 2010; Imfeld et al. 2009; Hoffmann et al. 2011; Vymazal 2005, 2007; Sheoran and Sheoran 2006; Brisson and Chazarenc 2009). However, the technology of constructed wetlands for wastewater treatment has not developed to its maximum, and various problems are still present with regard to its best management and sustainability. This chapter discusses the role of plants in constructed wetlands in order to remediate the wastewaters from various sources, different types of plants used in constructed wetlands, types of CWs, removal of various pollutants, constraints, and future of CWs. The main aim of this chapter is to provide a concise discussion of the constructed wetlands and its phytoremediation aspects as a plant-based cleanup technique for the remediation of wastewater.

I. Herath, M.Sc. • M. Vithanage, Ph.D. (✉)
Chemical and Environmental Systems Modeling Research Group, National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka
e-mail: meththikavithanage@gmail.com

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## 21.2 Phytoremediation: An Emerging Technology to Remediate the Polluted Environment

### 21.2.1 General Aspects of Phytoremediation

Phytoremediation is being recognized as an integrated economically viable technology using green plants for the degradation, removal, and detoxification of chemical pollutants from contaminated soils, sediments, or waters (Clayton 2007). Phytoremediation is considered as a subcategory of bioremediation in which biological organisms, processes, or products are used for environmental detoxification with the help of microbes such as bacteria and fungi. The term phytoremediation is formed from the Greek "phyto" for "plant" and the Latin "remedium" for "to heal again." This technology has garnered increasing scientific and commercial interest as a more environmentally compatible and less expensive method of site remediation relative to engineering-based methods such as excavation, soil washing, or soil incineration (Clayton 2007).

In the past two decades, phytoremediation of wetlands has been a significant technology to remediate wastewaters, since wetland sites act as a sink of various toxic contaminants including heavy metals, radionuclides, pesticides, organic carbon, particulate matter, and nutrients. Constructed wetlands for waste treatment are one existing practice that is increasingly a vital part of phytoremediation (McCutcheon and Schnoor 2003).

There are several processes associated with phytoremediation depending on the contaminant to be treated and site-specific conditions. Based on the physiological action of plants, at least ten different processes have been identified that assist in the management of polluted soil, water, and air (McCutcheon and Schnoor 2003). Major processes include phytoextraction, phytodegradation, phytostimulation, phytostabilization, rhizofiltration, and phytovolatilization (Clayton 2007). Figure 21.1 illustrates the major processes of phytoremediation involved in the treatment of wastes.

*Phytoextraction* is the use of metal-tolerant plants or hyperaccumulators, to acquire elevated levels of inorganic contaminants in their aboveground tissues with subsequent harvest, recovery, and disposal or recycling of the metals. *Phytodegradation* describes the use of plants capable of enzymatic breakdown of organic, or carbon-containing, compounds to simpler and less toxic chemicals either alone or in combination with soil microbes. *Phytostabilization* refers to the use of plants to immobilize contaminants within the soil profile to minimize pollutant escape or biological exposure. *Rhizofiltration* is the use of plant root systems to intercept or degrade waterborne contaminants. *Phytovolatilization* attributes to plants to take up organic contaminant-rich water and release the contaminants into the atmosphere through transpiration (Clayton 2007).

At present, phytoextraction and phytofiltration processes are being best developed for toxic metal phytoremediation nearing commercialization (Raskin et al. 1997). Phytostabilization technology is relatively less developed for treating pollutants compared to the other processes of

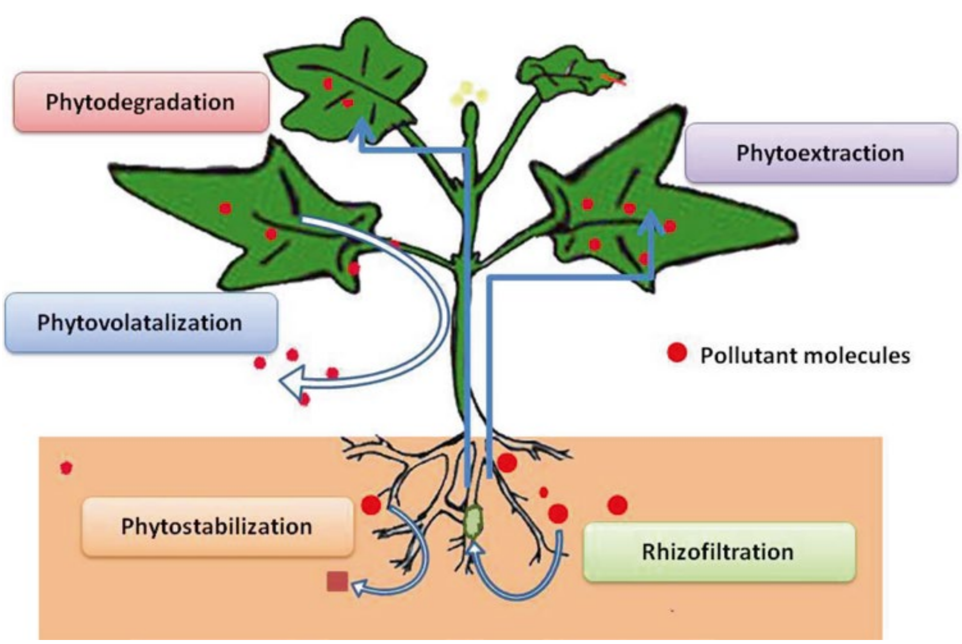


**Fig. 21.1** Major processes of phytoremediation involved in the treatment of wastes

**Table 21.1** Types of phytoremediation and their significance in the removal of contaminants

| Process | Significance | Target toxicants | Media |
|---|---|---|---|
| Phytoextraction | Contaminants taken up, transported, and translocated above ground shoots | Heavy metals radionuclides, perchlorate, BTEX, PCP, and other organic compounds | Soil only |
| Phytodegradation | Plants take up, store, and biochemically degrade or convert harmful contaminants to harmless by-products | Chlorinated solvents, DDT, atrazine, nitriles, TNT, DNT, Cl- and P-based pesticides, anilines, and nitromethane | Soil and Sediment<br>Wetlands<br>Wastewater<br>Surface and groundwater |
| Phytovolatilization | Plants extract volatile metals and organic compounds from soil and volatilize them from foliage | Se, tritium, As, Hg, m-xylene, and chlorinated solvents | Soil and sludges<br>Wetlands<br>Groundwater |
| Rhizofiltration | Plant roots grown in aerated water precipitate and concentrate toxic pollutants | Metals, radionuclides, organic chemicals, nutrients, and pathogens | Wetlands<br>Wastewater<br>Landfill leachates<br>Surface and groundwater |
| Phytostabilization | Plants stabilize the pollutants in soils rendering them harmless and control soil pH, gas, redox causing speciation, precipitation, and sorption. Humification and lignifications of organic compounds | Metals, phenols, and chlorinated solvents | Soil<br>Mine tailings<br>Wetlands<br>Leachate pond sediments |

phytoremediation (Raskin et al. 1997). Phytoextraction is used in large scale, because of its low cost. A rhizofiltration approach has been successfully used to remove radionuclides such as uranium from groundwater on sites at Ashtabula and Oak Ridge and to remove toxic metals including cesium and strontium from a pond near the Chernobyl reactor (Raskin and Ensley 2000). Table 21.1 lists major processes of phytoremediation and their significance in the removal of toxicants from the environment (McCutcheon and Schnoor 2003).

### 21.2.2 Factors Affecting Phytoremediation

The influence of environmental factors on phytoremediation process is of particular concern. The success of phytoremediation depends mainly on a variety of environmental factors including soil structure, texture, and organic matter, water and oxygen availability, temperature, nutrients, solar radiation, and weathering. These factors tend directly to enhance the bioavailability of contaminants and ability of plants to take up, translocate, and accumulate contaminants in shoots and plant–microbe interactions (Hooda 2007).

Soil type is determined by various features such as structure, texture, and organic matter content. Soil structure greatly influences the growth and survivability of plants. A study revealed that the toxic phenanthrene may be trapped and sorbed to the surface of nanopores (soil pores with diameters <100 nm) and hence is not bioavailable (Alexander et al. 1997). Soil texture also affects the bioavailability of contaminants. For example, clay is capable of binding molecules more readily than silt or sand (Brady and Weil 1996). Since soil structure and texture are involved in controlling the bioavailability of contaminants, the selection of a suitable soil type becomes an important factor for the success of a particular phytoremediation mechanism. It has been found that high organic carbon content (>5 %) in soil usually leads to strong adsorption which reduces the availability and a moderate organic carbon content (1–5 %) may lead to limit the availability (Otten et al. 1997) and, hence, soil type may directly affect on the phytoremediation efforts.

Phytoremediation mechanisms take place in maximum rate at a particular temperature. In general, the rate of microbial degradation or transformation doubles for every 10 °C increase in temperature (Yu et al. 2007). In an experiment, pre-rooted weeping willows (*Salix babylonica* L.) were used to study uptake and metabolism of cyanide in response to change in temperature (Yu et al. 2007). The results revealed that the rate of cyanide metabolism for weeping willows was found at 32 °C with a value of 2.78 mg CN/(kg · d), whereas the lowest value was 1.20 mg CN/(kg · d) at 11 °C. In conclusion, changes in temperature have considerably influenced on the uptake and metabolism of cyanide by plants.

Nutrients are considered as an essential component for the growth of plants and their associated microorganisms when the plants are growing under high stress conditions from toxic contaminants. The effect of fertilizers has been significant on phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum-contaminated wetlands (Lin and Mendelssohn 1998). The oil degradation rate in the soil is significantly enhanced by the application of fertilizer in conjunction with the presence of transplants. This vegetative transplantation can simultaneously restore oil-contaminated wetlands and accelerate oil degradation in the soil, when it is implemented with fertilization (Lin and Mendelssohn 1998).

The effect of solar radiation on the phytoremediation process also is of particular concern. Photomodifications of PAHs by ultraviolet light can occur in contaminated water or on the surface of soil, increasing the polarity, water solubility, and toxicity of the contaminants prior to uptake by the plant (McConkey et al. 1997; Huang et al. 1997; Ren et al. 1994). Researchers have investigated that PAHs which can be modified in this manner include anthracene, phenanthrene, fluoranthene, pyrene, and naphthalene. Enhanced toxic effects such as reduction of growth also can result from penetration of ultraviolet radiation into plant tissue, followed by photomodifications and photosensitizations of PAHs accumulated within these tissues (Duxbury et al. 1997).

Weathering processes that involve in phytoremediation technology are volatilization, evapotranspiration, photo-modification, hydrolysis, leaching, and biotransformation of the contaminant. These processes can selectively reduce the concentration of easily degradable contaminants with the more recalcitrant compounds remaining in the soil. The contaminants left behind are typically nonvolatile or semi-volatile compounds that preferentially partition to soil organic matter or clay particles, which limits their bioavailability and the degree of degradation (Cunningham and Ow 1996). It is clearly documented that the contaminant bioavailability is a major factor limiting the degradation of weathered (>60 years) PAHs (Carmichael and Pfaender 1997).

## 21.3 Constructed Wetlands

### 21.3.1 Introduction to Constructed Wetlands

Wetlands are highly diverse and specific ecosystems playing a vital role in the environment. They are identified as areas covered by water or that have waterlogged soils for significant periods during the growing season (Mejáre and Bülow 2001). Wetlands can be defined as land transitional between terrestrial and aquatic systems where the water table is usually at or near the surface of the land or the land is covered by shallow water (Mejáre and Bülow 2001). Ramsar convention has proposed that wetlands are areas of marsh, fen, peatland, or water, whether natural or artificial or permanent or temporary, with water that is static or flowing, fresh, brackish, or salty, including areas of marine water, the depth of which at low tide does not exceed 6 m (Mejáre and Bülow 2001). These definitions emphasized the ecological significance of wetlands.

Wetlands are often rich in a variety of resources that are highly valuable for different habitats including a wide variety of plant and animal life such as water birds, fish, shellfish, and other aquatic organisms. Coastal wetlands act as an ecotone between the sea and freshwater and/or freshwater and terrestrial habitats showing high species diversity. The ecological function of wetlands also is significant due to great performance to regulate water regime, act as natural filters, and display amazing nutrient dynamics (Mulligan et al. 2001).

Wetlands are found to be present as natural wetlands and constructed wetlands. Natural wetlands usually purify and improve the quality of water passing through the system acting as ecosystem filters (Cheng et al. 2002). Constructed wetlands are artificially engineered systems that are used to control or remove hazardous wastes from polluted waters under a more controlled environment. They are designed to take advantage of many of the same processes that occur in natural wetlands (Vymazal 2010), and the natural wetlands have been recognized to be a good volunteer to improve the quality of water over a million of years. Both natural and constructed wetlands are considered as a cost-effective and an alternative technology for wastewater treatment.

Wetlands are characterized by several factors including the presence of water, nature of soil, and the presence of vegetation (Cheng et al. 2002). The system around constructed wetlands is a pool of unlimited number of toxins and pollutants discharged from industrial, municipal, and domestic waste effluents. Therefore, the natural or constructed wetlands are best reserved for two purposes including polishing of already partially treated (oxidized) industrial or domestic waste and removal of specific pollutants, such as nitrogen, phosphorus, copper, lead, selenium, organic compounds, pesticides, viruses, or protozoan cysts from all wastes including agricultural and urban storm runoff (Banuelos and Terry 1999). Wetland plants facilitate major mechanisms in order to remove such contaminants, and hence, the use of wetlands for phytoremediation has been developed as a cost-effective and environmentally friendly remediation of contaminated water.

### 21.3.2 Hydrology of Constructed Wetlands

The ecological function of a constructed wetland depends on the hydrological factors including nutrient availability and physicochemical parameters such as soil, water pH, and soil within anaerobiosis (Scholz and Lee 2005). In constructed wetlands, all these factors are under controlled conditions to maintain the efficiency of biotic processes occurring within them. Water is the key of wetland ecosystems and the water budget of these systems directly affects the biological processors resulting in great seasonal variations. The hydrology defines the species diversity, productivity, and nutrient cycling of specific wetlands, and that is very important for the richness of flora and fauna or utilizing wetlands for pollution control (Scholz and Lee 2005).

The stability of particular constructed wetlands is directly linked with their hydroperiod. Hydroperiod of a wetland is the seasonal shift in surface and subsurface water levels. The hydrologic cycle is the main hydrologic process that is occurring in any wetland system. Major components of the hydrologic cycle include precipitation, surface water flow, groundwater flow, and evapotranspiration (ET). The water budget of a constructed wetland is the total of inflows and outflows of water within the system. A simple water budget of wetlands provides suitable tool for wastewater treatment in order to ensure their long-term sustainability (Hedges et al. 2008). The water balance is important for determining conformance with desired limits for hydraulic loading rate (HLR), hydraulic residence time (HRT), and mass balances. The hydraulic residence time (HRT) of a constructed wetland is the average time that water remains in the wetland. Hydraulic loading rate (HLR) is defined as the loading on a water volume per unit area basis. The components of a water budget are shown in the following equation (Scholz and Lee 2005):

$$\Delta V / \Delta t = P_{\mathrm{n}} + S_{\mathrm{i}} + G_{\mathrm{i}} - \mathrm{ET} - S_{\mathrm{o}} - G_{\mathrm{o}} \pm T$$

where $\Delta V$ = volume of water storage in a wetland, $\Delta V/\Delta t$ = change in volume of water storage in wetland per unit time ($t$), $P_{\mathrm{n}}$ = net precipitation, $S_{\mathrm{i}}$ = surface inflows including flooded streams, $G_{\mathrm{i}}$ = groundwater inflows, ET = evapotranspiration, $S_{\mathrm{o}}$ = surface outflows, $G_{\mathrm{o}}$ = groundwater outflows, and $T$ = tidal inflow (+) or outflow (−).

Precipitation is the main process that provides water directly or indirectly for natural wetlands as well as constructed wetlands. Precipitation is any form of water, such as rain, snow, sleet, hail, or mist that falls from the atmosphere and reaches the ground. The loss of water to the atmosphere is an important component of wetland water budget. Water is removed by evaporation from soil or surfaces of water bodies and by transpiration by plants. The combined loss of water by evaporation and transpiration is termed evapotranspiration (ET). Solar radiation, wind speed and turbulence, relative humidity, available soil moisture, and vegetation type and density affect the rate of ET. Surface water is supplied to wetlands through normal stream flow, flooding from lakes and rivers, overland flow, groundwater discharge, and tides. Groundwater originates as precipitation or as seepage from surface water bodies. The movement of water between a wetland and groundwater often affects the hydrology of the wetland.

### 21.3.3 Chemistry of Constructed Wetlands

Wetland chemistry is strongly influenced by the physicochemical variables that interacted with some elements such as oxygen, nitrogen, sulfur, phosphorus, iron, and aluminum. Physicochemical parameters include dissolved oxygen, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved organic sulfur (DOS), total Fe and Al concentration, and phosphorus concentration. The concentration of oxygen within sediments and the overlaying water is a critical factor. The lack of oxygen or limited oxygen conditions affect the aerobic respiration of plant roots and plant nutrient availability (Scholz and Lee 2005). Wetland plants can exist in anaerobic soils showing great adaptations for their survival.

The nutrient availability and toxicity of a wetland is determined by the state of oxidation and reduction of ions such as iron, manganese, nitrogen, and phosphorus that are present within waterlogged soil and sediments in wetlands. The decomposition (oxidation) of organic matter takes place in the presence of any electron acceptors including $O_2$, $NO_3^-$, $Mn^{2+}$, $Fe^{3+}$, and $SO_4^{2-}$, but the oxidation in the presence of oxygen is fast compared to other ions (Scholz and Lee 2005). A redox potential range between +400 and +700 mV is typical for environmental conditions associated with free dissolved oxygen. Below +400 mV, the oxygen concentration will begin to diminish and wetland conditions might become increasingly more reduced (>−400 mV) (Bradley 2001; Scholz and Lee 2005). In some wetlands, sulfur cycle is also involved in the degradation of organic matter (Bradley 2001). Low-molecular-weight organic compounds that result from fermentation (e.g., ethanol) are utilized as organic substrates by sulfur-reducing bacteria in the conversion of sulfate to sulfide (Bradley 2001).

Phosphorus that is within wetland soils exists in different forms as soluble or insoluble, organic or inorganic complexes. The physical, chemical, and biological characteristics of a wetland system depend on the solubility and reactivity of different forms of phosphorus. Phosphate solubility is regulated by temperature, pH, redox potential, interstitial soluble phosphorus level, and microbial activity (Scholz and Lee 2005). The phosphorus cycle is sedimentary rather than gaseous and predominantly forms complexes within organic matter in peatlands or inorganic sediments in mineral soil wetlands. It has been investigated that over 90 % of the phosphorus load in streams and rivers may be present in particulate inorganic form (Scholz and Lee 2005).

Biologically available orthophosphate is the soluble and reactive form of phosphorus which exits in primary inorganic form. The availability of phosphorus to plants and microconsumers is limited due to several effects. Under aerobic conditions, insoluble phosphates are precipitated with ferric iron, calcium, and aluminum limiting the availability of phosphorus to plants. Phosphates can also be adsorbed onto clay particles, organic peat, and ferric/aluminum hydroxides and oxides and bound up in organic matter through incorporation in bacteria, algae, and vascular macrophytes limiting the bioavailability (Scholz and Lee 2005).

Nitrogen within various oxidation states is also important to the biogeochemistry of wetlands. Its various oxidation states are capable of carrying out the oxidation of organic matter. The anoxic conditions in wetland environments lead the release of gaseous nitrogen from the lithosphere and hydrosphere to the atmosphere through denitrification (Bradley 2001). In flooded wetland soils, mineralized nitrogen exists in the form of ammonium ($NH_4^+$) that is formed through the process called ammonification. During this process, organically bound nitrogen is converted to ammonium nitrogen under aerobic or anaerobic conditions. Soil-bound ammonium can be taken up by plant root systems and reconverted to organic matter (Scholz and Lee 2005).

Sulfur and its various oxidation states also are critical in controlling the conditions of certain wetland systems. In wetlands, sulfur is transformed by microbiological processes and occurs in several oxidation stages. Reduction may occur between −100 and −200 mV on the redox potential scale (Bradley 2001). Sulfides provide the characteristic "bad egg" odor of some wetland soils. Assimilatory sulfate reduction is accomplished by obligate anaerobes such as *Desulfovibrio* spp. Bacteria may use sulfates as terminal electron acceptors in anaerobic respiration at a wide pH range but highest around neutral (Bradley 2001).

### 21.3.4 Plants in Constructed Wetlands

Plants that are morphologically tolerant to grow in wet soil under sufficient or insufficient water conditions are referred to as wetland plants or macrophytes. The vegetation within macrophytes increases the aesthetics of the site and enhances the landscape creating significant wildlife habitat for a variety of animals such as songbirds, insects, amphibians, waterfowl, etc. Most wetland plant communities consist of a highly diverse mix of grasses, sedges, forbs (broadleaf plants), ferns, shrubs, and trees. Wetland plants can be classified into general categories which include emergent, submerged, and floating plants based on their adaptations to life in water.

#### 21.3.4.1 Emergent Plants

Emergent wetland plants are rooted in soil with basal portions that typically grow beneath the surface of the water, but whose leaves, stems (photosynthetic parts), and reproductive organs are aerial (Fig. 21.2). Examples of emergent plants include cattail and rush species such as *Phragmites australis*, *Phalaris arundinacea*, *Typha domingensis*, *Typha latifolia*, *Phragmites karka*, *Juncus pallidus*, *Empodisma minus*, etc.

Emergent plants like cattails and rushes are adapted by developing more efficient cell structures with spaces between

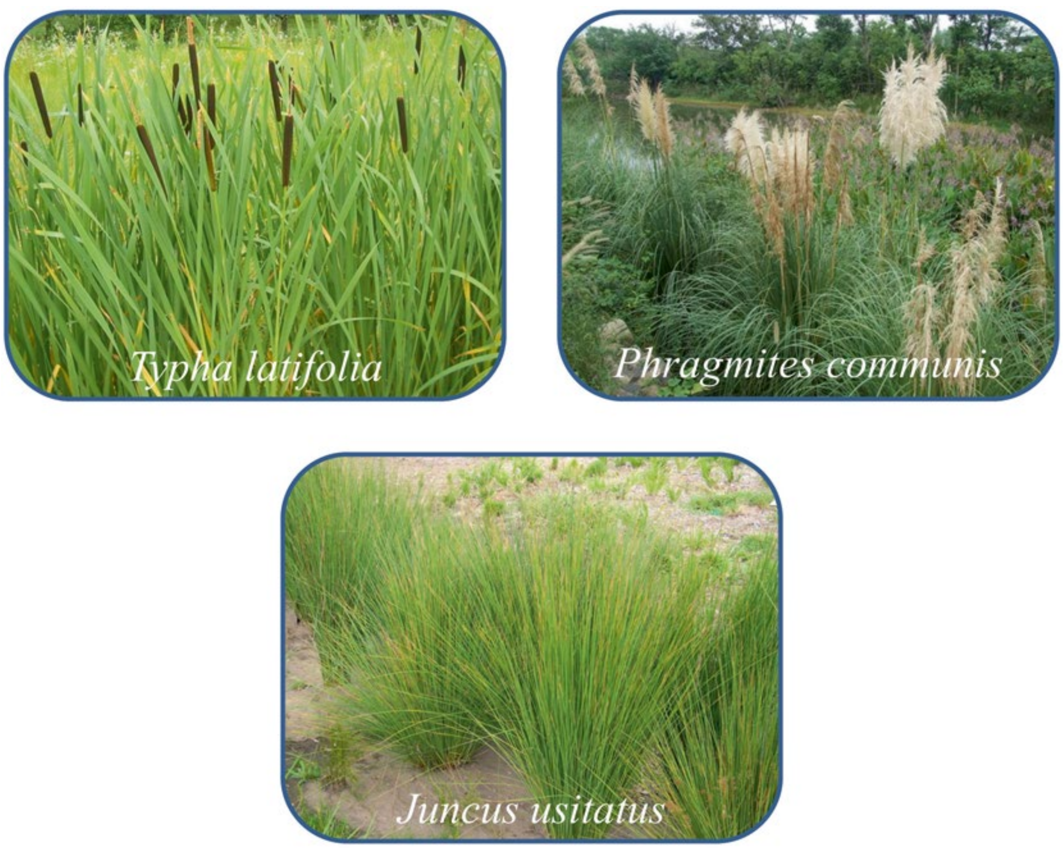


**Fig. 21.2** Emerged wetland plant species

cells to collect carbon dioxide. Photosynthesis moves carbon dioxide to the roots where it combines with other nutrients to produce root tissue, ethylene, and more aerenchyma tissues. Aerenchyma tissues contain large empty spaces that store oxygen and move it to different parts of the plant.

#### 21.3.4.2 Submerged Plants

Submerged plants spend their entire life cycle beneath the surface of the water and nearly all are rooted in the substrate (Fig. 21.3). Submerged plants can take up dissolved oxygen and carbon dioxide from the water column. Examples of submerged plants include *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Hydrilla verticillata*, *Heteranthera dubia*, etc.

Submerged plants have the greatest number of adaptations to life in water. They have little or no mechanical strengthening tissue in stems and leaf petioles, and thus, when these plants are removed from the water, they hang limply. Submerged plants lack the external protective tissues, and the entire surface cells appear to be able to absorb water, nutrients, and dissolved gases directly from the surrounding water. Roots can easily absorb nutrients and water from the substrate and their main function is anchorage. The leaves of submerged plants are often highly dissected or divided having the advantage of creating a very large surface area for absorption and photosynthesis. It also minimizes water resistance and hence potential damage to the leaves.

#### 21.3.4.3 Floating Plants

Plants whose leaves mainly float on the water surface while the roots are anchored in the substrate are known as floating plants. Much of the plant body is underwater and may or may not be rooted in the substrate (Fig. 21.4). Only small portions, namely, flowers, rise above water level. Stems connect the leaves, which are circular or oval and have a tough leathery texture to the bottom. Floating plants include free-floating and floating-leaved plants such as *Eichhornia*

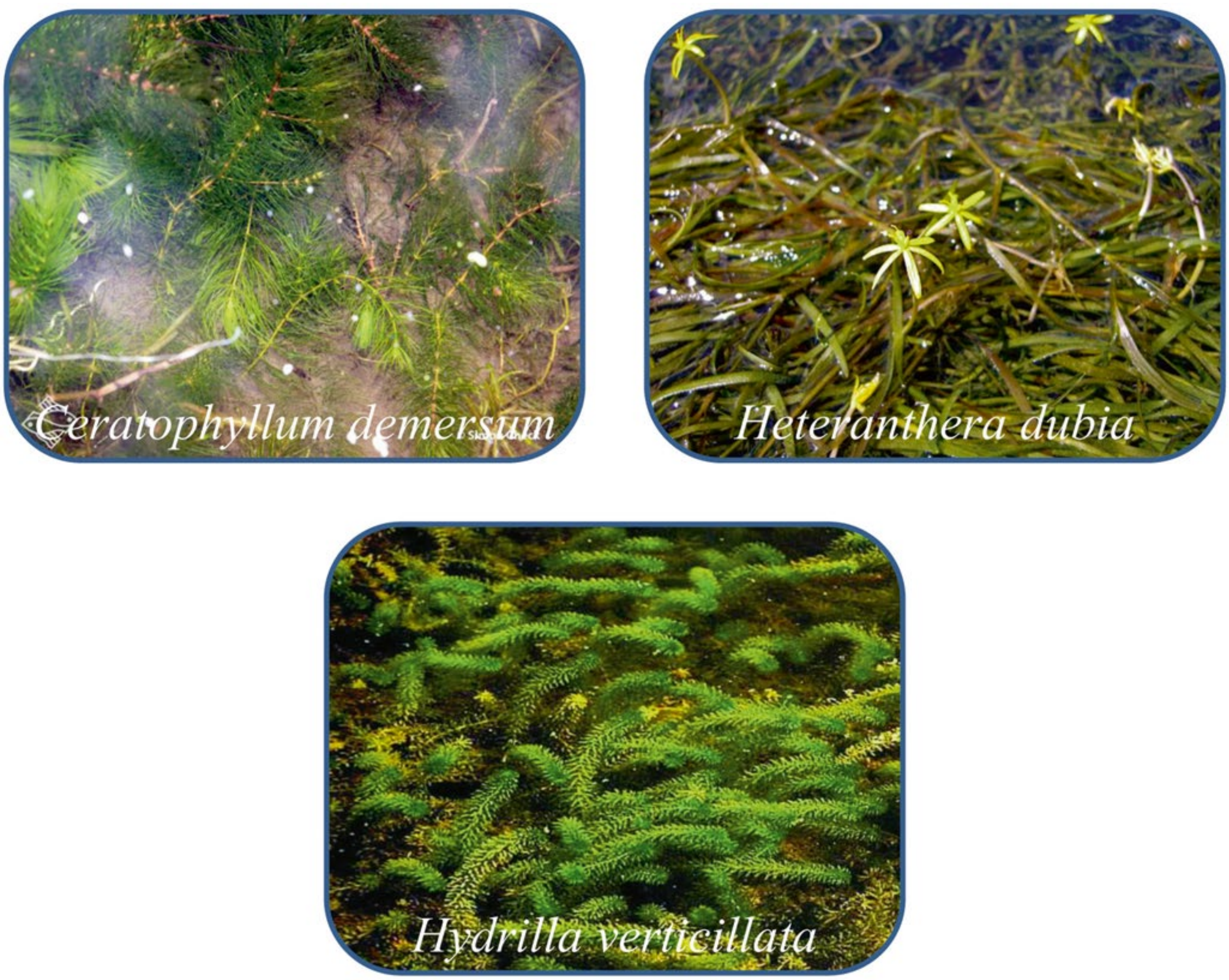


**Fig. 21.3** Submerged wetland plant species


**Fig. 21.4** Floating wetland plant species

*crassipes*, *Pistia stratiotes*, *Salvinia herzogii*, *Wolffia columbiana*, *Lemna valdiviana*, *Nymphaea* spp., *Nuphar advena*, *Juncus effusus*, etc.

Floating plants have structural adaptations that prevent them from sinking in water. By staying afloat, they are able to absorb maximum sunlight and can easily exchange gases with the atmosphere. Floating plants may heavily shade the water below, reducing the number of submersed species that compete with them for nutrients.

#### 21.3.4.4 Role of Plants in Constructed Wetlands

Wetland plants play a vital role in the removal and retention of pollutants in wastewater. They bring necessary physical effects for the treatment of wastewater. The plants are capable of stabilizing the surface of the beds which provide good conditions for physical filtration and a huge surface area for the attachment and growth of microbes, prevent vertical-flow systems from clogging, and insulate against frost during winter (Brix 1994). The selection of nice looking wetland plants like water lily, yellow flag, etc., makes constructed wetlands aesthetically pleasing while they are playing a significant role in water purification.

Aquatic plants should fulfill certain requirements to be selected for the use of constructed wetlands. The ecological adaptability of plants is very important to make sure that there is no any disease or weed risk to the surrounding natural ecosystems (Williams 2002). The capability of macrophytes to tolerate local conditions such as climate, pests, disease, and hypertrophic waterlogged conditions enables them to survive in great extent. High pollutant removal capacity is significant in order to achieve the goals of the technology. In addition, rapid propagation, establishment, spread, and growth also have to be taken into consideration when considering the suitability of different plant species in constructed wetlands (Williams 2002).

Wetland plants can take up nutrients through their well-developed root systems and accumulate significant amount of nutrients in the biomass (Brix 1994). Eutrophication of wetlands is mainly prevented by macrophytes due to their capabilities to tolerate and remove high concentrations of nutrients. The common reed (*Phragmites karka*) and cattail (*Typha angustifolia*) have a large biomass both above and below the surface of the substrate, and hence, they can take up and accumulate a large amount of nutrients from nutrient-rich wastewater. The uptake capacity of nutrients in emergent macrophytes is in the range of 50–150 kg P $ha^{-1}$ and 1,000–25,000 kg N $ha^{-1}$ $year^{-1}$ (Brix 1994).

Aquatic plants are able to release oxygen from their roots into the rhizosphere, which is very important in subsurface-flow constructed wetlands for aerobic degradation of oxygen-consuming substances and nitrification (Brix 1994). The presence of some hollow vessels in plant tissues provides oxygen to move from leaves to the root zone and to the surrounding soil facilitating the active microbial aerobic decomposition process and the uptake of pollutants from the water system (Brix 1994). Macrophytes have adaptations with suberized cell walls, and lignified layers in the hypodermis and outer cortex are capable of conserving internal oxygen minimizing the rate of oxygen leakage (Brix 1994).

## 21.4 Constructed Wetland Phytoremediation Attributes

### 21.4.1 Overview

Natural wetlands are not very much efficient for removal of pollutants from wastewater, since water often short-circuits through natural wetlands, giving little time for treatment (Terry and Bañuelos 1999). The first experiments using wetland macrophytes for wastewater treatment were carried out in Germany in the early 1950s (Vymazal 2010). Constructed wetlands have now been designed to increase the efficiency of phytoremediation process, targeting a specific pollutant or group of pollutants. The most important difference between constructed and natural wetlands is the isolation of the water regime from natural patterns (Terry and Bañuelos 1999). Physicochemical properties of wetlands provide many positive attributes for remediating contaminants.

Constructed wetlands are considered to be complex ecosystems due to variable conditions of hydrology, soil and sediment types, plant species diversity, growing season, and water chemistry. Constructed wetlands are being particularly designed to remove a wide variety of pollutants including bacteria, enteric viruses, suspended solids, nutrients (ammonia, nitrate, phosphate), metals and metalloids, volatile organic compounds (VOC), pesticides and other organohalogens, TNT and other explosives, and petroleum hydrocarbons and additives. These pollutants should be specific and manageable for the success of phytoremediation (Terry and Bañuelos 1999). Figure 21.5 summarizes the process of phytoremediation using constructed wetlands for the removal of pollutants from wastewater.

The vegetation of constructed wetlands is considered as a massive biofilm (Brix 1994). Different environmental parameters including wind velocity, light intensity, and insulation of snow are controlled within this biofilm. Macrophytes provide a large surface area for the growth of microbial biofilms that are responsible for important microbial processors including nitrogen reduction and decomposition of organic compounds. Indeed the vegetation is the heart of a wetland, since it plays a crucial role in the function of wastewater treatment.

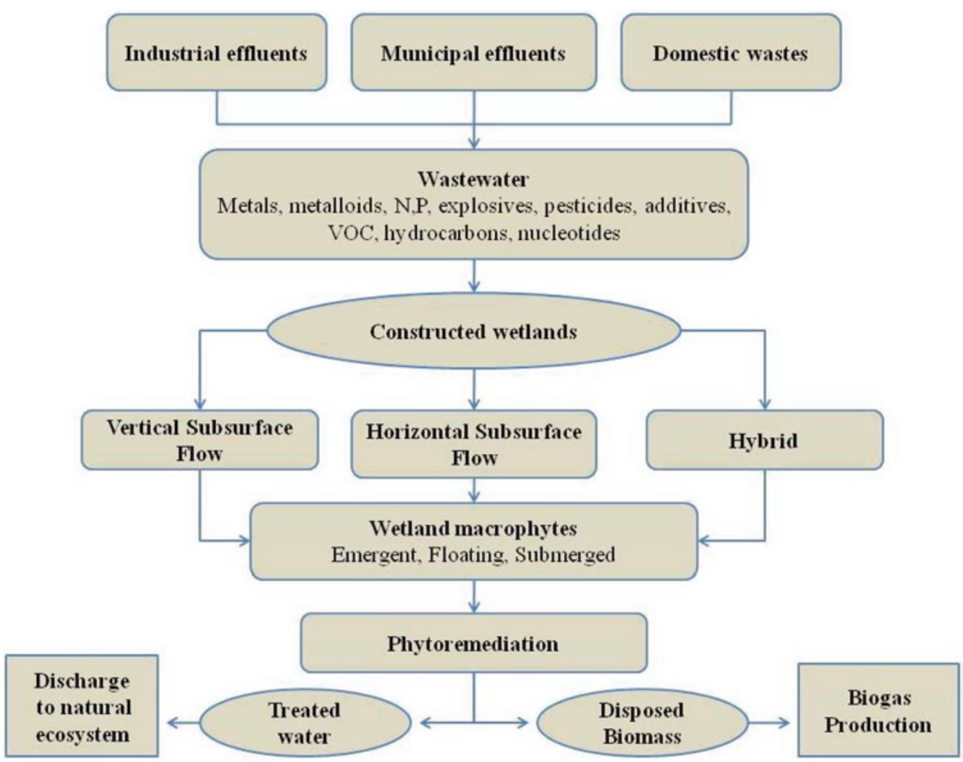


**Fig. 21.5** The big picture of phytoremediation using constructed wetlands

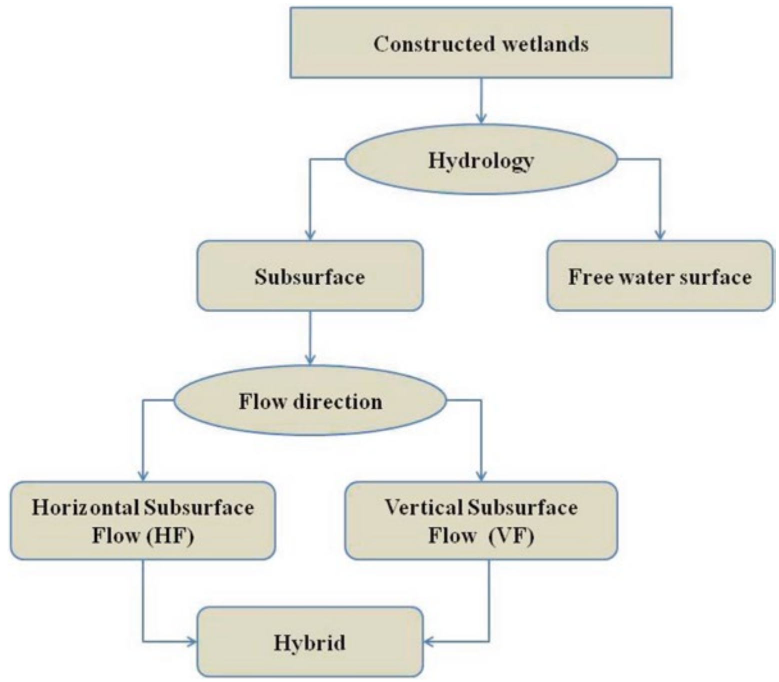


**Fig. 21.6** General classification of constructed wetland treatment systems

## 21.4.2 Types of Constructed Wetland Treatment Systems

### 21.4.2.1 General Classification

The classification of constructed wetlands (CWs) is based on vegetation type (emergent, submerged, floating leaved, free floating), hydrology, and flow direction (Vymazal 2010). According to the wetland hydrology, CWs are classified into two divisions, free-water-surface and subsurface system, whereas the subsurface-flow CWs are further classified into two general types based on the flow direction, horizontal subsurface flow (HF) and vertical subsurface flow (VF) (Vymazal 2010). Various types of constructed wetlands are now being combined into hybrid systems, in order to achieve better treatment performance for removal of pollutants. Figure 21.6 illustrates the general classification of constructed wetland treatment systems.

Most of the systems are designed with horizontal subsurface flow (HF CWs), but vertical-flow (VF CWs) systems are getting more popular at present. Constructed wetlands with free water surface (FWS CWs) are not used as much as the HF or VF systems despite being one of the oldest designs in Europe (Vymazal 2005).

### 21.4.2.2 Free-Water-Surface Constructed Wetlands (FWS CWs)

A typical FWS CW with emergent macrophytes is a shallow sealed basin or sequence of basins (Fig. 21.7), containing 20–30 cm of rooting soil, with a water depth of 20–40 cm (Vymazal 2010). Dense emergent vegetation covers a significant fraction (more than 50 %). Both planted and naturally occurring macrophytes may be present in these types of CWs. Plants are usually not harvested and the litter provides organic carbon for denitrification which may proceed in anaerobic pockets within the litter layer (Vymazal 2010). The free-water-surface wetland technology started in North America with the ecological engineering for wastewater treatment at the end of the 1960s and beginning of the 1970s. It has been reported that the IJssel Lake Polder Authority in Flevoland in the Netherlands constructed its first free-water-surface constructed wetland in 1967 (Kadlec and Wallace 2008).

The primary role of macrophytes is providing structure for enhancing flocculation, sedimentation, and filtration of suspended solids through hydrodynamic conditions, and plants provide essential solid surfaces for microbial activities to remove organic matter. The most commonly used species in Europe for FWS CWs are *Phragmites australis* (common reed) and *Scirpus* (*Schoenoplectus*) *lacustris*; in North America, *Typha* spp. (cattail), *Scirpus* spp. (bulrush), and *Sagittaria latifolia* (arrowhead); and in Australia and New Zealand, *Phragmites australis*, *Bolboschoenus* (*Scirpus*) *fluviatilis* (marsh club rush), *Eleocharis sphacelata* (tall spike rush), and *Scirpus tabernaemontani* (soft-stem bulrush). These plants may not work well in the tropics. FWS CWs are efficient in removal of organics through microbial degradation and settling of colloidal particles. Suspended solids are effectively removed via settling and filtration through the dense vegetation. Nitrogen is removed primarily through nitrification (in water column) and subsequent denitrification (in the litter layer) and ammonia volatilization

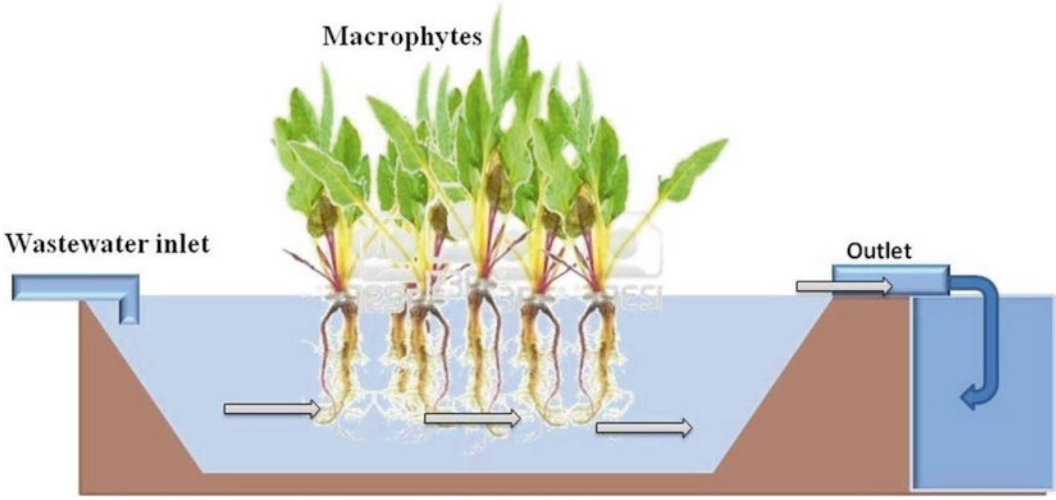


**Fig. 21.7** Layout of a free-water-surface constructed wetland

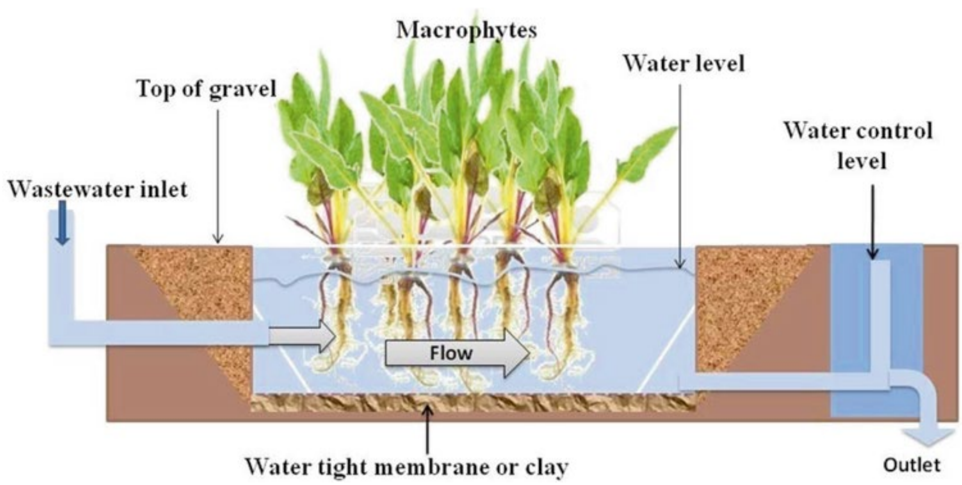


**Fig. 21.8** Layout of a horizontal subsurface-flow constructed wetland system

under higher pH values caused by algal photosynthesis. In this type of CWs, phosphorus retention is usually low because of limited contact of water with soil particles which adsorb and/or precipitate phosphorus. Plant uptake represents only temporal storage because the nutrients are released to water after the plant decay.

### 21.4.2.3 Horizontal Subsurface-Flow Constructed Wetlands (HF CWs)

A horizontal subsurface-flow constructed wetland (HF CW) is a large gravel- and sand-filled channel that is planted with aquatic vegetation (Fig. 21.8). Wastewater flows horizontally through the channel. The filter material filters out particles that are present in wastewater and microorganisms degrade organic materials. The water level in a HF CW wetland is maintained at 5–15 cm below the surface to ensure subsurface flow (Hoffmann et al. 2011). The flow path of the water is maximized due to the bed being often wide and shallow. A wide inlet zone is used to evenly distribute the flow. Pretreatment is essential to prevent clogging and ensure efficient treatment.

The removal efficiency of the constructed wetland depends on the surface area and the cross-sectional area which determines the maximum possible flow. A well-designed inlet that allows for even distribution is important to prevent short-circuiting and preferential pathways. The outlet should be variable so that the water surface can be adjusted to optimize treatment performance.

The filter media acts as a filter for removing solids as well as a base for the vegetation. Facultative and anaerobic bacteria within the system are capable of degrading most organic materials. The vegetation tends to transfer a small amount of

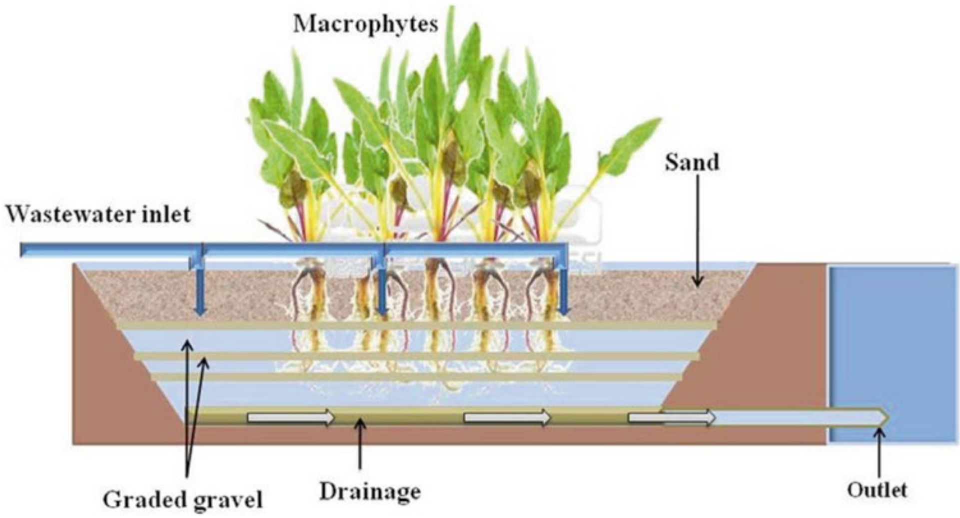


**Fig. 21.9** Layout of a vertical subsurface-flow constructed wetland system

oxygen to the root zone so that aerobic bacteria can colonize the area and degrade organics as well. The plant roots play an important role in maintaining the permeability of the filter. *Phragmites australis* (reed) is supposed to be a good choice for HF CWs, since it forms horizontal rhizomes that penetrate the entire filter depth (Vymazal 2007). In these systems, the major removal mechanism for nitrogen is denitrification, and the removal of ammonia is limited due to lack of oxygen in the filtration bed (Vymazal 2007). Phosphorus is removed primarily by ligand exchange reactions, where phosphate displaces water or hydroxyls from the surface of iron and aluminum hydrous oxides (Vymazal 2007).

#### 21.4.2.4 A Vertical Subsurface-Flow Constructed Wetlands (VF CW)

A vertical subsurface-flow constructed wetland (VF CW) is a planted filter bed for secondary or tertiary treatment of wastewater (Hoffmann et al. 2011). Pretreated wastewater is distributed over the whole filter surface and flows vertically through the filter. The water is treated by a combination of biological and physical processes. There is a drainage system on the bottom of the filter to collect the treated wastewater (Fig. 21.9). Sand and gravel are used to construct the filter body. The filtered water of a well-functioning constructed wetland can be used directly for irrigation, aquaculture, and groundwater recharge or is discharged in surface water.

In vertical filter beds, wastewater is supplied either by pump or self-acting siphon device onto the surface and then drains vertically down through the filter layers toward a drainage system at the bottom. The treatment process is characterized by intermittent short-term loading intervals (4–12 doses per day) and long resting periods during which the wastewater percolates through the unsaturated substrate and the surface dries out (Hoffmann et al. 2011). The intermittent batch loading enhances the oxygen transfer and leads to high aerobic degradation activities, so that VF CWs are more aerobic than HF CWs providing suitable conditions for nitrification. On the other hand, VF CWs do not provide any denitrification (Vymazal 2007). Vertical filters always need pumps or at least siphon pulse loading, whereas horizontal-flow constructed wetlands can be operated without pumps.

The treatment process of VF CWs is based on a number of biological and physical processes such as adsorption, precipitation, filtration, nitrification, predation, decomposition, etc. (Hijosa-Valsero et al. 2010), and hence, VF CWs are capable of removing organics and suspended solids effectively rather than HF CWs. Moreover, VF CW systems require less land compared to HF CWs that is usually about 1–3 $m^2$ $PE^{-1}$ (Vymazal 2010).

#### 21.4.2.5 Hybrid Constructed Wetlands

Hybrid constructed wetlands are highly engineered systems that are designed combining different types of constructed wetlands in order to achieve a higher treatment efficiency (Vymazal 2010). Hybrid systems are comprised most frequently of vertical-flow and horizontal-flow constructed wetlands arranged in a staged manner (Vymazal 2005), and they are now being used in many countries around the world. Hybrid systems are used particularly, when removal of ammonia-N and total N is required (Vymazal 2010). Horizontal-flow systems cannot provide nitrification because of their limited oxygen transfer capacity. Vertical-flow systems can provide good conditions for nitrification, but denitrification does not really occur in these systems. Therefore, in hybrid systems, the advantages of the horizontal- and vertical-flow systems can be combined to complement processes in each system to produce an effluent low in BOD, which is fully nitrified and partly denitrified and hence has much lower total N outflow concentrations (Vymazal 2005).

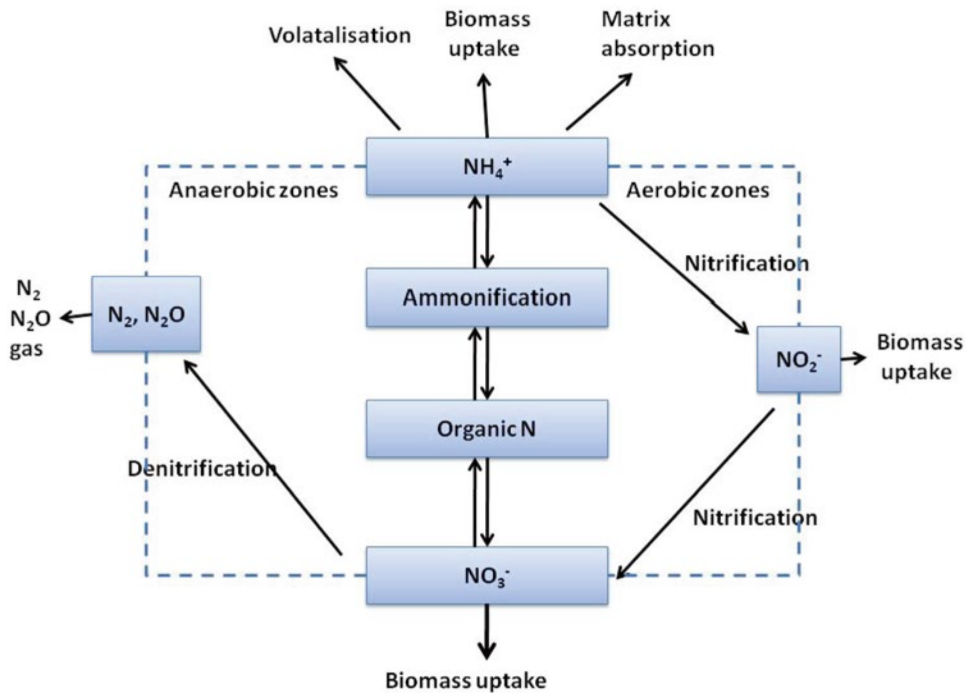


**Fig. 21.10** Nitrogen transformations in a constructed wetland system

### 21.4.3 Removal of Pollutants Through Constructed Wetland Phytoremediation

#### 21.4.3.1 Nutrient Removal

Nitrogen and phosphorus are the main pollutants present at high concentrations in effluents of sewage, agriculture, and urban storm runoff. Eutrophication of lakes, rivers, estuaries, and coastal oceans is mainly due to the presence of excess nutrients, and hence, there is considerable requirement to control and remove such nutrients from wastewater. Constructed wetlands are now being recognized as an economically viable wastewater treatment option for the removal of nitrogen and phosphorus in wastewater.

**Nitrogen Removal**

Constructed wetlands are widely being used for treating nitrogen-rich wastewaters, since they provide an attractive and economical alternative for denitrifying high-quality nitrified wastewater. The processes that are involved in the removal and retention of nitrogen during wastewater treatment in constructed wetlands include $NH_4$ volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake, mineralization (ammonification), nitrate reduction to ammonium (nitrate ammonification), anaerobic ammonia oxidation (ANAMMOX), fragmentation, sorption, desorption, burial, and leaching (Vymazal 2007). However, only few processes are capable of removing total nitrogen from wastewater at the end, whereas most processes just convert nitrogen to its various forms (Fig. 21.10). Removal of total nitrogen by constructed wetland system depends on the type of CWs and inflow loading (Vymazal 2007).

A study revealed that in the ammonia volatilization process, ammonium-N is in equilibrium between gaseous and hydroxyl forms, and volatilization of ammonia can result in nitrogen removal rates as high as 2.2 g N m$^{-2}$ day$^{-1}$ (Vymazal 2007). Ammonification (mineralization) is the process by which the biological conversion of organic N into ammonia takes place. The ammonification process is essentially a catabolism of amino acids and includes several types of deamination reactions such as oxidative deamination and reductive deamination (Vymazal 2007). The oxidative deamination can be written as

$$\text{Amino acids} \rightarrow \text{Imino acids} \rightarrow \text{Keto acids} \rightarrow \text{NH}_3.$$

This may be operative in the oxidized soil layer. The reductive deamination can be written as

$$\text{Amino acids} \rightarrow \text{Saturated acids} \rightarrow NH_3.$$

This happens within the reduced soil layer. Ammonification rates are dependent on temperature, pH, C/N ratio, available nutrients, and soil conditions such as texture and structure

(Vymazal 2007). Nitrification can be defined as the biological oxidation of ammonium to nitrate with nitrite as an intermediate in the reaction sequence. Nitrification depends on temperature, pH value, alkalinity of the water, inorganic C source, moisture, microbial population, and concentrations of ammonium-N and dissolved oxygen. Nitrification rates in wetlands were found to be in the range of 0.01–2.15 g N $m^{-2}$ $day^{-1}$ with the mean value of 0.048 g N $m^{-2}$ $day^{-1}$ (Vymazal 2007). Denitrification is the process in which nitrate is converted into dinitrogen via intermediates nitrite, nitric oxide, and nitrous oxide. Denitrification can be illustrated by the following equation (Vymazal 2007):

$$6\left(CH_2O\right)+4\,NO_3^- \rightarrow 6CO_2+2N_2+6H_2O.$$

Denitrification is a bacterial process in which nitrogen oxides act as terminal electron acceptors for respiratory electron transport. Electrons are carried from an electron-donating substrate through several carrier systems to a more oxidized N form. The resultant free energy is conserved in ATP, following phosphorylation, and then this energy is used by the denitrifying organisms for respiration (Vymazal 2007). Figure 21.10 summarizes the process of nitrogen transformations in a constructed wetland treatment system (Sim 2003).

In the recent decade, many field and laboratory studies have been increasingly carried out using various types of constructed wetland systems for the removal of total nitrogen from waste effluents (Beutel et al. 2009; Brisson and Chazarenc 2009; Ciria et al. 2005; Wießner et al. 2005; Ye and Li 2009; Vymazal 2005, 2007, 2010). The effects of vegetation and temperature in removing total nitrogen using a mixture of vegetation including bulrush, cattail, and mixed macrophytes and grasses have been studied well (Bachand and Horne 1999). They revealed that the average nitrate removal rates differ significantly between vegetation treatments and mixed treatment removes over three times more nitrate than the single treatment. The mass balance calculations determined that the denitrification is the dominant mechanism. Both water temperature and available organic carbon apparently affect denitrification rates (Bachand and Horne 1999). The efficiency of different CW types to remove total nitrogen was also studied by Vymazal (2005, 2007) and revealed that the removal of total nitrogen in studied types of constructed wetlands varies between 40 and 50 % with removed load ranging between 250 and 630 g N $m^{-2}$ $year^{-1}$ depending on CW type and inflow loading (Vymazal 2007).

A simple Vollenweider-type model, which can be used to predict wetland nitrate removal efficiency as the hydrologic and nutrient conditions change, has developed regarding nitrate retention based on seasonal temperature, hydraulic loading, and nitrate loading (Spieles and Mitsch 1999). Removal efficiency of N was studied using *Juncus effusus*, planted in a laboratory reactor where 82 and 97.6 % removal efficiencies were reported for ammonia and for nitrates (Wießner et al. 2005). In this study, the enrichment of ammonia closely linked to the light, particularly during summertime, indicated the existence of additional N turnover pathways in the rhizoplane involving $N_2$ produced by microbes or released by plants (Wießner et al. 2005). The results of this study highlighted the importance of macrophytes and their physiological specifics for removal of nitrates using constructed wetlands.

Rates of nitrate loss in wetlands are reported to be highly seasonal, generally peaking in the summer months (June–August), and it correlates with water temperature and dissolved oxygen (Beutel et al. 2009). The macrophyte *Typha latifolia* planted in constructed wetlands has high potential for phytoremediation of high organic matter and ammonia-N content present in wastewater (Ciria et al. 2005).

A novel CW configuration with three stages, towery hybrid constructed wetland (THCW), has been designed to enhance nitrogen removal (Ye and Li 2009). The first and third stages are rectangle HF CWs, and the second stage is a circular three-layer free-water flow CWs. This type of constructed wetlands enhances nitrification rates due to high dissolved oxygen concentration, and phytoremediation of nitrates is being efficient using macrophytes such as evergreen tree pond cypress (*Taxodium ascendens*), industrial plants mat rush (*Schoenoplectus triqueter*) and wild rice shoots (*Zizania aquatica*), and ornamental floriferous plants pygmy water lily (*Nymphaea tetragona*) and narrow-leaved cattail (*Typha angustifolia*) (Ye and Li 2009).

#### Phosphorus Removal

Phosphorus in wetlands occurs mainly as phosphate in organic and inorganic compounds. Free orthophosphate is the only form of phosphorus that is supposed to be utilized directly by algae and macrophytes (Vymazal 2007). The other inorganic phosphorus compounds are polyphosphates linearly condensed and cyclic. Organically bound phosphorus is present in phospholipids, nucleic acids, nucleoproteins, phosphorylated sugars, or organic condensed polyphosphates (coenzymes, ATP, ADP) (Vymazal 2007). The forms of organic P can be generally grouped into easily decomposable P (nucleic acids, phospholipids, or sugar phosphates) and slowly decomposable organic P (inositol phosphates or phytin) (Vymazal 2007). It has been observed that the wetlands provide an environment for the interconversion of all forms of phosphorus. Soluble reactive phosphorus can be taken up by plants and converted to tissue phosphorus or may become sorbed to wetland soils and sediments (Vymazal 2007). Main phosphorus retention mechanisms include uptake and release by vegetation, periphyton and microorganisms, sorption and exchange reactions with soils and sediments, chemical precipitation in the water column, and sedimentation and entrainment (Reddy et al. 1999). These mechanisms define the combined biological, physical, and chemical nature of P retention in wetlands and streams.

The macrophyte *Scirpus validus* is found to be the most effective plant species for phytoremediation of phosphorus compared to the removal efficiencies of *Carex lacustris*, *Phalaris arundinacea*, and *Typha latifolia* species planted in constructed wetlands (Fraser et al. 2004). The use of mixture of wetland plant species was found to be more effective than the use of individual plants for treating of both phosphorus and nitrogen present in nutrient-rich water effluents (Fraser et al. 2004). Floating macrophytes such as water hyacinth, water lettuce, and dwarf red-stemmed parrot feather that are planted under subsurface constructed wetland conditions have been found to be suitable plant species for the remediation of phosphorus from wastewater (Polomski et al. 2009).

#### 21.4.3.2 Metal and Metalloid Removal

Constructed wetlands have been used successfully for treating heavy metals and metalloids present in wastewaters. It has been investigated that some of macrophytes such as *Typha*, *Phragmites*, *Eichhornia*, *Azolla*, *Lemna*, *Glyceria grandis*, *Scirpus validus*, *Spartina pectinata*, etc., are capable of uptake and accumulate a variety of heavy metals (e.g., Cd, Pb, Cr, Zn, Hg, Ni, etc.) and metalloids (e.g., Se) that are present within high concentrations in wastewater (de Souza et al. 1999; Lin and Terry 2003; Cheng et al. 2002; Deng et al. 2004; Karathanasis and Johnson 2003; Liao and Chang 2004; Maine et al. 2009; Stottmeister et al. 2003; Türker et al. 2014; Vymazal and Šveha 2012; Weis and Weis 2004; Ye et al. 2001). Some macrophytes can be used to treat more than one metal, and these plants can accumulate heavy metals in concentrations 100,000 times greater than in the associated water (Marchand et al. 2010). Hyperaccumulators can tolerate, take up, and translocate high levels of certain metals that would be toxic to most organisms (Marchand et al. 2010). There are four mechanisms involved in heavy metal ion removal in wetlands, adsorption to fine-textured sediments and organic matter, precipitation as insoluble salts, absorption and induced changes in biogeochemical cycles by plants and bacteria, and deposition of suspended solids due to low flow rates (Lesage et al. 2007).

Several emergent plants have been used in constructed wetlands to remove heavy metals successfully. *Phragmites australis* has been identified as the most suitable emergent plant species for toxic metal ion removal (Marchand et al. 2010), but the performance of *Phalaris arundinacea* is very much similar to *Phragmites australis* as do *Typha domingensis*, *Typha latifolia*, and *Phragmites karka* (Maine et al. 2009; Marchand et al. 2010; Vymazal 2007). *Phragmites australis* and *Phalaris arundinacea* species have also been used for treating alkali metals such as Na, Mg, K, and Ca, and the results of this study provide comprehensive information on the retention and sequestration of such alkali metals in vegetation during municipal wastewater treatment in constructed wetlands with subsurface horizontal flow (Vymazal and Šveha 2012). *Phragmites australis* is an invasive species in the Northeast USA that sequesters more metals belowground than the native *Spartina alterniflora* (Weis and Weis 2004). Vertical-flow constructed wetlands within *Cyperus alternifolius* have been found to be an effective tool for the phytoremediation of heavy metals including Cd, Cu, Pb, and Zn (Cheng et al. 2002).

Water hyacinth plants (*Eichhornia crassipes*) in Erh-Chung constructed wetlands in Taiwan have been recognized as a good hyperaccumulator for Cd, Cu, Zn, Ni, and Pb in the order of Cu > Pb > Cd > Ni > Zn, and they have a high bioconcentration of these trace elements when grown in water environments with low concentrations of the five heavy metal ions (Liao and Chang 2004). In addition, CWs with floating aquatic plants such as *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia herzogii* also provide good metal uptake and accumulation (Marchand et al. 2010).

The concentrations of Pb, Zn, Cu, and Cd accumulated by some emergent-rooted wetland plant species including *Leersia hexandra*, *Juncus effusus*, and *Equisetum ramosissimum* have been investigated in field conditions of China, and concentrations of Pb and Cu in both aboveground and underground tissues of the plants are significantly positively related to their total and/or DTPA-extractable fractions in substrata, while negatively to soil N and P, respectively (Deng et al. 2004). The factors affecting metal accumulation by wetland plants are metal concentrations, pH, and nutrient status in substrata, and metal accumulation by wetland plants differs among species, populations, and tissues (Deng et al. 2004).

Most constructed wetlands in the USA and Europe are soil- or gravel-based horizontal-flow systems planted with macrophytes such as *Typha latifolia* and *Phragmites australis*, and they are widely used to treat storm runoff, domestic and industrial wastewater, and mine wastewater drainage (Scholz and Lee 2005). In the recent years, acid mine drainage has become a significant environmental problem facing the mining industry worldwide. Water infiltrating through the metal sulfide minerals, effluents of mineral processing plants, and seepage from tailing dams becomes acidic, and this acidic nature of the solution allows the metals to be transported in their most soluble form (Sheoran and Sheoran 2006). The conventional treatment technologies used in the treatment of acid mine drainage are expensive. The use of wetland treatment systems is now being attractive for treating acid mine water, since it is an economically viable and environmentally friendly technology. These wetlands can absorb and bind heavy metals and make them concentrated in the sedimentary deposits to take part with the geological cycle (Sheoran and Sheoran 2006).

Three wetland plant species, cattail (*Typha latifolia*), bulrush (*Scirpus validus*), and tickseed sunflower (*Bidens aristosa*), planted in an acid mine drainage wetland in McCreary County, Kentucky, USA, have shown capability to bioaccumulate metals such as Al, Fe, and Mn from a coal mine effluent (Karathanasis and Johnson 2003). *Scirpus validus* shows high tolerance to Al, and Fe accumulation is similar in all plant species (Karathanasis and Johnson 2003). A flow-through wetland treatment system within macrophytes, cattail (*Typha latifolia* L.), and wetland cells that is constructed to treat coal combustion by-product leachate from an electrical power station at Springdale, Pennsylvania (Ye et al. 2001), can effectively remove and bioaccumulate Fe and Mn from the inlet water. It is also revealed that the Fe and Mn concentrations are decreased by an average of 91 % in the first year and by 94 and 98 % in the second year, respectively. Cobalt (Co) and nickel (Ni) are decreased by an average of 39 and 47 % in the first year and 98 and 63 % in the second year, respectively (Ye et al. 2001).

Constructed wetlands are being of increasing interest to remove metalloids as well. The major metalloids that are found to be treated using constructed wetlands are boron (B) and selenium (Se). Turkey possesses approximately 70 % of the world's total B reserves, and B contamination can be seen in both natural and cultivated sites particularly in the northwest of Turkey (Türker et al. 2014). *Typha latifolia* and *Phragmites australis* planted in HF CWs in Turkey have been recognized as effective macrophytes to treat wastewater from a borax reserve, and the *T. latifolia* was able to take up a total of 1,300 mg $kg^{-1}$ B, whereas *P. australis* was able to uptake only 839 mg $kg^{-1}$ (Türker et al. 2014).

Macrophytes *Typha latifolia* L. and *Phragmites australis* are effective for Se removal from wastewater (Shardendu-Salhani et al. 2003). In addition, saltmarsh bulrush (*Scirpus robustus* Pursh) and rabbit-foot grass (*Polypogon monspeliensis* (L.) Desf.) with *rhizosphere* bacteria also are capable of removing Se and Hg from wastewater (de Souza et al. 1999). The vegetated wetlands that are constructed in Corcoran, California, are capable of reducing Se from the inflow drainage water with an average of 69.2 % (Lin and Terry 2003).

Several studies have been reported even in Sri Lanka for removal of toxic metals using constructed wetlands (Jayaweera et al. 2008; Kularatne et al. 2009; Weerasinghe et al. 2008; Mahatantila et al. 2008). Phytoremediation efficiencies of water hyacinth grown under different nutrient conditions were investigated for removal of Fe from Fe-rich wastewaters in batch-type constructed wetlands (Jayaweera et al. 2008). The results revealed that plants grown in the setup without any nutrients have the highest phytoremediation efficiency of 47 % during optimum growth at the 6th week with a highest accumulation of 6707 Fe mg $kg^{-1}$ dry weight (Jayaweera et al. 2008). Iron removal was largely due to phytoremediation mainly through the process of rhizofiltration and chemical precipitation of $Fe_2O_3$ and $Fe(OH)_3$ followed by flocculation and sedimentation (Jayaweera et al. 2008). It has been found to be that the constructed wetlands comprising water hyacinth are effective in removing Mn through phytoextraction as the main mechanism (Kularatne et al. 2009).

#### 21.4.3.3 Organic Contaminant Removal

Volatile organic compounds (VOCs), organochlorines, PAHs, and some pharmaceuticals are particularly concerned for phytoremediation in constructed wetlands. Organic chemicals exhibit a wide range of physicochemical properties, numerous specific toxicity effects, and often a degree of recalcitrance rarely encountered in common contaminants of domestic and agricultural sewage. Therefore, evaluating the physicochemical properties and biological effects of specific groups of organic chemicals with respect to their potential and observed fate in constructed wetlands may help refining artificial wetland design and operation modes (Imfeld et al. 2009). Phytoremediation of organic pollutants takes place through several mechanisms which include accumulation into biomass, phytovolatilization, cellular degradation, and rhizosphere degradation (Williams 2002).

##### Volatile Organic Compounds (VOCs) and Hydrocarbons

Direct volatilization and phytovolatilization processes are mainly used to remediate hydrophilic compounds such as acetone and phenol (Imfeld et al. 2009). Volatilization is supposed to be an important removal process for volatile hydrophobic compounds such as lower chlorinated benzenes, chlorinated ethenes, and BTEX (benzene, toluene, ethylbenzene, xylene) (Imfeld et al. 2009). The removal of MTBE compounds using constructed wetlands is based on the Henry coefficient, high water solubility, and strong recalcitrance under anaerobic conditions (Imfeld et al. 2009).

Several large-scale wetland projects currently exist at oil refineries, and numerous pilot studies of constructed treatment wetlands have been conducted at terminals, gas and oil extraction and pumping stations, and refineries. Petroleum industry wetland studies indicate that treatment wetlands are more effective at removing pollutants from petroleum industry wastewaters. A pilot-scale VF CW system was constructed at the former BP Refinery in Casper, Wyoming, in order to determine BTEX degradation rates in a cold-climate application (Wallace and Kadlec 2005). The removal rates for petroleum hydrocarbons in aerated subsurface-flow wetlands are considerably higher than in non-aerated wetlands (Wallace and Kadlec 2005). Low-molecular-weight hydrocarbons such as benzene are treated by vertical-flow constructed wetlands achieving efficiencies between 88–89 and 72–80 % for indoor and outdoor constructed wetlands, respectively (Tang et al. 2009).

Trichloroethylene (TCE) has been remediated effectively by phytoremediation of wetland species including cattails (*Typha latifolia*), cottonwoods (*Populus deltoides*), and hybrid poplars (*Populus trichocarpa x P. deltoides*) (Gordon et al. 1998; Williams 2002; Bankston et al. 2002; Amon et al. 2007). Mineralization of TCE in the range of 73–96 % by cattails and cottonwoods was reported suggesting natural attenuation as a potential bioremediative strategy for TCE-contaminated wetlands (Bankston et al. 2002). Hybrid poplars (*Populus trichocarpa x P. deltoides*) were used to remove TCE from wastewaters (Gordon et al. 1998). This study investigated that the cells of hybrid poplars are capable of metabolizing 95 % of the TCE present in influent water and 70–90 % of the TCE is transpired (Gordon et al. 1998). Perchloroethylene (PCE)-contaminated groundwater was successfully treated by an upward-flowing subsurface constructed wetland through sequential dechlorination (Amon et al. 2007).

### Pesticides and Pharmaceuticals

The capability of constructed, restored, and natural wetlands has been evaluated developing design guidelines to assimilate and process pesticides associated with agricultural runoff from croplands (Rodgers Jr and Dunn 1992). Wetland plants, such as *Ceratophyllum demersum*, *Elodea canadensis*, and *Lemna minor*, have found evidence that phytoremediation may accelerate the removal and biotransformation of metolachlor and atrazine from herbicide-contaminated waters (Williams 2002). Bulrush (*Scirpus validus*) planted in subsurface-flow constructed wetlands showed effective removal of pesticides including simazine and metolachlor in runoff water (Stearman et al. 2003). A field-scale evaluation of a constructed wetland has been performed for removal of pesticides in the Lourens River watershed of Cape Town, South Africa. Results indicated that the wetland is able to reduce the concentration of chlorpyrifos and suspended sediment entering the receiving Lourens River (Moore et al. 2002).

The efficiency of a surface-flow constructed wetland for the removal of pharmaceutical and personal care products (PPCPs) and herbicides that are discharged from a wastewater treatment plant (WWTP) into a small tributary of the River Besos in northeastern Spain has been studied (Reddy and D'Angelo 1997). This study demonstrated that the removal efficiencies are often higher than 90 % for all compounds, with the exception of carbamazepine and clofibric acid (30–47 %). Furthermore, a seasonal trend of pollutant removal in the wetland was observed for several compounds having low biodegradation and moderate photodegradation rates (e.g., naproxen and diclofenac) (Reddy and D'Angelo 1997).

### Explosives

Contamination of soil and groundwater by trinitrotoluene (TNT) is becoming a serious problem. In the USA, TNT is largely discharged through military-based activities and ammunition manufacturing industries (Medina and McCutcheon 1996). Phytoremediation by constructed wetlands provides a promising treatment of TNT-contaminated groundwater and wastewater since many wetland plant species contain the necessary enzymes to degrade explosives such as TNT (Medina and McCutcheon 1996). The first quantitative demonstration of 2,4,6-trinitrotoluene (TNT) transformation by aquatic plants was conducted using common wetland species *Myriophyllum spicatum* and *Myriophyllum aquaticum* (Williams 2002). Phytoremediation of 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in groundwater was successfully tried out using constructed wetlands vegetated with some macrophytes and aquatic plants such as elodea, pondweed, water star grass, parrot feather, sweet flag, reed canary grass, and wool grass (Best et al. 1999). TNT was completely removed in plant treatments, and removal rates varied from 0.001 mg TNT g total $FW^{-1}$ $day^{-1}$ in the emergent wool grass to 0.05 mg TNT g $FW^{-1}$ $day^{-1}$ in the submersed water star grass, whereas RDX removal rates varied from 0.001 mg RDX g total $FW^{-1}$ $day^{-1}$ in the emergent reed canary grass to 0.004 mg RDX g $FW^{-1}$ $day^{-1}$ in the submersed elodea (Best et al. 1999).

### Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are aromatic hydrocarbons having two or more fused benzene rings arise from natural as well as anthropogenic sources (Haritash and Kaushik 2009). They are the one of widely distributed environmental contaminants bringing serious hazardous effects such as detrimental biological effects, toxicity, mutagenicity, and carcinogenicity in the environment, and hence, PAHs are of particular concern in the environment (Haritash and Kaushik 2009). The constructed wetland technology has been widely extended for the removal of polycyclic aromatic hydrocarbons (PAHs) as well (Haritash and Kaushik 2009; Terzakis et al. 2008; Fountoulakis et al. 2009).

The USEPA has identified 16 PAH compounds as priority pollutants, and urban runoff is considered as an important pathway of releasing PAHs to water environments and aquatic ecosystems (Terzakis et al. 2008). Free-water-surface (FWS) and subsurface-flow (SSF) pilot-size constructed wetlands have been used for treating highway runoff (HRO) in the central Mediterranean region, and it was found to be 59 % removal efficiencies of total PAHs in runoff (Terzakis et al. 2008). Aquatic weeds *Typha* spp. and *Scirpus lacustris* have been used in horizontal–vertical macrophyte-based wetlands to treat PAHs (Haritash and Kaushik 2009). Removal efficiencies of polycyclic aromatic hydrocarbons (PAHs) and linear alkyl benzene sulfonates (LAS) were evaluated in a pilot-scale constructed wetland (CW) system combining a free-water-surface wetland, a subsurface wetland, and a gravel filter in parallel (Fountoulakis et al. 2009). It was reported that the average removal of PAHs and LAS is 79.2 and 55.5 % for the SSF (subsurface-flow) constructed wetland and 68.2 and 30.0 % for the FWS (free-water-surface) constructed wetland, whereas 73.3 and 40.9 % was observed for the gravel filter, respectively (Fountoulakis et al. 2009).

#### 21.4.3.4 Removal of Pathogens

Constructed wetlands are shown to be capable of removing a wide variety of pathogens including bacteria, viruses, and protozoan cysts (Greenway 2005). Wetlands act as biofilters through a combination of physical, chemical, and biological factors which all participate in the reduction of a number of bacteria (Ottová et al. 1997). Harmful microorganisms such as fecal coliform bacteria and *Enterobacteriaceae* can be effectively removed using constructed wetlands planted with *Glyceria* and *Phragmites* (Ottová et al. 1997).

Sedimentation is one of the mechanisms of microbial reduction from wetlands used for wastewater treatment (Karim et al. 2004). Sediments of constructed wetlands are able to accumulate significant concentrations of pathogens (Karim et al. 2004). It was investigated that the die-off rates of fecal coliforms in the water and sediment are 0.256 $\log_{10}\text{day}^{-1}$ and 0.151 $\log_{10}\text{day}^{-1}$, *Salmonella typhimurium* in the water and sediment are 0.345 $\log_{10}\text{day}^{-1}$ and 0.312 $\log_{10}\text{day}^{-1}$, and the die-off rates of naturally occurring coliphage in water column and sediment are 0.397 $\log_{10}\text{day}^{-1}$ and 0.107 $\log_{10}\text{day}^{-1}$, respectively (Karim et al. 2004). *Typha angustifolia* has been tested for fecal coliform in free-water-surface (FWS) constructed wetlands (Khatiwada and Polprasert 1999). Major mechanisms influencing the removal of fecal microorganisms in constructed wetlands treating sewage in tropical regions include the effects of temperature, solar radiation, sedimentation, adsorption, and filtration (Khatiwada and Polprasert 1999). A kinetic model for the removal of bacteria has been developed to evaluate the kinetics of fecal coliform removal vegetated with cattails (*Typha angustifolia*).

## 21.5 Limitations and Areas of Uncertainty in Wetland Phytoremediation

Phytoremediation in constructed wetlands is still new and not fully developed. Phytoremediation is generally restricted by limitation of rooting depth due to the shallow distribution of plant roots. Phytoremediation of soil or water requires that the contaminants be within the zone of influence of the plant roots. Only a little regulatory experience is present with wetland phytoremediation and it is site specific. The inherent characteristics of phytoremediation limit the size of the niche that it occupies in the site remediation market. Phytoremediation requires longer time period and it is climate dependent. Hence, more research is needed in terms of achieving the maximum phytoremediation in constructed wetlands. In most of constructed wetlands, phytoremediation of pollutants takes place effectively, when the contaminants are present in shallow water. This process usually favors nutrient addition and mass transfer is limited. High initial concentrations of contaminants may be phytotoxic inhibiting the growth of wetland plants. Harvesting and proper disposal plans are required for plant biomass that accumulates hazardous contaminants, since the contaminants being treated by phytoremediation may be transferred and bioaccumulated in animals through food chains. The analysis of risk pathways also is necessary to ensure that the degree of risk is lessened through the use of wetlands for phytoremediation that will benefit to livestock and the general public. Phytoremediation of mixed contaminants including both organic and inorganic pollutants present in wetlands is practically unsuccessful, and to make it a success, more than one phytoremediation method or an integrated approach may be required. The requirement of a greater land area for wetland phytoremediation competes with other remedial methods.

## 21.6 Current Knowledge and the Future of Wetland Phytoremediation

The relatively brief history of phytoremediation using constructed wetlands has been endeavored for most field applications in order to remediate hazardous pollutants and heal the Earth. The current knowledge and well understanding upon the limitations will enrich the future goals of phytoremediation in constructed wetlands. Post-harvest strategies are essential with pre-harvest approaches for developing a sustainable phytoremediation technology. The knowledge, experience, and field trials are necessary to forecast and certify that the wetland plants have almost detoxified contaminants attaining minimal residual risks to humans and the environment. Residual management is particularly necessary to alleviate some restrictions arising from the public.

Fundamental researches based on wetland phytoremediation will not be enough to solve the problem, so that it should be developed in large scale within new strategies and approaches together with integrated technology. Understanding the interactions of microbes and their symbiotic effects on wastewater treatment is also a necessity. Researchers have used biotechnological strategies to either enhance existing traits or confer novel capabilities to plants through genetic engineering. The use of genetic engineering technology is being an existing practice in moving phytoremediation into the forefront of remediation technology. Recent achievements in plant genomic and proteomic research significantly enrich the potential of phytoremediation (McCutcheon and Schnoor 2003). The range and reproducibility of phytotechnologies will be improved with additional study and refinement of such field trials. For a wide array of pollutants in wastewaters, wetland phytoremediation attributes are an ecologically compatible, cost-effective alternative, or complementary option for standard engineered approaches. Extensive efforts are ongoing to extend the range of biological capabilities and technical application of phytoremediation systems. The successful transfer of the constructed wetland technologies from the laboratory to the field is a crucial step for the future of wetland phytoremediation, and the investment of this technology in the development is also an urgent necessity.

## References


Aksu Z (2002) Determination of the equilibrium, kinetic and thermodynamic parameters of the batch biosorption of nickel(II) ions onto Chlorella vulgaris. Process Biochem 38:89–99

Alexander M, Hatzinger PB, Kelsey JW, Kottler BD, Nam K (1997) Sequestration and realistic risk from toxic chemicals remaining after bioremediation. Ann N Y Acad Sci 829:1–5

Amarasinghe BMWPK, Williams RA (2007) Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. Chem Eng J 132:299–309

Amon JP, Agrawal A, Shelley ML, Opperman BC, Enright MP, Clemmer ND, Slusser T, Lach J, Sobolewski T, Gruner W, Entingh AC (2007) Development of a wetland constructed for the treatment of groundwater contaminated by chlorinated ethenes. Ecol Eng 30:51–66

Bachand PAM, Horne AJ (1999) Denitrification in constructed free-water surface wetlands: II. Effects of vegetation and temperature. Ecol Eng 14:17–32

Bankston JL, Sola DL, Komor AT, Dwyer DF (2002) Degradation of trichloroethylene in wetland microcosms containing broad-leaved cattail and eastern cottonwood. Water Res 36:1539–1546

Banuelos G, Terry N (eds) (1999) Phytoremediation of contaminated soil and water. Lewis Publishers, Boca Raton, FL

Best EPH, Sprecher SL, Larson SL, Fredrickson HL, Darlene BF (1999) Environmental behavior of explosives in groundwater from the Milan army ammunition plant in aquatic and wetland plant treatments. Removal, mass balances and fate in groundwater of TNT and RDX. Chemosphere 38:3383–3396

Beutel MW, Newton CD, Brouillard ES, Watts RJ (2009) Nitrate removal in surface-flow constructed wetlands treating dilute agricultural runoff in the lower Yakima Basin, Washington. Ecol Eng 35:1538–1546

Bradley C (2001) Wetlands (third edition) by W.J. Mitsch and J.G. Gosselink. John Wiley & Sons, New York, 2000. No. of pages: 920. Price: £60.95. ISBN 0 471 29232 X. Regul Rivers Res Manage 17:295–295

Brady NC, Weil RR (eds) (1996) The nature and properties of soils. Prentice-Hall, Upper Saddle River, USA

Brisson J, Chazarenc F (2009) Maximizing pollutant removal in constructed wetlands: should we pay more attention to macrophyte species selection? Sci Total Environ 407:3923–3930

Brix H (1994) Function of macrophytes in constructed wetlands. Water Sci Technol 29:71–78

Carmichael LM, Pfaender FK (1997) Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and pre-exposure. Environ Toxicol Chem 16:666–675

Cheng S, Grosse W, Karrenbrock F, Thoennessen M (2002) Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Ecol Eng 18:317–325

Ciria MP, Solano ML, Soriano P (2005) Role of macrophyte Typha latifolia in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. Biosystems Eng 92:535–544

Clayton LR (2007) Phytoremediation. Encyclopedia of plant and crop science. Taylor & Francis, London, UK

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719

DE Souza MP, Huang CPA, Chee N, Terry N (1999) Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. Planta 209:259–263

Deng H, Ye ZH, Wong MH (2004) Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environ Pollut 132:29–40

Duxbury CL, Dixon DG, Greenberg BM (1997) Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemna gibba. Environ Toxicol Chem 16:1739–1748

Fountoulakis MS, Terzakis S, Kalogerakis N, Manios T (2009) Removal of polycyclic aromatic hydrocarbons and linear alkylbenzene sulfonates from domestic wastewater in pilot constructed wetlands and a gravel filter. Ecol Eng 35:1702–1709

Fraser LH, Carty SM, Steer D (2004) A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. Bioresour Technol 94:185–192

Gordon M, Choe N, Duffy J, Ekuan G, Heilman P, Muiznieks I, Ruszaj M, Shurtleff BB, Strand S, Wilmoth J, Newman LA (1998) Phytoremediation of trichloroethylene with hybrid poplars. Environ Health Perspect 106:1001–1004

Greenway M (2005) The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. Ecol Eng 25:501–509

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

Hedges P, Fermor P, Dušek J (2008) The hydrological sustainability of constructed wetlands for wastewater treatment. In: Vymazal J (ed) Wastewater treatment, plant dynamics and management in constructed and natural wetlands. Springer, Netherlands

Hijosa-Valsero M, Matamoros V, Martín-Villacorta J, Bécares E, Bayona JM (2010) Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. Water Res 44:1429–1439

Hoffmann H, Platzer C, Von Münch E, Winker M (2011) Technology review of constructed wetlands—subsurface flow constructed wetlands for greywater and domestic wastewater treatment. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ),GmbH, Eschborn, Germany

Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. J Environ Biol 28:367–376

Huang XD, Krylov SN, Ren L, Mcconkey BJ, Dixon DG, Greenberg BM (1997) Mechanistic quantitative structure–activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons: II. An empirical model for the toxicity of 16 polycyclic aromatic hydrocarbons to the duckweed Lemna gibba L. G-3. Environ Toxicol Chem 16:2296–2303

Imfeld G, Braeckevelt M, Kuschk P, Richnow HH (2009) Monitoring and assessing processes of organic chemicals removal in constructed wetlands. Chemosphere 74:349–362

Jayaweera MW, Kasturiarachchi JC, Kularatne RKA, Wijeyekoon SLJ (2008) Contribution of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown under different nutrient conditions to Fe-removal mechanisms in constructed wetlands. J Environ Manage 87: 450–460

Jomjun N, Siripen T, Maliwan S, Jintapat N, Prasak T, Somporn C, Petch P (2010) Phytoremediation of Arsenic in submerged soil by wetland plants. Int J Phytoremediation 13:35–46
Kadlec RH, Wallace SD (eds) (2008) Treatment wetlands. CRC Press, Boca Raton, USA
Karathanasis AD, Johnson CM (2003) Metal removal potential by three aquatic plants in an acid mine drainage wetland. Mine Water Environ 22:22–30
Karim MR, Manshadi FD, Karpiscak MM, Gerba CP (2004) The persistence and removal of enteric pathogens in constructed wetlands. Water Res 38:1831–1837
Khatiwada NR, Polprasert C (1999) Kinetics of fecal coliform removal in constructed wetlands. Water Sci Technol 40:109–116
Kularatne RKA, Kasturiarachchi JC, Manatunge JMA, Wijeyekoon SLJ (2009) Mechanisms of manganese removal from wastewaters in constructed wetlands comprising water hyacinth (Eichhornia crassipes (Mart.) Solms) grown under different nutrient conditions. Water Environ Res 81:165–172
Lesage E, Rousseau DPL, Meers E, Tack FMG, De-Pauw N (2007) Accumulation of metals in a horizontal subsurface flow constructed wetland treating domestic wastewater in Flanders, Belgium. Sci Total Environ 380:102–115
Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquat Plant Manage 42:60–68
Lin Q, Mendelssohn IA (1998) The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. Ecol Eng 10:263–274
Lin ZQ, Terry N (2003) Selenium removal by constructed wetlands: quantitative importance of biological volatilization in the treatment of selenium-laden agricultural drainage water. Environ Sci Technol 37:606–615
Mahatantila K, Chandrajith R, Jayasena HAH, Ranawana KB (2008) Spatial and temporal changes of hydrogeochemistry in ancient tank cascade systems in Sri Lanka: evidence for a constructed wetland. Water Environ J 22:17–24
Maine MA, Suñe N, Hadad H, Sánchez G, Bonetto C (2009) Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J Environ Manage 90:355–363
Marchand L, Mench M, Jacob DL, Otte ML (2010) Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ Pollut 158:3447–3461
Mcconkey BJ, Duxbury CL, Dixon DG, Greenberg BM (1997) Toxicity of a pah photooxidation product to the bacteria Photobacterium phosphoreum and the duckweed Lemna gibba: effects of phenanthrene and its primary photoproduct, phenanthrenequinone. Environ Toxicol Chem 16:892–899
Mccutcheon SC, Schnoor JL (eds) (2003) Phytoremediation transformation and control of contaminants. Wiley, Hoboken, NJ
Medina VF, Mccutcheon SC (1996) Phytoremediation: modeling removal of TNT and its breakdown products. Remediation J 7:31–45
Mejáre M, Bülow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19:67–73
Mohan D, Pittman CU Jr (2007) Arsenic removal from water/wastewater using adsorbents—a critical review. J Hazard Mater 142:1–53
Moore MT, Schulz R, Cooper CM, Smith JRS, Rodgers JH Jr (2002) Mitigation of chlorpyrifos runoff using constructed wetlands. Chemosphere 46:827–835
Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207
Otten A, Alphenaar A, Pijls C, Spuij F, DE Wit H (eds) (1997) In situ soil remediation. Kluwer Academic Publishers, Boston
Ottová V, Balcarová J, Vymazal J (1997) Microbial characteristics of constructed wetlands. Water Sci Technol 35:117–123
Polomski R, Taylor M, Bielenberg D, Bridges W, Klaine S, Whitwell T (2009) Nitrogen and phosphorus remediation by three floating aquatic macrophytes in greenhouse-based laboratory-scale subsurface constructed wetlands. Water Air Soil Pollut 197:223–232
Rai PK (2008) Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an ecosustainable approach. Int J Phytoremediation 10:133–160
Raskin I, Ensley BD (eds) (2000) Phytoremediation of toxic metals, using plants to clean up the environment. Wiley, New York
Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
Reddy KR, D'Angelo EM (1997) Biogeochemical indicators to evaluate pollutant removal efficiency in constructed wetlands. Water Sci Technol 35:1–10
Reddy KR, Kadlec RH, Flaig E, Gale PM (1999) Phosphorus retention in streams and wetlands: a review. Crit Rev Environ Sci Technol 29:83–146
Ren L, Huang XD, Mcconkey BJ, Dixon DG, Greenberg BM (1994) Photoinduced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene, and naphthalene) to the duckweed Lemna gibba L. G-3. Ecotoxicol Environ Saf 28:160–171
Rodgers JH Jr, Dunn A (1992) Developing design guidelines for constructed wetlands to remove pesticides from agricultural runoff. Ecol Eng 1:83–95
Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668
Scholz M, Lee BH (2005) Constructed wetlands: a review. Int J Environ Stud 62:421–447
Shardendu-Salhani N, Boulyga SF, Stengel E (2003) Phytoremediation of selenium by two helophyte species in subsurface flow constructed wetland. Chemosphere 50:967–973
Sheoran AS, Sheoran V (2006) Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. Miner Eng 19:105–116
Sim CH (2003) The use of constructed wetlands for wastewater treatment. Wetlands International, Malaysia Office, Selangor, Malaysia
Spieles DJ, Mitsch WJ (1999) The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: a comparison of low- and high-nutrient riverine systems. Ecol Eng 14:77–91
Stearman GK, George DB, Carlson K, Lansford S (2003) Pesticide removal from container nursery runoff in constructed wetland cells. J Environ Qual 32:1548–1556
Stottmeister U, Wiessner A, Kuschk P, Kappelmeyer U, Kästner M, Bederski O, Müller RA, Moormann H (2003) Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol Adv 22:93–117
Tang X, Eke PE, Scholz M, Huang S (2009) Processes impacting on benzene removal in vertical-flow constructed wetlands. Bioresour Technol 100(1):227–234
Terry N, Bañuelos G (eds) (1999) Phytoremediation of contaminated soil and water. Library of Congress Cataloging, US
Terzakis S, Fountoulakis MS, Georgaki I, Albantakis D, Sabathianakis I, Karathanasis AD, Kalogerakis N, Manios T (2008) Constructed wetlands treating highway runoff in the central Mediterranean region. Chemosphere 72:141–149
Türker OC, Türe C, Böcük H, Yakar A (2014) Constructed wetlands as green tools for management of boron mine wastewater. Int J Phytoremediation 16(6):537–553
Vymazal J (2005) Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol Eng 25:478–490

Vymazal J (2007) Removal of nutrients in various types of constructed wetlands. Sci Total Environ 380:48–65

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549

Vymazal J, Šveha J (2012) Removal of alkali metals and their sequestration in plants in constructed wetlands treating municipal sewage. Hydrobiologia 692:131–143

Wallace S, Kadlec R (2005) BTEX degradation in a cold-climate wetland system. Water Sci Technol 51(9):165–172

Weerasinghe A, Ariyawnasa S, Weerasooriya R (2008) Phytoremediation potential of Ipomoea aquatica for Cr(VI) mitigation. Chemosphere 70:521–524

Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ Int 30:685–700

Wießner A, Kappelmeyer U, Kuschk P, Kästner M (2005) Influence of the redox condition dynamics on the removal efficiency of a laboratory-scale constructed wetland. Water Res 39:248–256

Williams JB (2002) Phytoremediation in wetland ecosystems: progress, problems, and potential. Crit Rev Plant Sci 21:607–635

Ye F, Li Y (2009) Enhancement of nitrogen removal in towery hybrid constructed wetland to treat domestic wastewater for small rural communities. Ecol Eng 35:1043–1050

Ye ZH, Whiting SN, Lin ZQ, Lytle CM, Qian JH, Terry N (2001) Removal and distribution of iron, manganese, cobalt, and nickel within a Pennsylvania constructed wetland treating coal combustion by-product leachate. J Environ Qual 30:1464–1473

Yu XZ, Trapp S, Zhou PH, Chen L (2007) Effect of temperature on the uptake and metabolism of cyanide by weeping willows. Int J Phytoremediation 9:243–255

# 22 Phytoremediation Using Algae and Macrophytes: I

Qaisar Mahmood, Nosheen Mirza, and Shahida Shaheen

## 22.1 Heavy Metal Contamination

Consequent to global industrialization, heavy metal pollution is a widespread problem which has become a major environmental concern due to hazardous effects on human and environmental health. Phytoremediation is an emerging technology that aims at metal extraction from soil, water, and air (Meagh 2000). Heavy metals are the global environmental contaminants. Air or water pollution by metals varies from soil pollution, because heavy metals persevere in soil for a longer time period as compared with the other compartment of the biosphere (Lasat 2002). In the latest decades, the yearly global release of heavy metals attained 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead, and 1,350,000 t for zinc (Singh et al. 2003).

Industrial activities like production of energy and fuel, mining and smelting of metalliferous ores, and post production exploitation of the materials contained in wastes result in heavy metal pollution. Because of the industrial revolution, a pompous increase of heavy metal accumulation was seen in the soil (Nriagu 1979). By and large, the majority widespread heavy metal pollutants are lead, cadmium, chromium, copper, mercury, and zinc (Kabata-Pendias and Pendias 1989). Although cadmium (Cd) is less toxic than mercury, it is more itinerant in soil-plant system than others (Alloway and Ayres 1997).

Besides the other noxious substances in the industrial wastes, heavy metals are considered to be one of the major pollutants in the environment, as they have a noteworthy effect on its ecological quality. Human activities escort toward the ever-increasing level of heavy metal pollution in the environment. Heavy metals due to atmospheric and industrial pollution amass in the soil and affect the nearby ecosystem. Metal contamination is also caused by ground transportation. Highway traffic, repairing, and deicing operations serve as constant surface and groundwater pollution causes. Among the well-recognized heavy metal sources associated with highway traffic are the tread ware, brake abrasion, and corrosion (Ho and Tai 1988; García and Millán 1998; Sánchez et al. 2000). Heavy metal contaminants in roadside soils are derived from engine and brake pad wear (e.g., Cd, Cu, and Ni) (Viklander 1998; Turer et al. 2001), exhaust emissions (e.g., Pb) (Gulson et al. 1981; Al-Chalabi and Hawker 2000; Sutherland et al. 2003), lubricants (e.g., Cd, Cu, and Zn) (Birch and Scollen 2003), and tire abrasion (e.g., Zn) (Smolders and Degryse 2002).

As mentioned earlier, cadmium is an environment pollutant which may be found in all ecosystems, water, air, and plants. Consequently, different living species including humans, animals, and plants are always in danger of being polluted by this metallic element. Cadmium concentration inside the body of living animals can reach an amount higher than its standard value because of its accumulative characteristic. As a result different symptoms may appear (Alloway 1990; Okoronkwo et al. 2005).

Accumulation of heavy metals in soils and their transport through the food chains are potential threats to human health, especially to children's health by ingestion of Pb-contaminated soil (Melamed et al. 2003). Three special approaches may be implemented for the renovation of extremely metal-contaminated sites (Zwonitzer et al. 2003). The most precise key is to eradicate the contaminated substrates and to restore with clean soil. On a large level, conversely, this kind of elucidation is not practicable owing to sky-scraping overheads. A subsequent doable remediation approach is soil cleanup by means of chemical, physicochemical, or biological methods, which take away the metals from the soil. On the other hand, these practices may generate new problems, like the increased level of metal mobility and its availability to the biota, redeployment of the contaminants to sludge, and alteration in physicochemical characters of the treated soils. A third form of remediation method is the in situ control of

Q. Mahmood, Ph.D. (✉) • N. Mirza, Ph.D. • S. Shaheen, M.S., Ph.D.
Department of Environmental Sciences, COMSATS IIT, Abbottabad, Pakistan
e-mail: mahmoodzju@gmail.com

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the metals using sequestering means such as minerals (e.g., apatite, zeolite) or industrial by-products (e.g., steel shots). This approach is a smart substitute to a lot of modern remediation techniques, particularly for outsized industrialized localities and discarding grounds. Immobilization of metals lessens both discharge and availability to plant. Experimentations have been accepted out by using extremely soluble types of phosphate (P, e.g., $K_2HPO_4$, DAP) (Hettiarachchi et al. 2001). Soluble P added to polluted soil may stimulate the formation of pyromorphite, which has very slight solubility. However, the accumulation of well-soluble P heightens the possibility of eutrophication owing to the appliance of huge amount of such modifications. Laboratory trials by means of phosphate rock (PR) have also been doing well (Basta and McGowan 2004; Zhu et al. 2004), but PR apatite is highly insoluble and thus may not liberate P adequately swiftly to remediate infected soil within an adequate timescale. Using finely powdered synthetic hydroxyapatite in soils and solutions results in the formation of pyromorphite (Nriagu 1984; Zhang and Ryan 1999; Basta and McGowan 2004), but the use of synthetic hydroxyapatite on a field scale is economically unfavorable. It has recently been suggested that poorly crystalline apatite, such as bone char apatite, might represent a low-cost, readily available phosphate source that could be used to remediate metal-contaminated soils without causing excessive P runoff (Ma et al. 1995; Ma and Rao 1997). Bone char is a mixed compound adsorbent in which carbon is distributed throughout a porous structure of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ or CaHAP). It contains around 76 % of CaHAP, which is not only a major inorganic constituent of teeth and bones but also the main inorganic constituent of phosphate rock (Cheung et al. 2001). The physical and chemical properties of the CaHAP have been widely reported; the removal mechanism has been suggested to be not only an adsorption effect but also a type of ion-exchange reaction between the ions in solution and the calcium ions of the apatites (Danny et al. 2004). However bone char application to metal-contaminated soils has rarely been reported. In this form, the major compound, CaHAP, in bone char has been developed as a treatment for decontaminating polluted water (Gabaldon et al. 1996; Dhabi et al. 1999; Wilson et al. 2001). In particular, its potential to adsorb both cationic and anionic metal species including radionuclides from radioactive wastes and contaminated water supplies is now being examined. Recently, the ability of bone meal addition to immobilize pollutant metals in soils has also been reported (Hodson et al. 2000).

The extraordinary swift alteration in environmental situation is expected to override the adaptive potential of plants. Heavy metal contagion caused by natural processes or through a variety of industrial wastes, in addition to the diverse anthropogenic activities such as metal working industries, mining, and smelting, and the use of mineral fertilizers as well as pesticides are the most severe environmental troubles (Grant and Loake 2000).

## 22.2 Remediation of Metal-Contaminated Soils

Reclamation of metal-contaminated soil can be done by chemical, physical, or biological methods (McEldowney et al. 1993). Numerous new physicochemical techniques are also used, but they are pretty costly and complicated to do where phytoremediation is incredibly viable to carry out. Among various technologies, existing so far, phytoremediation is considered to be the most environmentally friendly and cost-effective (Zhu and Rosen 2009):

- Physicochemical methods
- Biological methods

### 22.2.1 Physicochemical Methods

The most common treatment processes used include chemical precipitation, oxidation/reduction, ion exchange, reverse osmosis, and solvent extraction. Physical methods include processes where no gross chemical or biological changes are carried out and strictly physical phenomena are used to improve or treat the contaminated soils or wastewater:

1. Examples would be coarse screening to remove larger entrained objects and sedimentation (or clarification). Nanofiltration is a known and efficient method for the separation of pollutants from water which allows us to close the circulation of water used in dyehouses (Ciardelli et al. 2002; Tang and Chen 2002; Chakraborty et al. 2003; Kim and Park 2005). However, after the process of nanofiltration, a concentrated mixture of dyes and auxiliary substances (salts, acids, alkalies, organic compounds) remains, which causes a serious problem.
2. In the present work, $Pb^{2+}$ and $Cd^{2+}$ adsorption onto a natural polysaccharide has been studied in membrane reactors. The process involves a stirred semi-batch reactor for the adsorption step and a microfiltration (MF) process in order to confine the particles. Due to their lower affinity for the biosorbent, $Cd^{2+}$ ions were found to break through the process faster than $Pb^{2+}$ cations. The experimental results showed the technical feasibility of the pilot. A mass balance model based on the Langmuir equilibrium isotherm was used to describe the adsorption process. This relation is able to predict experimental data under different operating conditions: the adsorbent and metal concentrations and the permeate flow rate. Based on these results, it is demonstrated that the biosorbent studied represents an interesting low-cost solution for the treatment of metal ion-polluted waters (Reddad et al. 2003).

### 22.2.2 Disadvantages of Physicochemical Methods

There are some disadvantages for traditional physicochemical methods to treat metal-polluted wastewater, such as:

- Expensive cost.
- Low efficiency.
- Labor-intensive operation.
- Lack of selectivity in the treating process (Tarley and Arruda 2004).
- Physicochemical treatment processes remain unaffected by the presence of toxic substances such as metals, whereas biological systems fail to operate in case of wastes predominantly inorganic or nonbiodegradable in nature (Mohan et al. 2008).

Scientists are paying much attention to searching the substitutable materials, which can enhance the removal efficiency and reduce the treatment cost (Eccles 1999; Liu et al. 2007).

### 22.2.3 Biological Methods

Biological methods of metal removal employ various microbes or plant species and are cost-effective as compared to physicochemical methods. Many new materials have been reported to be able to remove cadmium from water system, such as white-rot fungus (Arıca et al. 2001), dead biomass (Cruz et al. 2004; Zhou et al. 2007), rice or wheat milling by-products (Tarley and Arruda 2004), brown seaweed biomass (Vannela and Verma 2006), chitin (Benguella and Benaissa 2002), and so on. These biomaterials are of low cost, or even the waste from industrial or agricultural by-product. Biological methods are generally considered as environmentally friendly. Furthermore, they can lead to complete mineralization of organic pollutants at relatively low costs (Minke and Rott 1999; Sen and Demirer 2003).

Mónica et al. (2005) has elucidated in her study that the use of biological processes with sulfate-reducing bacteria (SRB) has great potential within environmental biotechnology. The aim of this study was to develop a bioremediation system, using a mixed culture of SRB, for the treatment of AMD from São Domingos mine as acid mine drainage (AMD) causes several environmental problems in many countries. Sulfate and heavy metal (Fe, Cu, and Zn) concentrations, pH, and Eh were monitored during 243 days. The process that was developed consisted of two stages that proved highly efficient at AMD neutralization and the removal of sulfates and the heavy metals iron, copper, and zinc.

#### 22.2.3.1 Aerobic Methods

Aerobic treatment systems such as the conventional activated sludge (CAS) process are widely adopted for treating low strength wastewater (<1,000 mg COD/L) like municipal wastewater. CAS process is energy intensive due to the high aeration requirement, and it also produces large quantity of sludge (about 0. 4 g dry weight $g^{-1}$ COD removed) that has to be treated and disposed of. As a result, the operation and maintenance cost of a CAS system is considerably high. Anaerobic processes for domestic wastewater treatment are an alternative, which is potentially more cost-effective, particularly in the subtropical and tropical regions where the climate is warm consistently throughout the year. Anaerobic wastewater purification processes have been increasingly used in the last few decades. These processes are important because they have positive effects: removal of higher organic loading, low sludge production and high pathogen removal, methane gas production, and low energy consumption (Nykova et al. 2002).

#### 22.2.3.2 Anaerobic Methods

Anaerobic digestion is characterized by the complete biodegradation of organic matter into methane and carbon dioxide in discrete steps, by the combined action of numerous groups of microbes (McInerney and Bryant 1981). In the first step, fermentative bacteria (FB) hydrolyze the polymeric substrates, such as polysaccharides, proteins, and lipids, and ferment the hydrolysis products to acetate and longer chain fatty acids, $CO_2$, formate, $H_2$, $NH_4$, and $H_2S$. In the next step, a group of organisms, called proton-reducing acetogenic bacteria, degrade propionate and longer chain fatty acids, alcohols, amino acids, and aromatic compounds to the methanogenic substrates, $H_2$, formate, and acetate. The degradation of these compounds with hydrogen production is thermodynamically unfavorable unless the concentration of $H_2$ or formate is kept low by $H_2$-utilizing bacteria as methanogens (McInerney and Bryant 1981).

#### 22.2.3.3 Advantages of Anaerobic Treatment

1. A high degree of waste stabilization is possible.
2. Low production of waste biological sludge.
3. Low nutrient requirements.
4. No oxygen requirements.
5. Methane is a useful end product (McCarty 1964).

The feasibility of high-rate anaerobic wastewater treatment (AnWT) systems for cold wastewater depends primarily on:

1. The quality of the seed material used and its development under sub-mesophilic conditions.
2. An extremely high sludge retention time under high hydraulic loading conditions because little if any viable biomass can be allowed to wash out from the reactor.
3. An excellent contact between retained sludge and wastewater to utilize all the available capacity within the bioreactor.
4. The types of the organic pollutants in the wastewater.
5. The reactor configuration, especially its capacity to retain viable sludge. It is not clear, as yet, whether high-rate

psychrophilic anaerobic wastewater treatment requires the development of psychrophilic or psychro-tolerant subpopulations, nor to what extent mesophilic sludges can become psychro-tolerant (McCarty 1964).

### 22.2.4 Plant-Based Metal Adsorbents

The adsorption process is one of the proficient methods to eliminate pollutants from effluents (Imagawa et al. 2000; Mohan et al. 2001; Malik 2003). During adsorption, there is the amassing of certain element or substance in the interface between two phases, i.e., between the solid surface and the adjacent solution (Sousa et al. 2007; Karnitz et al. 2009). Biosorption has become known as a potential and cost-effective alternative for heavy metal exclusion from aqueous solution. Volesky (1990) has long been reviewing the use of algae. Agricultural by-products have also offered a potential alternative as biosorbents for heavy metals among the existing techniques and are yet a subject of broad studies. Agricultural biosorbents including soybean hulls, peanut hulls, almond hulls, cottonseed hulls, and corncobs have also been proven to take out heavy metal ions (Wartelle and Marshall 2000; Marshall et al. 2000).

Lignocellulosic materials (e.g., agricultural by-products) are currently used in many different technological applications (Álvarez et al. 2007). Metal ion binding to lignocellulosic adsorbents occurs through chemical functional groups, such as carboxyl, amino, or phenolic groups. Gardea-Torresdey et al. (1990) demonstrated that carboxyl groups found on the cell walls of dead algal biomass are partially responsible for Cu binding. The characteristics of each adsorbent depend on its physical and chemical properties. Some of these materials need chemical activation to be used as adsorbents, for example, peach pits (Molina et al. 1996).

#### 22.2.4.1 Bark

The efficacy of the bark of *Eucalyptus tereticornis* (Smith) as an adsorbent for the removal of metal ions and sulfate from acid mine water was assessed. About 96 % of Fe, 75 % of Zn, 92 % of Cu, and 41 % of sulfate removal were achieved from the acid mine water of pH 2.3 with a concomitant increase in pH value by about 2 units after interaction with the tree bark, under appropriate conditions (Chockalingam and Subramanian 2009).

#### 22.2.4.2 Husk

Rice husk, an undesirable agriculture mass residue in Egypt, is a by-product of the rice milling industry. It is one of the most important agricultural residues in quantity. It represents about 20 % of the whole rice produced, on weight basis of the whole rice (Daifullah et al. 2003). The estimated annual rice production of 500 million tons in developing countries, approximately 100 million tons of rice husks is available for utilization in these countries alone. Traditionally, rice husks have been used in manufacturing block employed in civil construction as panels and were used by the rice industry itself as a source of energy for boilers (Della et al. 2001). However, the amounts of rice husk available are so far in excess of any local uses and have posed disposal problems. It was chosen because of its granular structure, chemical stability, and its local availability at very low cost and there is no need to regenerate them due to their low production costs.

Tarley and Arruda (2004) reported that adsorption of $Cd^{2+}$ increases by almost double when rice husk as an adsorbent was treated with NaOH. The reported adsorption capacities of $Cd^{2+}$ were 7 and 4 mg $g^{-1}$ for NaOH-treated and unmodified rice husk, respectively. Liu et al. (2006) demonstrated that $Pb^{2+}$, $Cr^{3+}$, and $Cu^{2+}$ could be effectively adsorbed by sulfuric acid-modified peanut husk.

#### 22.2.4.3 Activated Carbon

Activated carbon is the most widely used adsorbent for this purpose because of its extended surface area, microporous structure, high adsorption capacity, and high degree of surface reactivity. However, commercially available activated carbons are very expensive. In addition, the laboratory preparation of activated carbons has been accompanied by a number of problems such as combustion at high temperature, pore blocking, and hygroscopicity. Bilal et al. (2013) have reviewed various low-cost adsorbents for the removal of various metals from aqueous solutions.

#### 22.2.4.4 Maize Tassel

The possibility of using maize tassel as an alternative adsorbent for the removal of chromium (VI) and cadmium (II) ions from aqueous solutions was investigated by Zvinowanda et al. (2009). The effect of pH, solution temperature, contact time, initial metal ion concentration, and adsorbent dose on the adsorption of chromium (VI) and cadmium (II) by tassel was investigated using batch methods. Adsorption for both chromium (VI) and cadmium (II) was found to be highly pH dependent compared to the other parameters investigated. Obtained results gave an adsorption capacity of 79.1 % for chromium (VI) at pH 2, exposure time of 1 h at 25 °C. Maximum capacity of cadmium of 88 % was obtained in the pH range of 5–6 at 25 °C after exposure time of 1 h. The adsorption capacities of tassel for both chromium (VI) and cadmium (II) were found to be comparable to those of other commercial adsorbents currently in use for the removal of heavy metals from aqueous wastes. These results have demonstrated the immense potential of maize tassel as an alternative adsorbent for toxic metal ion remediation in polluted water and wastewater (Zvinowanda et al. 2009).

#### 22.2.4.5 Nutshells

Ajmal et al. (2006) has developed new low-cost adsorbent using ground nutshells, an agricultural waste, for the removal of cadmium and lead ions from aqueous solution. Almond is the seed of almond tree and classified into two categories: sweet (*Prunus amygdalus* var. *dulcis*) and bitter (*Prunus amygdalus* var. *amara*). In comparing the parameters of the models, it was observed that the affinity of almond shells for adsorption of lead is stronger than affinity for adsorption of cadmium.

Peanut shells of mesh size 10 ± 20 were modified by combinations of treatments following a 32 factorial design. Treatments consisted of either no wash, water wash, or base wash followed by no modification or modification with 0.6 M citric acid or 0.6 M phosphoric acid. The nine samples were evaluated for their uptake of five metal ions (Cd (II), Cu (II), Ni (II), Pb (II), and Zn (II)) from solution. The results were compared with metal ion adsorption by three commercial cation exchange resins, namely, Amberlite1 200, Amberlite1 IRC 718, and Duolite1 GT-73. The percent of metal ions adsorbed per gram of adsorbent was significantly increased by each of the acid treatments; average values ranged from 19 to 34 % compared with nonacid-treated samples at 5.7 %. The percent of metal ions adsorbed for base-washed samples was higher than water-washed or unwashed shells. Interaction between wash and acid treatment was not significant for most of the experimental conditions used. Acid-treated samples were as effective as Duolite1 GT-73 in the adsorption of Cd (II) and almost twice as effective in the adsorption of Zn (II) from solutions containing a single metal ion. In solutions containing multiple metal ions, citric acid samples were found to be most effective and selective for Cu (II) compared with Cd (II), Ni (II), and Zn (II). In general, phosphoric acid-modified shells removed the most metals from solution for the experimental samples and were more effective in removing Cd (II) and Zn (II) than two of the three commercial resins. Acid-modified peanut shells are promising as metal ion adsorbents (Wafwoyo et al. 1999).

#### 22.2.4.6 Bagasse Fly Ash

The bagasse fly ash, an industrial solid waste of sugar industry, was used for the removal of cadmium and nickel from wastewater. As much as 90 % removal of cadmium and nickel is possible in about 60 and 80 min, respectively, under the batch test conditions. Effect of various operating variables, viz., solution pH, adsorbent dose, adsorbate concentration, temperature, particle size, etc., on the removal of cadmium and nickel has been studied. Maximum adsorption of cadmium and nickel occurred at a concentration of 14 and 12 mg $L^{-1}$ and at a pH value of 6.0 and 6.5, respectively. A dose of 10 g $L^{-1}$ of adsorbent was sufficient for the optimum removal of both the metal ions (Vinod et al. 2003).

Grape bagasse generated in the wine production process was characterized through X-ray diffractometry, Fourier transform infrared spectroscopy, scanning electron microscopy, nuclear magnetic resonance, and thermogravimetric analysis. The efficiency of this natural material for Cd (II) and Pb (II) adsorption was evaluated using a batch adsorption technique. Factors affecting metal adsorption such as pH and contact time were investigated. Maximum adsorption was found to occur at pH 7.0 and 3.0 for Cd (II) and Pb (II), respectively, and a contact time of 5 min was required to reach equilibrium for both metals. With these conditions, adsorption studies were performed using a single solution. In addition, to calculate the adsorption capacities for each metal, the Langmuir isotherm model was used. The adsorption capacities were found to be 0.479 and 0.204 mmol g $L^{-1}$ for Cd (II) and Pb (II), respectively. The results showed that grape bagasse could be employed as a low-cost alternative adsorbent for effluent treatment (Farinella et al. 2007).

Low et al. (1995) used nitric acid-modified banana pith as an adsorbent and reported the maximum adsorption capacity of 13.46 mg $g^{-1}$ for $Cu^{2+}$. Karnitz et al. (2007) showed that sugarcane bagasse pretreated with sodium bicarbonate could adsorb heavy metals effectively. The reported adsorption capacities of $Cu^{2+}$, $Pb^{2+}$, and $Cd^{2+}$ were 114, 196, and 189 mg $g^{-1}$, respectively.

## 22.3 Phytoremediation

Phytoextraction is the cheapest method to remediate the polluted soils. The only thing that could be done with is to develop an appropriate gainful biological soil remediation method to eliminate pollutants devoid of affecting soil productiveness. For metal remediation phytoremediation may possibly offer sustainable techniques. In phytoremediation plants are used to eliminate, transport, stabilize, and/or degrade noxious pollutants in soil, sediment, and water (Hughes et al. 1997). The inspiration of using plants for environmental remediation is very ancient and cannot be traced to any meticulous source. In the course of progressively mesmerizing scientific innovations, pooled with interdisciplinary explorations, phytoremediation is considered to be environmentally friendly technology. The word phytoremediation ("phyto" meaning plant and the Latin suffix "remedium" meaning to clean or restore) ascribes to a variety of technologies based on plants that may use naturally occurring as well as the genetically engineered plants to clean up the contaminated environments (Cunningham et al. 1997; Flathman and Lanza 1998). Various plants which are raised on metalliferous soils have adopted the capacity to mount up substantial amounts of native metals in their tissues without any indication of toxicity (Baker and Brooks 1989; Baker et al. 1991; Entry et al. 1999). Utsunamyia (1980) and Chaney et al. (1997) have reintroduced the phytoremediation technology to remove metals from contaminated soil. Baker et al. (1991) has conducted the first field tryout on Zn and Cd phytoextraction. Several comprehensive reviews have been written, summarizing many important aspects of this novel plant-based technology, giving general guidance and recommendations for applying phytoremediation, and highlighting the processes associated with applications and underlying biological mechanisms (Salt et al. 1995, 1998; Chaney et al. 1997; Raskin et al. 1997; Chaudhry et al. 1998; Wenzel et al. 1999a, b; Meagh 2000; Navari-Izzo and Quartacci 2001; Garbisu and Alkorta 2001; Lasat 2002; McGrath et al. 2002; McGrath and Zhao 2003a, b; McIntyre 2003; Singh et al. 2003; Prasad and Freitas 2003; Alkorta et al. 2004; Ghosh and Singh 2005; Pilon-Smits 2005).

Different plant species have different capacities for uptaking and tolerating the heavy metals like cadmium and others (Roosens et al. 2003; Bert et al. 2002). The metal hyperaccumulators show an extra aptitude for accumulating the large quantity of metals in their aerial parts (Baker and Walker 1990). This special characteristic of the metal hyperaccumulators makes them extremely appropriate for phytoremediation, i.e., to use plants for cleaning up the polluted soils. In the preceding decade, many studies have been accomplished to explore the mechanisms liable for the better metal uptake and tolerance via natural hyperaccumulators as model plant species (Lombi et al. 2001a, b). In general the metal hyperaccumulation in plants is acknowledged as a mishmash of high metal uptake coupled with an improved tissue tolerance against the detrimental effects of higher metal concentrations through a better antioxidative response and sequestration at the cellular level (Rauser 2002; Shaw et al. 2004). Remediation of heavy metal-contaminated soil may possibly be carried out using physicochemical processes such as ion exchange, precipitation, reverse osmosis, evaporation, and chemical reduction, however, the procedures require man-made resources and expensive chemicals (Lasat 2002).

Phytoremediation has been considered recently as useful technology in which plant is applied to absorb, renovate, and detoxify heavy metals. The phytoremediation method was simple, efficient, cost-effective, and environment friendly (Schnoor and McCutcheon 2003). Research on the capability of plants in eliminating heavy metals from soil has been strengthened by using *Polygonum hydropiper* L., *Rumex acetosella* L. (Wang et al. 2003), *Lolium perenne* (O'Connor et al. 2003), *Brassica juncea Helianthus annuus* and *Brassica napus* (Solhi et al. 2005), *Streptanthus polygaloides*, *Sebertia acuminata*, *Armeria maritima*, *Aeollanthus biformifolius*, grass, water hyacinth, and sunflower (Ghosh and Singh 2005).

Currently phytoremediation has grabbed more attention due to low cost of implementation and environmental benefits. The elevated levels of heavy metals in the environment would affect soil and water quality which consequently hampers plant growth. Conventional cleanup technologies are generally too expensive to be used for restoration of contaminated soils and are often harmful to the normal properties of soil (Holden 1989). The emerging phytoremediation techniques, with their low cost and environmental friendly nature, have received increasing attention in the last decades (Salt et al. 1998). Over 400 hyperaccumulation plant species from all over the world can accumulate high concentrations of metals from contaminated soils (Baker et al. 2000).

### 22.3.1 Categories of Phytoremediation

Different phytoremediation technologies can be used for the containments (phytoimmobilization and phytostabilization) or elimination (phytoextraction and phytovolatilization) depending on the contaminants, the site conditions, the level of cleanup required, and the types of plant phytoremediation technology (Thangavel and Subhuram 2004). The four different plant-based technologies of phytoremediation, each having a different mechanism of action for remediating metal-polluted soil, sediment, or water:

1. Phytostabilization, where plants stabilize, rather than remove contaminants by plant root metal retention
2. Phytofiltration, involving plants to clean various aquatic environments
3. Phytovolatilization, utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization
4. Phytoextraction, in which plants absorb metals from soil and translocate them to harvestable shoots where they accumulate

Ecological issues also need to be evaluated when developing a phytoremediation strategy for a polluted site. In particular, one has to consider how the phytoremediation efforts might affect local ecological relationships, especially those involving other crops. Since the phytoremediation plants will be grown under contaminated soil/water conditions, where other crops may not thrive because of contaminant toxicities, the competition problem is unlikely to arise.

### 22.3.2 Phytostabilization

Phytostabilization uses certain plant species to immobilize contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone, and physical stabilization of soils. This process reduces the mobility of contaminants and prevents migration to groundwater or air. This can reestablish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations (Tordoff et al. 2000). Thorough planning is essential for successful revegetation, including physical and chemical analyses, bioassays, and field trials. Metal-tolerant species may be used to restore vegetation to such sites, thereby decreasing the potential migration of contaminants through wind, transport of exposed surface soils, leaching of soil, and contamination of groundwater (Stoltz and Greger 2002). Unlike other phytoremediative techniques, phytostabilization is not intended to remove metal contaminants from a site, but rather to stabilize them by accumulation in roots or precipitation within root zones, reducing the risk to human health and the environment. It is applied in situations where there are potential human health impacts, and exposure to substances of concern can be reduced to acceptable levels by containment. The disruption to site activities may be less than with more intrusive soil remediation technologies. Phytostabilization is most effective for fine-textured soils with high organic-matter content, but it is suitable for treating a wide range of sites where large areas are subject to surface contamination (Cunningham et al. 1997; Berti and Cunningham 2000). However, some highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is impossible (Berti and Cunningham 2000). Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, easier to implement, and preferable aesthetically (Berti and Cunningham 2000; Schnoor 2000). When decontamination strategies are impractical because of the extent of the contaminated area or the lack of adequate funding, phytostabilization is advantageous (Berti and Cunningham 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique. Characteristics of plants appropriate for phytostabilization at a particular site include: tolerance to high levels of the contaminant(s) of concern; high production of root biomass able to immobilize these contaminants through uptake, precipitation, or reduction; and retention of applicable contaminants in roots, as opposed to transfer to shoots, to avoid special handling and disposal of shoots. Yoon et al. (2006) evaluated the potential of 36 plants (17 species) growing on a contaminated site and found that plants with a high bio-concentration factor (BCF, metal concentration ratio of plant roots to soil) and low translocation factor (TF, metal concentration ratio of plant shoots to roots) have the potential for phytostabilization. The lack of appreciable metals in shoot tissue also eliminates the necessity to treat harvested shoot residue as a hazardous waste (Flathman and Lanza 1998). In a field study, mine wastes containing copper, lead, and zinc were stabilized by grasses (*Agrostis tenuis* cv. Goginan for acid lead and zinc mine wastes, *Agrostis tenuis* cv. Parys for copper mine wastes, and *Festuca rubra* cv. Merlin for calcareous lead and zinc mine wastes) (Smith and Bradshaw 1992). The research of Smith and Bradshaw (1992) led to the development of two cultivars of *Agrostis tenuis* Sibth and one of *Festuca rubra* L which are now commercially available for phytostabilizing Pb-, Zn-, and Cu-contaminated soils. Stabilization also involves soil amendments to promote the formation of insoluble metal complexes that reduce biological availability and plant uptake, thus preventing metals from entering the food chain (Adriano et al. 2004; Berti and Cunningham 2000; Cunningham et al. 1997). One way to facilitate such immobilization is by altering the physicochemical properties of the metal–soil complex by introducing a multipurpose anion, such as phosphate, that enhances metal adsorption via anion-induced negative charge and metal precipitation (Bolan et al. 2003). Addition of humified organic matter (OM) such as compost, together with lime to raise soil pH (Kuo et al. 1985), is a common practice for immobilizing heavy metals and improving soil conditions, to facilitate revegetation of contaminated soils (Williamson and Johnson 1981).

### 22.3.3 Phytofiltration

Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from water and aqueous waste streams (Prasad and Freitas 2003). Plant roots or seedlings grown in aerated water absorb, precipitate, and concentrate toxic metals from polluted effluents (Dushenkov and Kapulnik 2000; Elless et al. 2005). Mechanisms involved in biosorption include chemisorption, complexation, ion exchange, micro precipitation, hydroxide condensation onto the biosurface, and surface adsorption (Gardea-Torresdey et al. 2004). Rhizofiltration uses terrestrial plants instead of aquatic plants because the former feature much larger fibrous root systems covered with root hairs with extremely large surface areas. Metal pollutants in industrial-process water and in groundwater are most commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge (Ensley 2000). The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov et al. 1995; Salt et al. 1995; Flathman and Lanza 1998; Zhu et al. 1999). Root exudates and changes in rhizosphere pH may also cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza 1998; Zhu et al. 1999). Dushenkov et al. (1995), Salt et al. (1995), and Flathman and Lanza (1998) contend that plants for phytoremediation should accumulate metals only in the roots. Dushenkov et al. (1995) explain that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. However, Zhu et al. (1999) suggest that the efficiency of the process can be increased by using plants with a heightened ability to absorb and translocate metals. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes*, Zhu et al. 1999), pennywort (*Hydrocotyle umbellata* L., Dierberg et al. 1987), and duckweed (*Lemna minor* L., Mo et al. 1989). However, these plants have limited potential for rhizofiltration because they are not efficient in removing metals as a result of their small, slow-growing roots (Dushenkov et al. 1995). The high water content of aquatic plants complicates their drying, composting, or incineration. In spite of limitations, Zhu et al. (1999) indicated that water hyacinth is effective in removing trace elements in waste streams. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for removing metals from water. The roots of Indian mustard are effective in capturing Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al. 1995), whereas sunflower removes Pb (Dushenkov et al. 1995), U (Dushenkov et al. 1997a), 137 Cs, and 90 Sr (Dushenkov et al. 1997b) from hydroponic solutions. A novel phytofiltration technology has been proposed by Sekhar et al. (2004) for removal and recovery of lead (Pb) from wastewaters. This technology uses plant-based biomaterial from the bark of the plant commonly called Indian sarsaparilla (*Hemidesmus indicus*). The target of their research was polluted surface water and groundwater at industrially contaminated sites. Cassava waste biomass was also effective in removing two divalent metal ions, Cd (II) and Zn (II), from aqueous solutions (Horsfall and Abia 2003). Modification of the cassava waste biomass by treating it with thioglycolic acid resulted in increased adsorption rates for Cd, Cu, and Zn (Abia et al. 2003). Several species of Sargassum biomass (nonliving brown algae) were effective biosorbents for heavy metals such as Cd and Cu (Davis et al. 2000). Plants used for phytofiltration should be able to accumulate and tolerate significant amounts of the target metals, in conjunction with easy handling, low maintenance costs, and a minimum of secondary waste requiring disposal. It is also desirable for plants to produce significant amounts of root biomass or root surface area (Dushenkov and Kapulnik 2000).

### 22.3.4 Phytovolatilization

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in the environment. In recent years, researchers have sought naturally occurring or genetically modified plants capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization. Volatilization of Se from plant tissues may provide a mechanism of selenium detoxification. As early as 1894, Hofmeister proposed that selenium in animals is detoxified by releasing volatile dimethyl selenide from the lungs, based on the fact that the odor of dimethyl telluride was detected in the breath of dogs injected with sodium tellurite. Using the same logic, it was suggested that the garlicky odor of plants that accumulate selenium may indicate release of volatile selenium compounds. This is the most controversial of phytoremediation technologies. Hg and Se are toxic (Suszcynsky and Shann 1995), and there is doubt about whether the volatilization of these elements into the atmosphere is desirable or safe (Watanabe 1997). The volatile selenium compound released from the selenium accumulator *Astragalus racemosus* was identified as dimethyl diselenide (Evans et al. 1968). Selenium released from alfalfa, a selenium non-accumulator, was different from the accumulator species and was identified as dimethyl selenide. Lewis et al. (1966) showed that both selenium non-accumulator and accumulator species volatilize selenium. Selenium phytovolatilization has received the most attention to date (Lewis et al. 1966; Terry et al. 1992; Banuelos et al. 1993; McGrath 1998) because this element is a serious problem in many parts of the world where there are Se-rich soils. According to, the release of volatile Se compounds from higher plants was first reported by Lewis et al. (1966). Terry et al. (1992) report that members of the Brassicaceae are capable of releasing up to 40 g Se $ha^{-1}$ $day^{-1}$ as various gaseous compounds. Some aquatic plants, such as cattail (*Typha latifolia* L.), have potential for Se phytoremediation (Pilon-Smits et al. 1999). Volatile Se compounds such as dimethyl selenide are 1/600 to 1/500 as toxic as inorganic forms of Se found in soil (DeSouza et al. 2000). The volatilization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed, and gaseous species are not likely to redeposit at or near the site (Atkinson et al. 1990; Heaton et al. 1998). Furthermore, sites that utilize this technique may not require much management after the original planting. This remediation method has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al. 1998). Heaton et al. (1998) suggest that the transfer of Hg (O) to the atmosphere would not contribute significantly to the atmospheric pool. This technique appears to be a promising tool for remediating Se- and Hg-contaminated soils. Volatilization of arsenic as dimethylarsenite has also been postulated as a resistance mechanism in marine algae. However, it is not known whether terrestrial plants also volatilize arsenic in significant quantities. Studies on arsenic uptake and distribution in higher plants indicate that arsenic predominantly accumulates in roots and that only small quantities are transported to shoots. However, plants may enhance the biotransformation of arsenic by rhizospheric bacteria, thus increasing the rates of volatilization

(Salt et al. 1998). Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Some authors suggest that the addition to atmospheric levels through phytovolatilization would not contribute significantly to the atmospheric pool, since the contaminants are likely to be subject to more effective or rapid natural degradation processes such as photodegradation (Azaizeh et al. 1997). However, phytovolatilization should be avoided for sites near population centers and at places with unique meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al. 1998). Hence the consequences of releasing the metals to the atmosphere need to be considered carefully before adopting this method as a remediation tool.

### 22.3.5 Phytoextraction

Phytoextraction, also called phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into aboveground components of the plants. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept, whereas phytoextraction is a specific cleanup technology (Prasad and Freitas 2003). Certain plants, called hyperaccumulators, absorb unusually large amounts of metals compared to other plants and the ambient metal concentration. Natural metal hyperaccumulators are plants that can accumulate and tolerate greater metal concentrations in shoots than those usually found in non-accumulators, without visible symptoms. According to Baker and Brooks (1989), hyperaccumulators should have a metal accumulation exceeding a threshold value of shoot metal concentration of 1 % (Zn, Mn), 0.1 % (Ni, Co, Cr, Cu, Pb, and Al), 0.01 % (Cd and Se), or 0.001 % (Hg) of the dry weight shoot biomass. Over 400 hyperaccumulator plants have been reported, including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae. Recently Environment Canada has released a database "Phytorem" which contains a worldwide inventory of more than 750 terrestrial and aquatic plants, both wild and cultivated species and varieties, of potential value for phytoremediation. These plants are selected and planted at a site based on the metals' present and site conditions. After they have grown for several weeks or months, the plants are harvested. Planting and harvesting may be repeated to reduce contaminant levels to allowable limits (Kumar et al. 1995). The time required for remediation depends on the type and extent of metal contamination, the duration of the growing season, and the efficiency of metal removal by plants, but it normally ranges from 1 to 20 years (Kumar et al. 1995; Blaylock and Huang 2000). This technique is suitable for remediating large areas of land contaminated at shallow depths with low to moderate levels of metal contaminants (Kumar et al. 1995; Blaylock and Huang 2000).

Two basic strategies of phytoextraction are being developed: chelate-assisted phytoextraction, which we term induced phytoextraction, and long-term continuous phytoextraction. If metal availability is not adequate for sufficient plant uptake, chelates or acidifying agents may be added to the soil to liberate them (Cunningham and Ow 1996; Huang et al. 1997; Lasat et al. 1998). However, side effects of the addition of chelate to the soil microbial community are usually neglected. It has been reported (Wu et al. 1999) that many synthetic chelators capable of inducing phytoextraction might form chemically and microbiologically stable complexes with heavy metals, threatening soil quality and groundwater contamination. Several chelating agents, such as EDTA (ethylenediaminetetraacetic acid), EGTA (ethylene glycol-O,O′-bis-[2-amino-ethyl] *N,N,* *N′*,*N′*,-tetra acetic acid), EDDHA (ethylenediamine di o-hydroxyphenylacetic acid), EDDS (ethylene diamine disuccinate), and citric acid, have been found to enhance phytoextraction by mobilizing metals and increasing metal accumulation (Tandy et al. 2006; Cooper et al. 1999). The increase in the phytoextraction of Pb by shoots of *Z. mays* L. was more pronounced than the increase of Pb in the soil solution with combined application of EDTA and EDDS (Luo et al. 2006). Although EDTA was, in general, more effective in soil metal solubilization, EDDS, less harmful to the environment, was more efficient in inducing metal accumulation in *B. decumbens* shoots (Santos et al. 2006). However, there is a potential risk of leaching of metals to groundwater and a lack of reported detailed studies regarding the persistence of metal-chelating agent complexes in contaminated soils (Lombi et al. 2001a, b).

Use of high biomass crops such as the willow *Salix viminalis* to extract metals for soil remediation has been proposed as an alternative to the low biomass-producing hyperaccumulating plants. High yields compensate for the moderate heavy metal concentrations in the shoots of such species. The first long-term trials reported were using *Salix viminalis* to extract heavy metals from two contaminated soils: one calcareous (5 years) and one acidic (2 years). Total metals extracted by the plants were 170 g Cd ha ±1 and 13.4 kg Zn ha ±1 from the calcareous soil after 5 years and 47 g Cd ha ±1 and 14.5 kg Zn ha ±1 from the acidic soil after 2 years; in the first year outputs were negligible. After 2 years, Salix had performed better on the acidic soil because of larger biomass production and higher metal concentrations in shoots. Addition of elemental sulfur to the soil did not yield any additional benefit (Hornung 1997). In the long term, application of Fe chelate improved the biomass production. Cd and Zn concentrations were significantly higher in leaves than stems, highlighting the necessity to collect leaves as well as shoots. On both soils, concentration in shoots decreased with time, indicating a decrease in extraction efficiency (Hammer et al. 2003).

### 22.3.6 Successful Factors for Phytoextraction of Heavy Metals

As a plant-based technology, the success of phytoextraction is inherently dependent on several plant characteristics, the two most important being the ability to accumulate large quantities of biomass rapidly and the capacity to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar et al. 1995; Cunningham and Ow 1996; McGrath 1998; Pilon-Smits 2005). Effective phytoextraction requires both plant genetic ability and the development of optimal agronomic practices, including:

1. Soil management practices to improve the efficiency of phytoextraction
2. Crop management practices to develop a commercial cropping system

Ebbs et al. (1997) reported that *B. juncea*, while having one-third the concentration of Zn in its tissue, is more effective at removing Zn from soil than *Thlaspi caerulescens*, a known hyperaccumulator of Zn. The advantage is due primarily to the fact that B. *juncea* produces ten times more biomass than *T. caerulescens*. Plants for phytoextraction should be able to grow outside their area of collection, have profuse root systems, and be able to transport metals to their shoots. They should have high metal tolerance, be able to accumulate several metals in large amounts, exhibit high biomass production and fast growth, resist diseases and pests, and be unattractive to animals, minimizing the risk of transferring metals to higher trophic levels of the terrestrial food chain (Thangavel and Subhuram 2004). Phytoextraction is applicable only to sites containing low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow normal cultivation practices, utilizing agricultural equipment. Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth. Basic et al. (2006a, b) investigated the parameters influencing the Cd concentration in plants, as well as the biological implications of Cd hyperaccumulation in nine natural populations of *T. caerulescens*. Cd concentrations in the plant were positively correlated with plant Zn, Fe, and Cu concentrations. The physiological and/or molecular mechanisms for uptake, transport, and/or accumulation of these four heavy metals interact with each other. They specified a measure of Cd hyperaccumulation capacity by populations and showed that *T. caerulescens* plants originating from populations with high Cd hyperaccumulation capacity had better growth, by developing more and bigger leaves, taller stems, and produced more fruits and heavier seeds. Liu et al. (2006) conducted a survey of Mn mine tailing soils and eight plants growing on Mn mine tailings. The concentrations of soil Mn, Pb, and Cd and the metal-enrichment traits of these eight plants were analyzed. It was found that *Poa pratensis*, *Gnaphalium affine*, *Pteris vittata*, *Conyza canadensis*, and *Phytolacca acinosa* possessed specially good metal-enrichment and metal-tolerant traits. In spite of the high concentration of Mn *in P. pratensis*, its life cycle was too short, and its shoots were too difficult to collect for it to be suitable for soil remediation. The effectiveness of phytoextraction of heavy metals in soils also depends on the availability of metals for plant uptake (Liu et al. 2006). The rates of redistribution of metals and their binding intensity are affected by the metal species, loading levels, aging, and soil properties (Han et al. 2003).

Understanding the mechanisms of rhizosphere interaction, uptake, transport, and sequestration of metals in hyperaccumulator plants will lead to designing novel transgenic plants with improved remediation traits (Eapen and D'Souza 2005). Moreover, the selection and testing of multiple hyperaccumulator plants could enhance the rate of phytoremediation, giving this process a promise for bioremediation of environmental contamination (Suresh and Ravishankar 2004). Phytoremediation has been combined with electrokinetic remediation, applying a constant voltage of 30 V across the soil. The combination of both techniques could represent a very promising approach to the decontamination of metal-polluted soils (O'Connor et al. 2003).

### 22.3.7 Translocation of Absorbed Pollutants

Translocation from root to shoot requires a membrane transport step from root symplast into xylem apoplast. The impermeable suberin layer in the cell wall of the root endodermis (Casparian strip) prevents solutes from flowing straight from the soil solution or root apoplast into the root xylem (Taiz and Zieger 1999). Organic pollutants pass the membrane between root symplast and xylem apoplast via simple diffusion. Transpiration stream concentration factor (TSCF) is the ratio of the concentration of a compound in the xylem fluid relative to the external solution and is a measure of uptake into the plant shoot. Entry of organic pollutants into the xylem depends on similar passive movement over membranes as their uptake into the plants. Mass flow in the xylem from the shoot creates negative tension in the xylem that pulls up water and solutes (Taiz and Zieger 1999). Plant transpiration depends on plant properties and environmental conditions. Plant species differ in transpiration rate, due to metabolic differences (e.g., C3/C4/CAM photosynthetic pathway) and anatomical differences (e.g., surface to volume ratio, stomatal density, rooting depth) (Taiz and Zieger 1999). Transpiration is maximal at high temperature, moderate wind, low relative air humidity, as well as light intensity (Taiz and Zieger 1999).

Plant can tolerate wide range of environmental conditions. Enzyme and protein constitution of plants are of immense benefit for phytoremediation. Sedentary nature of most plants is of advantage since they can over time develop mechanisms to acquire nutrients, detoxify pollutants, and control local geochemical conditions. Infiltration is a primary pathway in contaminant migration to groundwater, and plants play an important role in regulating water content in soil. Roots of plants supplement microbial nutrient and provide aeration to the soil, consequently increasing microbial population compared to non-vegetated area. Above all, phytoremediation gives better aesthetic appeal than other physical means of remediation (Nwoko 2010).

### 22.3.8 Mechanism of Metal Uptake by Plant

Heavy metal pollution of the environment is increasing manyfold day by day, due to industrialization and drastic increase in population; anthropogenic input of heavy metals into the environment such as synthetic fertilization, fossil fuel burning, pesticide use, smelting industries, mining, and exhaust from automobiles; and emissions from municipal wastes (Revathi and Venugopal 2013; Sebastiani et al. 2004). Due to the persistent nature and biomagnification potential of heavy metal, their contamination in environment gets increased many a times (Dalvi and Bhalera 2013; Revathi and Venugopal 2013).

The toxicity of heavy metals threatens the existence and prevalence of life on Earth. Higher concentrations of essential as well as nonessential heavy metals that adversely affect the biochemical and physiological processes within the plant may result in appearance of toxicity symptoms, inhibited growth, and ultimately death of the plant (Dalvi and Bhalera 2013; Revathi and Venugopal 2013; Hall 2002). However, many of the heavy metals such as Fe, Zn, Cu, Ni, and Co are essential micronutrients and are integral part of various proteins and enzymes (Dalvi and Bhalera 2013). Considering the threads of heavy metal pollution, an effective, viable, and affordable solution is needed.

Phytoremediation is viable, economical, aesthetically pleasant, and environment friendly which has proved an effective mean of remediating heavy metal contamination (Sebastiani et al. 2004). Worldwide, there are about 400 plant species that are capable of accumulating metal contamination and can be used for remediating metal-contaminated soil and water (Memon and Schröder 2009; Smits 2005). Various plants such as *Pteris vittata* L., *Piricum sativum* L. (As), *Brassica juncea* L. (Cr), *Acanthopanax sciadophylloides*, *Maytenus founieri* (Mn), *Sasa borealis*, *Alyssum* sp. (Ni), *Clethra barbinervis* (Co), *Chenopodium album* L., *Vetiveria zizanioides* L., *Thlaspi rotundifolium* ssp. *cepaeifolium*, *T. caerulescens*, *Sesbania drummondii* (Pb), *Clethra barbinervis*, *Ilex crenata*, *Thlaspi caerulescens*, *Arabidopsis halleri* (Cd, Zn), *Marrubium vulgare* L. (Hg), and *Brassica juncea* Czern L. (Se) (Memon and Schröder 2009; Gonzaga et al. 2006; Jonnalagadda and Nenzou 1997; Zhang et al. 2007; Assunçao et al. 2003; McGrath et al. 2006; Baker et al. 2000; Reeves et al. 2001; Memon et al. 2001; Rotkittikhun et al. 2007; Sahi et al. 2002; Celestino et al. 2006; Fernando et al. 2007; Jimenez et al. 2006) have proved their potential in remediating the heavy metal-contaminated sites. Phytoremediation also reduces the risk of exposure of hazardous contamination and thus is expanding rapidly (Maiti et al. 2004).

Various plant species have evolved a variety of in vitro and in vivo tolerance and accumulation mechanisms. Some plants effectively prevent metal from entering in their aerial parts and contain them in their roots. Some plants transport and accumulate metals in their aerial parts/aboveground tissues, and metal concentration in their tissues is almost the same as in the soil. These plant species accumulate metal concentration in their aboveground tissues far higher than the concentration in the soil.

Several heavy metal tolerance mechanisms within the plants have been suggested by various researchers (Mazen 2004; Hall 2002) including: (1) synthesis of metal-binding compounds such as amino acids, citric acid, malic acid, and phytochelatins (PCs) (Reilly 1972; Thurman and Rankin 1982; Brookes et al. 1981; Grill et al. 1985, 1987, Rtiegsegger and Brunold 1992; Cobbett and Goldsbrough 2002; Hall 2002); (2) alterations of membrane structures (De Vos et al. 1988); (3) complex formation in vacuoles (Fernando and Fernando 1994); and (4) synthesis of stress metabolites including proteins (Neumann et al. 1994). It is also evident that of the abovementioned mechanisms more than one mechanism may be working together in the same species (Hall 2002).

The following aspects of metal uptake by the plants are being discussed in the subsequent sections:

1. Rhizosphere
2. Transport through membranes
3. Translocation within plant

#### 22.3.8.1 Mechanisms Wherein Rhizosphere Helps in Metal Uptake

1. Exudation and/or complex formation in rhizosphere
2. pH alteration of rhizosphere
3. Mycorrhizal association

#### Exudation and/or Complex Formation in Rhizosphere

Generally the bioavailability of metals in the soil solution is thought to be as free metal and/or free ion or radical. Plants in their rhizosphere (0.2 mm near roots) solubilize and facilitate their speciation of metals through exudation or chelate formation (Dalvi and Bhalera 2013). Mostly two strategies are observed in different plant species, i.e., dicots and nongraminaceous monocots reduce the metals and bring them in the radical form and later release/excrete organic acids (histidine and citrate) with which these metal radicals form complexes and plants either absorb the free metal radicals or metal chelators like malate and citrate and/or metal complexes (Revathi and Venugopal 2013; Hall 2002), e.g., buckwheat under Al stress secreted/exudated oxalic acid and accumulated Al-oxalate (nontoxic) in the leaves (Hall 2002), whereas graminaceous monocots excrete mugineic and avenic acids (chelates), with which metal forms complexes and plants absorb these complexes (Reichman 2002). Most common root exudates are protons, bicarbonates, $CO_2$, allelopathic compounds, siderophores, mucilage, sugars, $H_2O$, and organic and inorganic acids (Dalvi and Bhalera 2013).

#### pH Alteration of Rhizosphere

Depending upon plant species, age of the plant, nutrient concentration, and the buffering capacity of the soil, plant can alter the pH of its rhizosphere by 2.5 units from the bulk soil solution (Reichman 2002). This is done by imbalance uptake of anions/cations, $H^+$ and/or $OH^-$excretion, enzyme, amino acid and/or organic acid production, and/or $CO_2$ production by the microbes present in the rhizosphere of the plant (Revathi and Venugopal 2013). As a result, metal solubilization, speciation, and availability to the plant get ensured in the rhizosphere as compared to the soil solution. It is evident that plants alter the pH of rhizosphere to increase or decrease the bioavailability of metal of concern (Reichman 2002).

#### Mycorrhizal Association

Under heavy metal stress mutualistic association of fungi and plant roots has been reported (Dalvi and Bhalera 2013). For plants capable of growing on heavy metal contamination, two most common mycorrhizal associations are arbuscular mycorrhizas (AM) and ectomycorrhizas (ECM) (Dalvi and Bhalera 2013). The mycorrhizal fungi avail the benefits of shelter and photosynthesis from the plant and in return increase the bioavailability of metal to the plant, by producing chelators and/or by altering the pH and by increasing the surface area of roots for plant. Such associations are found in *Vaccinium macrocarpon*, *Trifolium pratense* (Mn), and *Betula* sp. (Zn). These associations are plant specific, metal contamination type, and fungal species specific (Reichman 2002).

It is observed that overexpression of metal transporters and overproduction of metal-chelating agents such as citrate, metallothioneins, and phytochelatins facilitate metal tolerance and accumulation within the plant. Plants under metal stress form more phytochelatins (PC) and metallothioneins (MT) and, hence, are widely studied by many scientists.

### 22.3.8.2 Transport Through Membranes

Transport and uptake of each metal through cell membrane depends upon electrochemical potential gradient for each metal ion between root cells and rhizosphere, across the plasma membrane. Mostly, it is found in the range of 0.12 and 0.18 mV. Secondly, low activity of metal ions within the cytoplasm is maintained to avoid harmful redox reactions which can occur due to the prevalence of free ionic forms of metals. These two factors maintain a balanced passive gradient for metal uptake (Reichman 2002; Jabeen et al. 2009). Figure 22.1 shows a hypothetical scheme of cellular transport of metal within plant.

Various metal- and protein-specific transporters are involved in influx and efflux of heavy metals, details of which are shown in Table 22.1.

For metal tolerance, plants sequester the metal concentrations away at places within the cell where the metals cannot interact with metabolically active cellular substances.

### 22.3.8.3 Translocation Within Plant

1. Cellular exclusion
2. Complex formation at the cell wall–plasma membrane interface
3. Metal distribution within plant parts
4. Cellular complexation and compartmentation:
   (a) Phytochelatin complexation
   (b) Metallothionein complexation
   (c) Organic and amino acids
   (d) Vacuole compartmentation

Various metal tolerance mechanisms in plants have been illustrated in Fig. 22.2.

#### Cellular Exclusion

Metals translocate within the plant by two routes, either through apoplastic free spaces, e.g., in Al-resistant grasses, or through symplastic route, i.e., in Al-sensitive *T. aestivum* (Reichman 2002), while in some plant species, both apoplastic and symplastic transports of metals have been observed.

#### Complex Formation at the Cell Wall–Plasma Membrane Interface

Significant proportions of metal contamination have been found accumulated within the space of cell wall–plasma membrane interface, i.e., Cu was found bounded by the cell wall and plasma membrane in *T. pratense* and *Lolium multiflorum* and higher concentrations of Zn, Pb, Fe, Si, and Cu with no accumulation of these metals in the cytoplasm were found in the cell walls of *Minuartia verna* ssp. (Reichman 2002). Pectic sites, extracellular carbohydrates, and histidyl

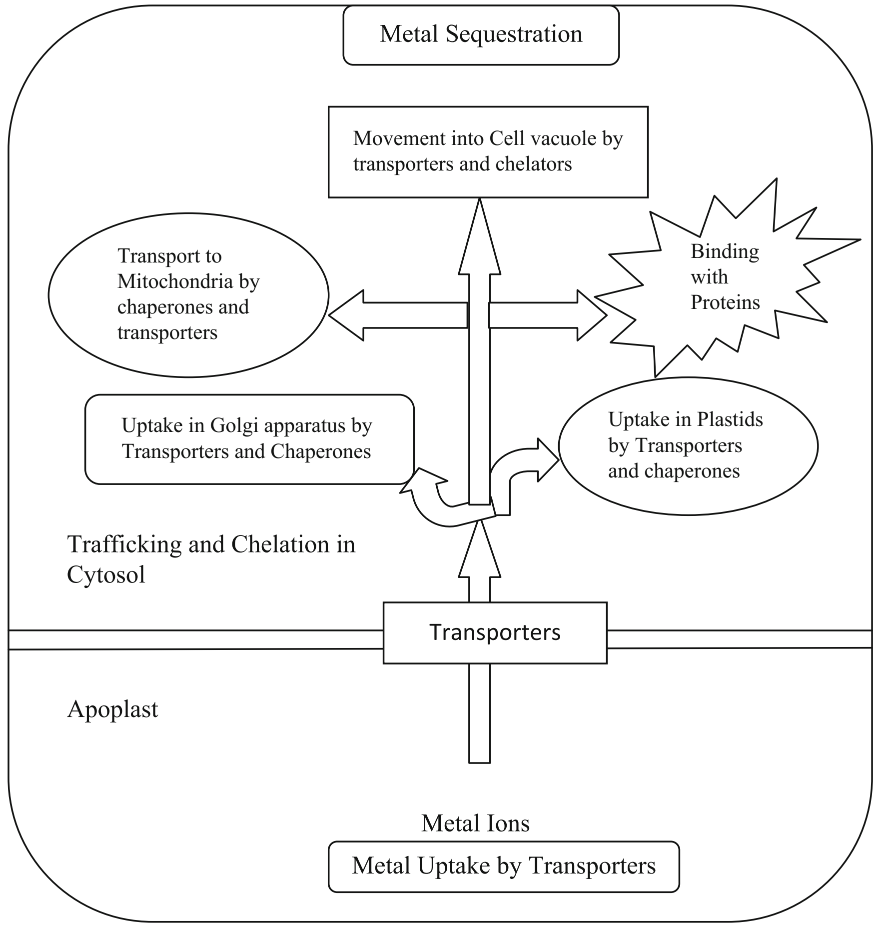


**Fig. 22.1** Schematic diagram of cellular transport of metals within plant

**Table 22.1** Various transporters of metals reported in plants

| Metal | Transporter | Plant | Reference |
|---|---|---|---|
| Zn | ZIP | *A. thaliana*, *O. sativa* | (Filatov et al. 2006; Ishimaru et al. 2005; Roosens et al. 2008; Memon and Schröder 2009) |
| Cu, Zn, Cd, Co, Pb | P-type ATPase | *A. thaliana*, *A. halleri*, *L. esculentum* | (Memon and Schröder 2009; Bernal et al. 2007; Courbot et al. 2007; Lee et al. 2007; Roosens et al. 2008; Talke et al. 2006; Willems et al. 2007; Xing et al. 2008) |
| Cd, Zn | Fe-regulated transporter (IRT) | *A. thaliana*, *T. caerulescens*, *L. esculentum*, *O. sativa*, *N. tabacum* | (Hodoshima et al. 2007; Memon and Schröder 2009; Kerkeb et al. 2008; Plaza et al. 2007) |
| Zn | Cation diffusion facilitator (CDF) | *A. thaliana*, *A. halleri*, *T. goesingense*, *N. tabacum*, *P. trichocarpa*, *P. deltoides* | (Kawachi et al. 2008; Memon and Schröder 2009; Shingu et al. 2005; Willems et al. 2007) |
| Fe, Cd | Natural resistance-associated macrophage proteins (Nramp) | *A. thaliana*, *A. halleri*, *T. caerulescens*, *G. max*, *O. sativa* | (Memon and Schröder 2009; Lanquar et al. 2005) |
| Metal stress | ABC transporters | *A. thaliana*, *O. sativa* | (Memon and Schröder 2009) |

groups of cell wall prevent the translocation of heavy metal and prevent their flow toward cell membrane (Dalvi and Bhalera 2013; Memon and Schröder 2009), as polygalacturonic acid in the pectins of cell wall serves as cation exchanger. Although cell wall is thought as a potential site for heavy metal accumulation, their role in the tolerance against heavy metal stress is not fully understood (Dalvi and Bhalera 2013; Memon and Schröder 2009). Under Cd stress, cell membrane changes are reported in wheat, sunflower, and *Nutella* (Hall 2002). ABC transporters are considered as the main transporters responsible for controlling the translocation of heavy metals through cell membrane. Pleiotropic drug resistance (PDR) and multidrug resistance-associated protein (MRP) are the two active ABC subfamilies of ABC transporters responsible for the sequestration of heavy metals, after chelation (Dalvi and Bhalera 2013).

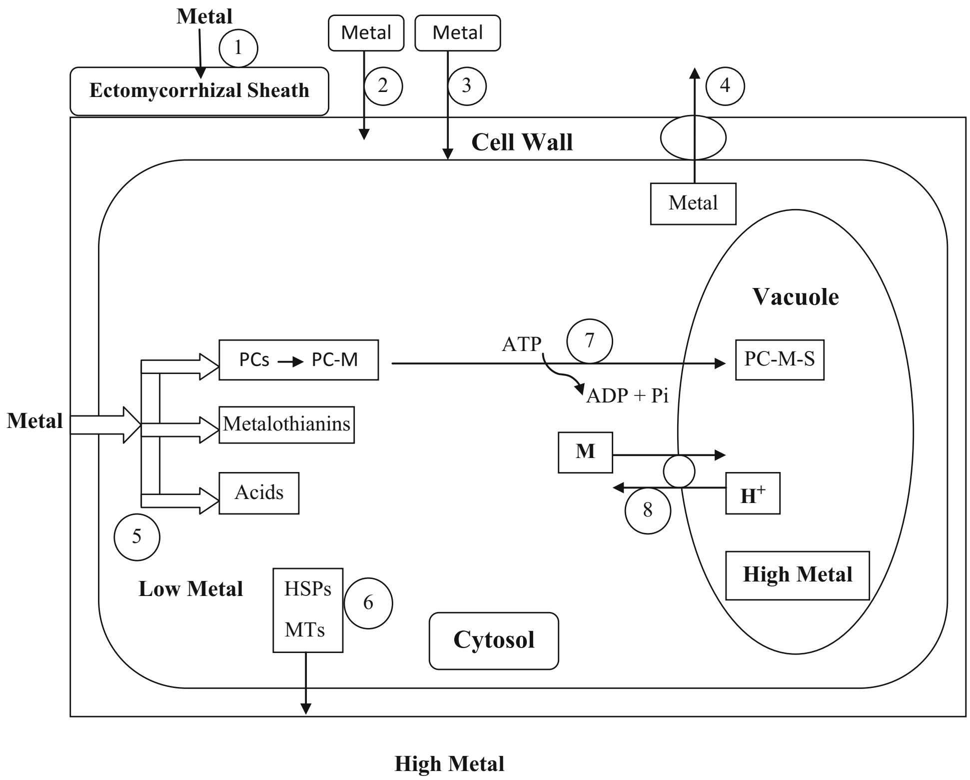


**Fig. 22.2** Summary of heavy metal avoidance and tolerance mechanisms (Redrawn after Dalvi and Bhalera 2013). (1) Restriction of metal movement to roots by mycorrhizas, (2) binding to cell wall and root exudates, (3) reduced influx across plasma membrane, (4) active efflux into apoplast, (5) chelation in cytosol by various ligands, (6) repair and protection of plasma membrane under stress conditions, (7) transport of PC–M complex into the vacuole, (8) transport and accumulation of metal in vacuole

### Metal Distribution Within Plant Parts

It is believed that depending upon the metal toxicity, extent of toxicity, and plant species, plant combats the metal contamination through its nonuniform distribution within oneself. Some plants accumulate high amounts of metal in roots (perhaps this is to keep contamination away from metabolism in the shoot), while some plants accumulate metals in their aerial parts and ultimately old leaf drop in deciduous plants may be the tolerance mechanisms adopted by such plant species (Reichman 2002; Hall 2002).

### Cellular Complexation and Compartmentation

#### Phytochelatins Complexation

Phytochelatins (PCs) are cysteine-rich nonprotein metal-binding peptides having structure (γ Glu-Cys)$_n$-Gly where $n=2$–11 is produced by plants. PCs are found in gymnosperms, dicots, monocots, and algae (Memon and Schröder 2009; Reichman 2002; Hall 2002; Memon et al. 2001; Jabeen et al. 2009; Clemens 2006) and are mostly studied under Cd stress, i.e., Cd is the strongest inducer of PCs (Dalvi and Bhalera 2013). They are synthesized non-transitionally on glutathione (enzyme) substrate. Glutathione synthesis is initiated due to the presence of metal ions. They are widely distributed and synthesized in *A. thaliana*, higher plants, and yeast under metal stress (Memon and Schröder 2009). Phytochelatins are the ones that belong to class 3 of MTs. They are involved both in the homeostasis of essential metals and in the detoxification of heavy metals within plant. After synthesis the PC–metal complex is transported by ABC transporters and/or $H^+$ to the vacuole, where this complex behaves resistant and upon favorable conditions (presence of sulfide/sulfite ions) PC–metal complex degrades and releases metal (Dalvi and Bhalera 2013). Under metal stress PC synthesis has been reported in *Zea mays* (Cd and Cu), *Silene vulgaris* (Cu), and *H. lanatus* (As) (Reichman 2002; Hall 2002). The gene of PCs synthesis has been identified in *Arabidopsis* and yeast (Clemens 2006).

#### Metallothionein Complexation

Metallothioneins (MTs) are low molecular weight, cysteine-rich, metal-binding proteins. Their function is the regulation of essential metals and the detoxification of metal contamination (Memon and Schröder 2009; Reichman 2002; Hall 2002; Clemens 2006; Jabeen et al. 2009). MTs originally were found in animals, and it initiated their search in plants as a detoxification mechanism. MT-like genes have been isolated from several plants species such as wheat, maize, soybean, rice, *Brassica napus*, and tobacco. However their role and function is not fully understood, as much information is not available about their role in the detoxification of heavy metals (Dalvi and Bhalera 2013; Memon and Schröder 2009; Memon et al. 2001).

Metallothionein are grouped into three classes:

1. Class 1 MTs are found in mammals, contain 61 amino acids, but are deficient of histidine or aromatic amino acid. Under metal stress conditions they predominantly are expressed in the roots.
2. Class 2 MTs are found in yeast or cyanobacteria. Under metal contamination, they are expressed in the leaves and/or aerial parts of the plant.
3. Class 3 MT phytochelatins belong to class 3 MTs (Reichman 2002; Hall 2002).

#### Organic and Amino Acids

Carboxylic and amino acids such as citric, malic, and histidine (His) are the ligands which have crucial role in the detoxification and tolerance of heavy metals especially Cd, Cu, Ni, and Zn, within plants (Hall 2002; Dalvi and Bhalera 2013) moving from roots to leaves, citrate, and His are the principal ligands responsible for the translocation of heavy metals in the xylem sap. Under Ni stress 36-fold increase of histidine concentration in the xylem of *Alyssum lesbiacum* was found (Clemens 2001; Hall 2002).

#### Vacuole Compartmentation

Compartmentation in the vacuole is the most viable and probable site (Memon and Schröder 2009; Jabeen et al. 2009). Excess of metal contamination results in the vacuolation of metal contamination using ABC transporters in the tolerant plants such as *F. rubra* and *D. caespitosa* (Hall 2002; Reichman 2002). Mostly metal contamination is pumped into the vacuole, e.g., Zn dumping into the vacuoles of grasses and *H. vulgare*, Cu and Al in *Zea mays*, and Zn and Cd in *Nicotiana tabacum* (Reichman 2002). Zn, Cd, and Mo are found in vacuole, whereas Ni accumulates in cytosol and causes leaf damage (Hall 2002). In *H. annuus* on exposure of Mn stress, plant developed leaf trichomes, whereas in the trichomes of *Vicia faba* under Mn stress, metallothionein gene was detected.

### 22.3.9 Future of Phytoremediation

Since the last decade phytoremediation has gained acceptance as a technology and has been acknowledged as an important area of research. Basic processes of phytoremediation are still largely not clear and hence require further basic and applied research to optimize its field performance. Information collected from basic research at physiological, biochemical, and genetic level in plants will be helpful in understanding the processes of passive adsorption, active uptake, translocation, accumulation, and chelation mechanisms. Research aimed at better understanding of the interactive roles among plant roots and microbes will help scientists to utilize their integrative capacity for soil decontamination. Further, genetic evaluation of hyperaccumulators growing in metal-contaminated soil and associated microbes would provide the researchers with a gene pool to be used in genetic manipulation of other non-accumulators and production of transgenics. However, new knowledge and plant material obtained from research is already being implemented for phytoremediation in the field. The first field tests with transgenics are showing promising results. As more results demonstrating the effectiveness of phytoremediation become available, its use may continue to grow, reducing cleanup costs and enabling the cleanup of more sites with the limited funds available.

Currently a great deal of research is in progress in this direction and its impact will soon be felt in phytoremediation market. An interesting development in phytoremediation could be the adoption of an integrated approach both for research and commercial purposes. Presently phytoremediation research is carried out by scientists with expertise in only certain fields, e.g. plant molecular biology, plant biochemistry, plant physiology, ecology, plant biochemistry, plant physiology, ecology, toxicology, or microbiology, but phytoremediation being an integrated technology will be benefited more by a team of researchers with different backgrounds. Commercially to enhance public acceptance, phytoremediation can be integrated with landscape architecture such as remediation of partially contaminated urban sites that may be combined with an attractive design so that the area may be used as a park or some other recreational place by the public after the remediation process (Pilon-Smits 2005). It is obvious that phytoremediation is an effective technology for removing and detoxifying metals and metalloids such as Cd, Se, and As from environment for re-cultivation and reclamation of polluted sites. Phytoremediation works best when supplemented by nonbiological remediation technologies for decontamination of most polluted sites. Because pollutant distribution and concentration are heterogeneous for sites, the most efficient and cost-effective remediation solution may be a combination of different technologies such as excavation of the most contaminated spots followed by polishing the site with the use of plants. The identification of unique genes from natural hyperaccumulators and their subsequent transfer to fast-growing species is another promising approach. To improve phytoremediation a number of agronomic enhancements are also possible ranging from traditional crop management techniques (use of pesticides, soil amendments, fertilizers, etc.) to approaches more specific to phytoremediation such as improving metal solubility in soils through the use of chelators. Finally, advances in optimizing plants for phytoremediation will depend on gaining new knowledge about the fate and transport of metals/metalloids in plants and innovative technologies to improve the acceptability of transgenic plants for phytoremediation.

## 22.4 Role and Mechanism of Algae in Heavy Metal Accumulation

There is an increased interest in employing algae for biomonitoring eutrophication and organic and inorganic pollutants. Chlorophyll produced during the growth of algae was estimated spectrophotometrically to judge the total nitrogen content in water collected from aquatic systems suggesting on eutrophication levels (Ben-Chekroun and Baghour 2013). A number of algal species have been employed in the phytoremediation of various pollutants including heavy metals.

Knowing the positive aspects and potential of phytoremediation, it has been commercialized for the decontamination of metal-contaminated soil and water. Role of rhizosphere in heavy metal reclamation cannot be undermined as it is well documented that rhizosphere is involved in various biogeochemical processes such as alleviation of biotic/abiotic stress in plants, nutrient uptake, and metal detoxification. Thus, plant-associated microbes, algae, and fungi which mostly are plant and metal contamination specific are playing their role in improving the efficiency of the phytoremediating plant (Rajkumar et al. 2012). They not only tolerate the metal contamination but provide numerous benefits to both the plant and soil (Rajkumar et al. 2012). The survival, existence, and population of rhizospheric algae, fungi, and/or microbes depend not only upon the properties of rhizosphere but also on the nutrients available which surely are metal contamination and plant specific.

Algae being aquatic organisms are found capable of the removal of heavy metal contamination (Perales-Vela et al. 2006; (Wenzel 2009; Gadd 2000; Rajkumar et al. 2010; Kidd et al. 2009; Ma et al. 2011; Khan et al. 2009; Uroz et al. 2009). Brown algae have been found efficient in hyperaccumulation of certain metals (Table 22.1). The direct function of rhizosphere-associated algae is the alteration of metal concentration and/or accumulation within plant tissues, and indirectly they are involved in the increase in the root/shoot biomass. Similarly, fungi through antioxidant enzymatic activities alter the biochemical and physiological processes of plant tolerance to heavy metals (Rajkumar et al. 2012; Yamane et al. 2004; Jiang and Zhang 2002; Glick 2010; Aafi et al. 2012; Miransari 2011; Ma et al. 2011; Rajkumar et al. 2010; Yang et al. 2012; Wenzel 2009).

Generally in organisms under heavy metal stress, various defense systems, such as exclusion, compartmentalization, complex formation, and binding protein synthesis, i.e., metallothioneins (MTs) and phytochelatins (PCs), and ultimately translocation of metal complexes to the vacuoles are operational (Ben-Chekroun and Baghour 2013; Grill et al. 1985, 1987, 1989). In *Thalassiosira weissflogii and Thalassiosira pseudonana*, PC synthesis was observed which forms glutathione and has great affinity for metal ions (Ben-Chekroun and Baghour 2013; Ahner et al. 2002; Payne 2000). Similarly, in *Chlorella vulgaris*, proline concentration was increased under Cu and Cr stress (Ben-Chekroun and Baghour 2013; Sharma and Dietz 2006; Krämer et al. 1996). During heavy metal remediation and/or metal removal process, algae undergo through two mechanisms, i.e., exclusion—restricted or no entry of metal ions—and, secondly, complex formation of metal ions, once they get entered with the cell of oneself. Complex formation helps in minimizing or decreasing the toxic effects of heavy metal contamination (Perales-Vela et al. 2006; Cobbett and Goldsbrough 2002). Metal extraction by algae mainly involves extracellular polymers especially carbohydrates, whereas metals complex formation by algae involves glutathione-derived peptides, i.e., class III metallothioneins (Mt III), are involved. Mt III is quick and energy requiring response of algae to heavy metal stress as a result of which heavy metal get transported to the vacuole of algae (Perales-Vela et al. 2006).

Algae are believed as exhibiting various heavy metal remediation and detoxification mechanisms, e.g., they deal with heavy metal stress either through low concentration uptake for metabolism or non-active adsorption–biosorption (Ben-Chekroun and Baghour 2013). Several green algae species, i.e., *Enteromorpha* and *Cladophora*, in various parts of the world have been identified for the phytoremediation of heavy metals (Ben-Chekroun and Baghour 2013; Al-Homaidan et al. 2011). Due to the phytoremediational capacity for heavy metals, they are being considered as biomonitor as they are found as both plant and metal specific (Ben-Chekroun and Baghour 2013; Gosavi et al. 2004; Rainbow 1995), e.g., *Cyanophyta* and *Chlorophyta* are hyperaccumulators and hyper-absorbents for arsenic (As) and boron (B). Similarly, an identified algal colony is found in phytoremediation of arsenic (As) contamination in symbiotic association with the roots of *Arundo donax* L. (Mirza et al. 2010a, b).

It is also believed that algae can synthesize heavy metal binding peptides, through gene encoding and enzyme synthesis with the appearance and prevalence of class II metallothioneins (Mt II) (although not well reported). But the presence of Mt III and expected Mt II makes algae an interesting potential for the remediation of heavy metal-contaminated wastewater (Gaur and Rai 2001). Isolation and identification

**Table 22.2** Metal detoxification mechanisms in algae

| Metal | Detoxification strategy | Reference |
|---|---|---|
| Cd, Cu, Ag, Hg, Zn, and Pb | Metallothioneins (MTs) and phytochelatins (PCs) | Clemens et al. (1999), Vatamaniuk et al. (1999), Ha et al. (1999), Volland et al. (2012) |
| Ni | Histidine | Krämer et al. (1996) |
| Pb, Cu, Cd, Zn, Ca | Cell wall components (alginates and guluronic acid, sulfated polysaccharides and alginates) | Sekabira et al. (2011), Davis et al. (2000), Gupta et al. (2013) |

of plant-associated algae is laborious as it would require isolation and identification of thousands of algal species and further research efforts are still needed to understand and explore the process completely. The metal detoxification mechanisms have been reported for various algal species which have been reported in Table 22.2.

### 22.4.1 Approximate Costs of Different Remediation Processes

Generally the chemical and physical managements permanently affect the soil properties, devastate biodiversity, and may perhaps leave the soil futile as a medium for plant development. These remediation methods can be costly. Cost of different remediation technologies has been summarized by Glass (1999). Vitrification has a process cost of US $75–425 ton$^{-1}$ and requires long-term monitoring, land filling requires US $100–500 ton$^{-1}$, and transport/excavation/monitoring of the project is also requisite. A lump sum of US $100–500 ton$^{-1}$ is needed in case of chemical treatment, whereas the recycling of contaminants is a major problem standing on its shoes. A constant monitoring and a process cost of US $20–200 ton$^{-1}$ are considered necessary for electrokinetics, whereas only US $5–40 ton$^{-1}$ is needed for phytoextraction and proper disposal of the generated phytomass.

## 22.5 Role of Rhizosphere

The prime goal of microbial association with metal-tolerant plants seems to be mutualistic relationship helping in cleaning up of metal-polluted environment as a result of the bioaccumulation (Jing et al. 2007; Glick 2010). Plant metal extraction potential from the metalliferous soil could be determined by the metal concentration in the shoots and leaves or biomass production (Singh et al. 2001; McGrath and Zhao 2003a, b). Application of synthetic chelators such as ethylenediaminetetraacetic acid and nitrilotriacetate and elemental sulfur is a commonly used soil practice in the field of phytoremediation (Puschenreiter et al. 2001; Chen et al. 2004).

In phytoremediation rhizosphere is the site from where plants absorb mineral nutrients where microorganisms act together with plant products like root exudates along with a mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions, molecules in gaseous form, enzymes, and root border cells (Dakora and Phillips 2002). Plants treated with metal-resistant rhizosphere bacteria have reported to reduce metal toxicity (Madhaiyan et al. 2007).

In phytoremediation the contaminated soil is utilized for plant growth where metal accumulation, harvesting, and removal of aboveground portions of the plants cause permanent elimination of metals from soils (Nandakumar et al. 1995). Some studies proposed that the volume of land filling material could be reduced by incineration of harvested plant tissue. Thus incineration is an important technique due to extraction of metals from the metal-rich ash and serves as a source of revenue and reduces the expense of remediation (Cunningham and Ow 1996).

The term “rhizosphere” was introduced by the German scientist Hiltner (1904). Rhizosphere is a borderline between soil and plants, which plays an important role in the agro-environmental structure (Wang et al. 2002), in which physio-chemical and biological characteristics of soil and biomass activity and community structure of microorganisms are significantly affecting each other (Sörensen 1997).

The rhizosphere is an environment around plants where plant growth and health could be enhanced by pathogenic and beneficial microorganisms (Lynch 1990). The rhizosphere is usually occupied by microbial groups and other microbes like protozoa, bacteria, fungi, nematodes, algae, and microarthropods (Lynch 1990; Raaijmakers 2001). Proper understanding of the ecosystem is necessary for the treatment through beneficial integration of microorganisms by a suitable selection of microbes (Díaz et al. 1996).

It has been proved plant roots attract soil microorganisms through their exudates which ultimately results in a variation between the rates of metabolic activity of the rhizosphere microbial communities from those of the non-rhizosphere soil (Brimecombe et al. 2001). Generally, the plants rhizobacteria migrate from the soil to the rhizosphere of living plant and colonize around plants roots (Kloepper and Schroth 1978). These rhizobacteria show symbiotic behavior of plants and act as plant growth-promoting microbes (Kapulnik 1991). Natural activities of colonizing and multiplying of free-living bacterial species along the surface of the roots of the inoculated plants enhance the growth rate of plants (Mishra et al. 2008).

The root exudation and related microbes increase the bioavailability of heavy metals by their rhizospheric mobilization (Wenzel et al. 1999a, b). Bacteria are the most common type of soil microorganism due to their rapid growth and extensive utilization of substances like carbon or nitrogen sources. Rhizospheric microbes may play a dual role in soil ecological potential both constructively and destructively. In the rhizosphere the activities and varieties of harmful and beneficial are closely related to the quality and quantity of rhizodeposits (Somers et al. 2004).

The careful application of appropriate heavy metal-tolerant, plant growth-promoting, and nitrogen-fixing rhizobacteria enhanced the efficiency of phytoremediation (Khan 2001). Many research works revealed the fact that high levels of heavy metals in the environment affected the soil and water quality retards plant growth enormously. Therefore the use of rhizospheric microorganisms may play an important role for the remediation of heavy metal toxicity (Burd et al. 2000). Heavy metals disturb the natural ecology of plants by causing dormant growth and reduce cell membrane activities, denaturation of protein, and functional disturbances (Leita et al. 1995).

Plants and microbes possess a strong valuable relationship but also possess strong competition for resources, including nutrients and water, and also can cause diseases (Kaye and Hart 1997). So many plant growth-promoting, beneficial, and free-living soil bacteria are usually referred to as rhizobacteria and are present in association with the roots of various plant species (Glick et al. 1999). In both natural and synthetic ecosystems, plant-associated bacteria play an important role in host variation for any change in environment. These microorganisms enable plants to tolerate high concentrations or stress of metals by causing modification in plant cell metabolism (Welbaum et al. 2004). Several authors have pointed out that bacterial plant growth could be enhanced by the activation of biosorption or bioaccumulation mechanism in heavy metal-contaminated soils, in addition to other plant growth-promoting factors that integrated the production of ACC deaminase and phytohormones for improved plant growth (Zaidi et al. 2006; Madhaiyan et al. 2007; Kumar et al. 2009).

It has been proved through experiments that seedling growth of *Triticum aestivum* grains could be improved by inoculation with two pseudomonad strains from rhizosphere, as compared with non-inoculated plants at different lead concentrations (Hasnain et al. 1993). Similarly, *Sedum alfredii*, a terrestrial hyperaccumulator of toxic heavy metals like zinc, cadmium, copper, and lead from wastewater, evidently improved the condition of phytoremediation in the presence of naturally occurring rhizospheric bacteria, also by using antibiotic ampicillin (Xiong et al. 2008).

## 22.6 Conclusions

In view of heavy metal contamination of the environments, phytoremediation is the cheapest method to remediate the polluted soils and waters. Plants are the natural biomaterials that can effectively uptake metals along their nutrients thus acting as natural biofilters rendering contaminated soils and waters fit for reuse. For metal remediation, phytoremediation may possibly offer sustainable techniques. Plants are used to eliminate, transport, stabilize, and/or degrade noxious pollutants in soil, sediment, and water. Different phytoremediation technologies can be used for the containments (phytoimmobilization and phytostabilization) or elimination (phytoextraction and phytovolatilization) depending on the contaminants, the site conditions, the level of cleanup required, and the types of plant phytoremediation technology.

Phytoremediation is inherently dependent on several plant characteristics, the two most important being the ability to accumulate large quantities of biomass rapidly and the capacity to accumulate large quantities of environmentally important metals in the shoot tissue. Effective phytoextraction of soils requires both plant genetic ability and the development of optimal agronomic practices, including soil management practices (to improve the efficiency of phytoextraction) and crop management practices (to develop a commercial cropping system).

There is an increased interest in employing algae for biomonitoring eutrophication and organic and inorganic pollutants. Brown algae have been found efficient in hyperaccumulation of certain metals due to their macroscopic nature. The direct function of rhizosphere-associated algae is the alteration of metal concentration and/or accumulation within plant tissues and indirect involvement in the increase in the root/shoot biomass. Algae are believed as exhibiting various heavy metal remediation and detoxification mechanisms, e.g., they deal with heavy metal stress either through low concentration uptake for metabolism or non-active adsorption–biosorption.

The prime goal of microbial association with metal-tolerant plants seems to be mutualistic relationship helping in cleaning up of metal-polluted environment as a result of the bioaccumulation. Plant roots attract soil microorganisms through their exudates which ultimately results in a variation between the rates of metabolic activity of the rhizosphere microbial communities from those of the non-rhizosphere soil. Future research is anticipated to be aimed at better understanding of the interactive roles among plants roots and microbes that will help scientists to utilize their integrative capacity for environmental remediation. Genetic evaluation of hyperaccumulators growing in metal-contaminated soil and associated microbes would provide the researchers with a gene pool to be used in genetic manipulation of other non-accumulators and production of transgenics.

## References


Aafi NE, Brhada F, Dary M, Maltouf AF, Pajuelo E (2012) Rhizostabilization of metals in soils using Lupinus luteus inoculated with the metal resistant rhizobacterium Serratia sp. MSMC 541. Int J Phytoremed 14:261–274

Abia AA, Horsfall M, Didi O (2003) The use of chemically modified and unmodified cassava waste for the removal of Cd, Cu and Zn ions from aqueous solution. Bioresour Technol 90:345–348

Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural remediation in environmental cleanup. Geoderm 122:121–142

Ahner BA, Wei L, Oleson JR, Ogura N (2002) Mar Ecol Prog Ser 232:93–103

Ajmal M, Rao RAK, Ahmad J, Ahmad R (2006) The use of ground nut shell (*Arachis hypogea*) for the adsorption of Ni (II) from the aqueous system. J Environ Sci Engine 48(3):221–224

Al-Chalabi AS, Hawker D (2000) Distribution of vehicular lead in roadside soils of major roads of Brisbane, Australia. Water Air Soil Pollut 118:299–310

Al-Homaidan AA, Al-Ghanayem AA, Areej AH (2011) Int J Water Resour Arid Environ 1:10

Alkorta I, Herna´ndez-Allica J, Becerril JM, Amezaga I, Albizu I, Garbisu C (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Environ Sci Biotechnol 3:71–90

Alloway BJ (1990) Heavy metal in soils. Wiley, New York, pp 20–27

Alloway BJ, Ayres DC (1997) Chemical principles of environmental pollution, 2nd edn. Blackie Academic and Professional, Chapman & Hall, London

Álvarez P, Blanco C, Granda M (2007) The adsorption of chromium (VI) from industrial wastewater by acid and base-activated lignocellulosic residues. J Hazard Mater 144(1–2):400–440

Arıca MY, Kacar Y, Genc O (2001) Entrapment of white-rot fungus Trametes versicolor in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresour Technol 80:121–129

Assunçao AGL, Schat H, Aarts MGM (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360

Atkinson R, Aschmann SM, Hasegawa D, Eagle-Thompson ET, Frankenberger JR (1990) Kinetics of the atmospherically important reactions of dimethyl selenide. Environ Sci Technol 24:1326–1332

Azaizeh HA, Gowthaman S, Terry N (1997) Microbial selenium volatilization in rhizosphere and bulk soils from a constructed wetland. J Environ Qual 26(3):666–672

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyper accumulate metallic elements – review of their distribution, ecology, and phytochemistry. Biorecover 1:81–126

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. CRC Press, Boca Raton, FL

Baker AJM, Reeves RD, McGrath SP (1991) In situ decontamination of heavy metal polluted soils using crops of metal accumulating plants – a feasibility study. In: Hinchee RE, Olfenbuttel RF (eds) In situ bioremediation, pp 539–544

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, FL, pp 155–177

Banuelos GS, Cardon G, Mackey B, Ben-Asher J, Wu LP, Beuselinck P (1993) Boron and selenium removal in B-laden soils by four sprinkler irrigated plant species. J Environ Qual 22(4):786–797

Basic N, Keller C, Fontanillas P, Vittoz P, Besnard G, Galland N (2006a) Cadmium hyperaccumulation and reproductive traits in natural Thlaspi caerulescens populations. Plant Biol 8:64–72

Basic N, Salamin N, Keller C, Galland N, Besnard G (2006b) Cadmium hyperaccumulation and genetic differentiation of Thlaspi caerulescens populations. Biochem Systemat Ecol 34(9):667–677

Basta NT, McGowan SL (2004) Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter contaminated soil. Environ Pollut 127:73–82

Ben-Chekroun K, Baghour M (2013) The role of algae in phytoremediation of heavy metals: a review. J Mater Environ Sci 4(6):873–880

Benguella B, Benaissa H (2002) Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies. Water Res 36:2463–2474

Bernal M, Testillano PS, Alfonso M, Del Carmen Risueno M, Picorel R, Yruela I (2007) Identification and subcellular localization of the soybean copper P1B-ATPase GmHMA8 transporter. J Struct Biol 158:146–158

Bert V, Bonnin I, Saumitou-Laprade P, de Laguerie P, Petit DD (2002) Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytolog 155:47–57

Berti WR, Cunningham SD (2000) Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York, pp 71–88

Bilal M, Shah JA, Ashfaq T, Gardazi SMH, Tahir AA, Pervez A, Haroon H, Mahmood Q (2013) Waste biomass adsorbents for copper removal from industrial wastewater – a review. J Hazard Mater http://dx.doi.org/10.1016/j.jhazmat.2013.07.071

Birch GE, Scollen A (2003) Heavy metals in road dust, gully pots and parkland soils in a highly urbanised subcatchment of Port Jackson, Australia. Aust J Soil Res 41:1329–1342

Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York, pp 53–70

Bolan NS, Adriano DC, Naidu R (2003) Role of phosphorus in (im) mobilization and bioavailability of heavy metals in the soil-plant system. Rev Environ Contamin Toxicol 177:1–44

Brimecombe MJ, DeLelj FA, Lynch JM (2001) The rhizosphere: the effect of root exudates on rhizosphere microbial populations. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp 95–140

Brookes AJ, Collins JC, Thurman DA (1981) The mechanism of zinc tolerance in grasses. J Plant Nutr 3:695–700

Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decreases heavy metal toxicity in plants. Can J Microbiol 46:237–245

Celestino MDR, Font R, Rojas RM, Bailon ADH (2006) Uptake of lead and zinc by wild plants growing in contaminated soils. Ind Crop Prod 24:230–237

Chakraborty S, Purkait MK, Gupta DS, De S, Basu JK (2003) Nanofiltration of textile plant effluent for color removal and reduction in COD. Separat Pur Technol 31:141–151

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–283

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation – focusing on accumulator plants that remediate metal-contaminated soils. Aust J Ecotoxicol 4:37–51

Chen BD, Shen H, Li XL, Feng G, Christie P (2004) Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (Zea mays L.) in soil experimentally contaminated with zinc. Plant Soil 261:219–229

Cheung CW, Chan CK, Porter JF, McKay G (2001) Combined diffusion model for the sorption of cadmium, copper, and zinc ions onto bone char. Environ Sci Technol 35:1511–1522

Chockalingam E, Subramanian S (2009) Utility of Eucalyptus tereticornis (Smith) bark and Desulfotomaculum nigrificans for the remediation of acid mine drainage. Bioresour Technol 100:615–621

Ciardelli G, Corsi L, Marcucci M (2002) Membrane separation for wastewater reuse in the textile industry. Resour Conservat Recycl 31:189–197

Clemens S (2001) Molecular mechanism of plant metal tolerance and homeostasis. Planta 212:475–486

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719

Clemens S, Kim EJ, Neumann D, Schroeder JI (1999) Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. EMBO J 18:3325–3333

Cobbett C, Goldsbrough P (2002) Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cooper EM, Sims JT, Cunningham SD, Huang JW, Berti WR (1999) Chelate-assisted phytoextraction of lead from contaminated soil. J Environ Qual 28:1709–1719

Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, Saumitou-Laprade P, Verbruggen N (2007) A major quantitative trait locus for cadmium tolerance in Arabidopsis halleri colocalizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiol 144:1052–1065

Cruz CCV, da Costa ACA, Henriques CA, Luna AS (2004) Kinetic modeling and equilibrium studies during cadmium biosorption by dead Sargassum sp. biomass. Bioresour Technol 91:249–257

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110(3):715–719

Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997) Phytoremediation of contaminated water and soil. In: Kruger EL, Anderson TA, Coats JR (eds) Phytoremediation of soil and water contaminants. ACS symposium, vol 664. American Chemical Society, Washington, DC, pp 2–19

Daifullah AAM, Girgis BS, Gad HMH (2003) Utilization of agro-residues (rice husk) in small wastewater treatment plans. Mater Lett 57:1723–1731

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47

Dalvi A, Bhalera S (2013) Response of plants towards heavy metal toxicity: an overview of avoidance, tolerance and uptake mechanism. Ann Plant Sci 02(09):362–368

Danny CKK, Cheung CW, Choy KKH, Porter JF, McKay G (2004) Sorption equilibria of metal ions on bone char. Chemosphere 54:273–281

Davis TA, Volesky B, Vieira RHSF (2000) Sargassum seaweed as biosorbent for heavy metals. Water Res 34:4270–4278

De Vos CH, Schat H, Ernst WHO (1988) Copper mediated changes in membrane permeability. J Cell Biochem 12A(Suppl):49

Della VP, Kuhn I, Hotza D (2001) Caracterização de cinza de casca de arroz para uso como matéria-prima na fabricação de refratários de sílica. Quim Nova 24:778–782

Desouza MP, Pilon-Smits EAH, Terry N (2000) The physiology and biochemistry of selenium volatilization by plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York, pp 171–190

Dhabi S, Azzi M, Guardia M (1999) Removal of hexavalent chromium from wastewaters by bone charcoal. Fresenius J Anal Chem 363:404–407

Díaz G, Azcón-Aguilar C, Honrubia M (1996) Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. Plant Soil 180:1201–1205

Dierberg FE, Débuts TA, Goulet NA Jr (1987) Removal of copper and lead using a thin-film technique. In: Reddy KR, Smith WH (eds) Aquatic plants for water treatment and resource recovery. Magnolia, Orlando, FL, pp 497–504

Dushenkov S, Kapulnik Y (2000) Phytofiltration of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals – using plants to clean-up the environment. Wiley, New York, pp 89–106

Dushenkov S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC (1997a) Phytoremediation: a novel approach to an old problem. In: Wise DL (ed) Global environmental biotechnology. Elsevier, Amsterdam, pp 563–572

Dushenkov S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC (1997b) Removal of uranium from water using terrestrial plants. Environ Sci Technol 31(12):3468–3474

Dushenkov V, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239–1245

Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23:97–114

Ebbs SD, Lasat MM, Brandy DJ, Cornish J, Gordon R, Kochian LV (1997) Heavy metals in the environment: phytoextraction of cadmium and zinc from a contaminated soil. J Environ Qual 26:1424–1430

Eccles H (1999) Treatment of metal-contaminated wastes: why select a biological process? Trends Biotechnol 17:462–465

Elless PM, Poynton YC, Williams AC, Doyle PM, Lopez CA, Sokkary AD (2005) Pilot-scale demonstration of phytofiltration for drinking arsenic in New Mexico drinking water. Water Res 39(16): 3863–3872

Ensley BD (2000) Rationale for use of phytoremediation. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York, pp 3–12

Entry JA, Watrud LS, Reeves M (1999) Accumulation of 137Cs and 90Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. Environ Pollut 104:449–457

Evans CS, Asher C, Johnson CM (1968) Isolation of dimethyl diselenide and other volatile selenium compounds from *Astragalus racemosus* (Pursh.). Aust J Biol Sci 21:13–20

Farinella NV, Matos GD, Arruda MAZ (2007) Grape bagasse as a potential biosorbent of metals in effluent treatments. Bioresour Technol 98:1940–1946

Fernando CL, Fernando SH (1994) Subcellular localization of copper and partial isolation of copper proteins in roots from rice plants exposed to excess copper. Aust J Plant Physiol 21:427–436

Fernando DR, Woodrow IE, Jaffre T, Dumontet V, Marshall AT, Baker AJM (2007) Foliar manganese accumulation by *Maytenus founieri* (Celastraceae) in its native New Caledonian habitats: populational variation and localization by X-ray microanalysis. New Phytol 177:178–185

Filatov V, Dowdle J, Smirnoff N (2006) Comparison of gene expression in segregating families identifies genes and genomic regions involved in a novel adaptation, zinc hyperaccumulation. Mol Ecol 15:3045–3059

Flathman PE, Lanza GR (1998) Phytoremediation: current views on an emerging green technology. J Soil Contamin 7(4):415–432

Gabaldon C, Marzal P, Ferrer J, Seco A (1996) Single and competitive adsorption of Cd and Zn onto a granular activated carbon. Water Res 30:3050–3060

Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr Opin Biotechnol 11:271–279

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236

García R, Millán E (1998) Assessment of Cd, Pb and Zn contamination in roadside soils and grasses from Gipuzkoa (Spain). Chemosphere 37:1615–1625

Gardea-Torresdey JL, Becker-Hapak MK, Hosea JM, Darnall DW (1990) Effect of chemical modification of algal carboxyl groups on metal ion binding. Environ Sci Technol 24:1372–1378

Gardea-Torresdey JL, de la Rosa G, Peralta-Videa JR (2004) Use of phytofiltration technologies in the removal of heavy metals: a review. Pure Appl Chem 76(4):801–813
Gaur JP, Rai LC (2001) Heavy metal tolerance in algae. In: Rai LC, Gaur JP (eds) Algal adaptation to environmental stresses. Physiological, biochemical and molecular mechanisms. Springer, Berlin, pp 363–388
Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its by-products. Appl Ecol Environ Res 3(1):1–18
Glass DJ (1999) U.S. and international markets for phytoremediation, 1999–2000. D. Glass Associates, Needham, MA, p 266
Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374
Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London
Gonzaga MIS, Santos JAG, Ma LQ (2006) Arsenic chemistry in the rhizosphere of *Pteris vittata* L. and *Nephrolepis exaltata* L. Environ Pollut 143:254–260
Gosavi K, Sammut J, Jankowski J (2004) Macroalgal biomonitors of trace metal contamination in acid sulfate soil aquaculture ponds. Sci Total Environ 324:25–39
Grant JJ, Loake GJ (2000) Role of active oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol 124:21–29
Grill E, Lo¨ffler S, Winnacker EL, Zenk MH (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific gamma-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc Natl Acad Sci 86:6838–6842
Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins, the principal heavy metal complexing peptides of higher plants. Science 230:674–676
Grill E, Winnacker EL, Zenk MH (1987) Phytochelatins, a class of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. Proc Natl Acad Sci 84:439–443
Gulson BL, Tiller KG, Mizon KJ, Merry RH (1981) Use of lead isotopes in soils to identify the source of lead contamination near Adelaide, South Australia. Am Chem Soc 15(6):691–696
Gupta DK1, Huang HG, Corpas FJ (2013) Lead tolerance in plants: strategies for phytoremediation. Environ Sci Pollut Res Int. 20(4):2150–2161
Ha SB, Smith AP, Howden R, Dietrich WM, Bugg S, O'Connell MJ, Goldsbrough PB, Cobbett CS (1999) Plant Cell 11:1153
Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11
Hammer D, Kayser A, Keller C (2003) Phytoextraction of Cd and Zn with Salix viminalis in field trials. Soil Use Manag 19:187–192
Han FX, Banin A, Kingery WL, Triplrtt GB, Zhou LX, Zheng SJ (2003) New approach to studies of heavy metal redistribution in soil. Adv Environ Res 8:113–120
Hasnain S, Yasmin S, Yasmin A (1993) The effects of lead-resistant Pseudomonads on the growth of Triticum aestivum seedlings under lead stress. Environ Pollut 81:179–184
Heaton ACP, Rugh CL, Wang N, Meagher RB (1998) Phytoremediation of mercury- and methyl mercury-polluted soils using genetically engineered plants. J Soil Contam 74:497–510
Hettiarachchi GM, Pierzynski GM, Ransom MD (2001) In situ stabilization of soil lead using phosphorus. J Environ Qual 30:1214–1221
Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb Deutsch Landwirt Ges 98:59–78
Ho YB, Tai KM (1988) Elevated levels of lead and other metals in roadside soil and grass and their use to monitor aerial metal depositions in Hong Kong. Environ Pollut 49(1):37–51
Hodoshima H, Enomoto Y, Shoji K, Shimada H, Goto F, Yoshihara T (2007) Differential regulation of cadmium-inducible expression of iron-deficiency-responsive genes in tobacco and barley. Physiol Plant 129:622–634
Hodson ME, Valsami-Jones E, Cotter-Howells JD (2000) Bone meal additions as a remediation treatment for metal contaminated soil. Environ Sci Technol 34:3501–3507
Holden Y (1989) How to select hazardous waste treatment technologies for soils and sludges: alternative, innovative, and emerging technologies. Noyes Data Corporation, Park Ridge, NJ
Hornung U (1997) Soil venting. http://cage.rug.ac.be/~ms/LHKW/lhkw.html. Accessed 12 July 2004
Horsfall M, Abia AA (2003) Sorption of cadmium (II) and zinc (II) ions from aqueous solutions by cassava waste biomass (Manihot sculenta Cranz). Water Res 37:4913–4923
Huang JW, Chen J, Berti WR, Cunningham SD (1997) Phytoremediation of lead contaminated soil: role of synthetic chelates in lead phytoextraction. Environ Sci Technol 31(3):800–805
Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31:266–271
Imagawa A, Seto R, Nagaosa Y (2000) Adsorption of chlorinated hydrocarbons from air and aqueous solutions by carbonized rice husk. Carbon 38:623–641
Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J Exp Bot 56:3207–3214
Jabeen R, Ahmad A, Iqbal M (2009) Phytoremediation of heavy metals: physiological and molecular mechanisms. Bot Rev 75:339–364
Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 53:2401–2410
Jing Y, He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci 8:192–207
Jonnalagadda SB, Nenzou G (1997) Studies on arsenic rich mine dumps. II. The heavy element uptake by vegetation. J Environ Sci Health A 32:455–464
Kabata-Pendias A, Pendias H (1989) Trace elements in the soil and plants. CRC Press, Boca Raton, FL
Kapulnik Y (1991) Plant-growth-promoting rhizobacteria. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots the hidden half. Marcel Dekker Inc., New York, pp 347–362
Karnitz O Jr, Gurgel LVA, De Freitas RP, Gil LF (2009) Adsorption of Cu(II), Cd(II), and Pb(II) from aqueous single metal solutions by mercerized cellulose and mercerized sugarcane bagasse chemically modified with EDTA dianhydride (EDTAD). Carbohydr Polymer 77:643–650
Karnitz O Jr, Gurgel LV, de Melo JC, Botaro VR, Melo TM, de Freitas Gil RP, Gil LF (2007) Adsorption of heavy metal ion from aqueous single metal solution by chemically modified sugarcane bagasse. Bioresour Technol 98:1291–1297
Kawachi M, Kobae Y, Mimura T, Maeshima M (2008) Deletion of a histidine-rich loop of AtMTP1, a vacuolar Zn2+/H+ antiporter of Arabidopsis thaliana, stimulates the transport activity. J Biol Chem 283:8374–8383
Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends Ecol Eng 12:139–143
Kerkeb L, Mukherjee I, Chatterjee I, Lahner B, Salt DE, Connolly EL (2008) Iron-induced turnover of the Arabidopsis iron-regulated transporter1 metal transporter requires lysine residues. Plant Physiol 146:1964–1973

Khan AG (2001) Relationships between chromium biomagnifications ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. Environ Int 26:417–423

Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19

Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259

Kim C, Park SK (2005) Water recycling from desalination and purification process of reactive dye manufacturing industry by combined membrane filtration. J Clean Prod 13:779–786

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Proceedings of 4th international conference plant pathogenic bacteria, pp 879–882

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Nature 379:635

Nanda Kumar PBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29(5):1232–1238

Kumar KV, Srivastava S, Singh N, Behl HM (2009) Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of Brassica juncea. J Hazard Mater 170:51–57

Kuo S, Jellum EJ, Baker AS (1985) Effects of soil type, liming, and sludge application on zinc and cadmium availability to Swiss chard. Soil Sci 139:122–130

Lanquar V, Lelievre F, Bolte S, Hames C, Alcon C, Neumann D, Vansuyt G, Curie C, Schröder A, Kramer U, Barbier-Brygoo H, Thomine S (2005) Mobilization of vacuolar iron by AtNramp3 and AtNramp4 is essential for seed germination on low iron. EMBO J 24:4041–4051

Lasat MM, Fuhrmann M, Ebbs SD, Cornish JE, Kochian LV (1998) Phytoremediation of a radio cesium contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. J Environ Qual 27(1):165–168

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120

Lee S, Kim Y-Y, Lee Y, An G (2007) Rice P1B-type heavy-metal ATPase, OsHMA9, is a metal efflux protein. Plant Physiol 145:831–842

Leita L, de Nobilim M, Muhlbachova G, Mondini C, Marchiol L, Zerbi G (1995) Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory conditions. Biol Fertil Soil 19:103–108

Lewis BG, Johnson CM, Delwiche CC (1966) Release of volatile selenium compounds by plants: collection procedures and preliminary observations. J Agric Food Chem 14:638–640

Liu YG, Wang X, Zeng GM, Qu D, Gu JJ, Zhou M, Chai LY (2007) Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in Bechmeria nivea (L.). Gaud Chem 69:99–107

Liu YG, Zhang HZ, Zeng GM, Huang BR, Li X (2006) Heavy metal accumulation in plants on Mn mine tailings. Pedosphere 16(1):131–136

Lombi E, Zhao FJ, Dunham SJ, MacGrath SP (2001a) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30:1919–1926

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001b) Cadmium accumulation in populations of Thlaspi caerulescens and Thlaspi geosingense. New Phytol 145:11–20

Low KS, Lee CK, Leo AC (1995) Removal of metals from electroplating wastes using banana pith. Bioresour Technol 51:227–231. doi:10.1016/0960- 8524(94)00123-I

Luo CL, Shen ZG, Li XD, Baker AJM (2006) Enhanced phytoextraction of Pb and other metals from artificially contaminated soils through the combined application of EDTA and EDDS. Chemosphere 63(10):1773–1784

Lynch J (1990) The rhizosphere. Wiley, London, UK, 458

Ma LQ, Logan TJ, Traina SJ (1995) Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks. Environ Sci Technol 29:1118–1126

Ma LQ, Rao GN (1997) Effects of phosphate rock on sequential chemical extraction of lead in contaminated soils. J Environ Qual 26:788–794

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Madhaiyan M, Poonguzhali S, Sa T (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (Lycopersicon esculentum L.). Chemosphere 69:220–228

Maiti RK, Pinero JLH, Oreja JAG, Santiago DL (2004) Plant based bioremediation and mechanisms of heavy metal tolerance of plants: a review. Proc Indian Natl Sci Acad. B70 No. 1:1–12

Malik PK (2003) Use of activated carbons prepared from sawdust and rice-husk for adsorption of acid dyes: a case study of acid yellow 36. Dyes Pigment 56:239–249

Maiti RK, Pinero JLH, Oreja JAG, Santiago DL (2004) Plant based bioremediation and mechanisms of heavy metal tolerance of plants. Appl Ecol Environ Res 5(1):27–35

Marshall WE, Wartelle LH, Boler DE, Tole CA (2000) Metal ion adsorption by soybean hulls modified with citric acid: a comparative study. Environ Technol 21:601–607

Mazen AMA (2004) Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. Biolog Planta 48(2):267–272

Mccarty PL (1964) Anaerob Waste Treat Fund 95:107–112

McEldowney S, Hardman DJ, Waite S (1993) Treatment technologies. In: McEldowney S, Hardman DJ, Waite S (eds) Pollution, ecology and biotreatment. Longman Singapore Publishers Pvt. Ltd., Singapore, pp 48–58

McGrath SP, Zhao FJ (2003a) Phytoextraction of metals and metalloids. Curr Opin Biotechnol 14:277–282

McGrath SP, Zhao FJ (2003b) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:1–6

McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, New York, pp 261–288

McGrath SP, Zhao FJ, Lombi E (2002) Phytoremediation of metals, metalloids, and radionuclides. Adv Agron 75:1–56

McGrath SP, Lombi E, Gray CW, Caille N, Dunham SJ, Zhao FJ (2006) Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators Thlaspi caerulescens and *Arabidopsis halleri*. Environ Pollut 141:115–125

McInerney MJ, Bryant MP (1981) Review of methane fermentation fundamentals. In: Wise DL (ed) Fuel gas production from biomass. CRC Press, 19/46

McIntyre T (2003) Phytoremediation of heavy metals from soils. Adv Biochem Eng Biotechnol 78:97–123

Meagh RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162

Melamed R, Cao XD, Chen M, Ma LQ (2003) Field assessment of lead immobilization in a contaminated soil after phosphate application. Sci Total Environ 305:117–127

Memon AR, Aktoprakligıl D, Özdemir A, veVertii A (2001) Heavy metal accumulation and detoxification mechanisms in plants. Turk J Bot 25:111–121

Memon AR, Schröder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Environ Sci Pollut Res 16:162–175

Minke R, Rott U (1999) Anaerobic treatment of split flow wastewater and concentrates from the textile processing industry. Water Sci Technol 40(1):169–176

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653

Mirza N, Mahmood Q, Pervez A, Ahmad R, Farooq R, Shah MM, Azim MR (2010a) Phytoremediation potential of *Arundo donax* L in arsenic contaminated synthetic wastewater. Bioresour Technol 101:5815–5819

Mirza N, Pervez A, Mahmood Q, Ahmad SS (2010b) Phytoremediation of arsenic (As) and mercury (Hg) contaminated soil. World Appl Sci J 1(8):113–118

Mishra PK, Mishra S, Selvakumar G, Bisht SC, Kundu S, Bisht JK, Gupta HS (2008) Characterization of a psychrotrophic plant growth promoting Pseudomonas PGERs17 (MTCC 9000) isolated from North Western Indian Himalayas. Ann Microbiol 58(4):1–8

Mo SC, Choi DS, Robinson JW (1989) Uptake of mercury from aqueous solution by duckweed: the effect of pH, copper, and humic acid. J Environ Health 24:135–146

Mohan D, Kunwar PS, Vinod KS (2008) Wastewater treatment using low cost activated carbons derived from agricultural byproducts – a case study. J Hazard Mater 152:1045–1053

Mohan SM, Rao NC, Prasad KK, Karthikeyan J (2001) Treatment of simulated reactive yellow 22 (Azo) dye effluents using Spirogyra species. Waste Manag 22:575–582

Molina SM, Rodrı´guez-Reinoso F, Caturla F, Selle´s MJ (1996) Development of porosity in combined phosphoric acid-carbon dioxide activation. Carbon 34:457–462

Mónica SFM, Santos ES, Barros RJJ, da Silva MCS (2005) Treatment of acid mine drainage with sulphate-reducing bacteria using a two stage bioremediation process

Moreno-Jimenez E, Gamarra R, Carpena-Ruiz RO, Millan R, Penalosa JM, Esteban E. (2006) Mercury bioaccumulation and phytotoxicity in two wild plant species of Almaden area. Chemosphere 63:1969–1973

Navari-Izzo F, Quartacci MF (2001) Phytoremediation of metals – tolerance mechanisms against oxidative stress. Minerva Biotecnol 13:73–83

Neumann D, Lichtenberger O, Gunther D, Tschiersch K, Nover L (1994) Heat-shock proteins induce heavy-metal tolerance in higher plants. Planta 194:360–367

Nriagu JO (1979) Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. Nature 279:409–411

Nriagu JO (1984) Formation and stability of base metal phosphates in soils and sediments. In: Nriagu JO, Moore PB (eds) Phosphate minerals. Springer, London, pp 318–329

Nwoko CO (2010) Trends in phytoremediation of toxic elements and organic pollutants. Afr J Biotechnol 9(37):6010–6016

Nykova N, Muller TG, Gyllenberg M, Timmer J (2002) Quantitative analyses of anaerobic wastewater treatment processes: identifiability and parameter estimation. Biotechnol Bioeng 78(1):89–103

O'Connor CS, Leppi NW, Edward R, Sunderland G (2003) The combined use of electrokinetic remediation and phytoremediation to decontaminate metal-pollute soils: a laboratory-scale feasibility study. Environ Monitor Assess 84:141–158

Okoronkwo NE, Igwe JC, Onwuchekwa EC (2005) Risk and health implications of polluted soils for crop production. Afr J Biotechnol 4:1521–1524

Payne R (2000) *Spirulina as* bioremediation agent: interaction with metals and involvement of carbonic anhydrase. M.Sc. thesis, Rhodes University

Perales-Vela HV, Pen˜a-Castro JM, Can˜izares-Villanueva RO (2006) Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64:1–10

Pilon-Smits EA (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39

Pilon-Smits EAH, Desouza MP, Hong G, Amini A, Bravo RC, Payabyab ST (1999) Selenium volatilization and accumulation by twenty aquatic plant species. J Environ Qual 28(3):1011–1017

Plaza S, Tearall KL, Zhao FJ, Buchner P, McGrath SP, Hawkesford MJ (2007) Expression and functional analysis of metal transporter genes in two contrasting ecotypes of the hyperaccumulator Thlaspi caerulescens. J Exp Bot 58(1717–1728):85–107

Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology. Elect J Biotechnol 6:275–321

Puschenreiter M, Stoger G, Lombi E, Horak O, Wenzel WW (2001) Phytoextraction of heavy metal contaminated soils with Thlaspi goesingense and Amaranthus hybridus: rhizosphere manipulation using EDTA and ammonium sulphate. J Plant Nutr Soil Sci 164:615–621

Raaijmakers JM (2001) Rhizosphere and rhizosphere competence. In: Maloy OC, Murray TD (eds) Encyclopedia of plant pathology. Wiley, New York, USA, pp 859–860

Rainbow PS (1995) Mar Pollut Bullet 31:183

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30:1562–1574

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226

Rauser W (2002) The role of glutathione in plant reaction and adaptation to excess metals. In: Grill D, Tausz M, de Kok LJ (eds) Significance of glutathione to plant adaptation to the environment. Springer, The Netherlands, pp 123–154

Reddad Z, Glerente C, Andres Y, Thibault J, Le Cloirec P (2003) Cadmium and lead adsorption by a natural polysaccharide in MF membrane reactor: experimental analysis and modeling. Water Res 37:3983–3991

Reeves RD, Schwartz C, Morel JL, Edmondson J (2001) Distribution and metal-accumulating behavior of Thlaspi caerulescens and associated metallophytes in France. Int J Phytoremediat 3:145–172

Reichman SM (2002) The responses of plants to metal toxicity: a review focusing on copper, manganese and zinc. Australian Minerals & Energy Environment Foundation, Melbourne

Reilly C (1972) Amino acids and amino acid copper complexes in water soluble extracts of copper tolerant and non tolerant *Becium homblei*. Z Pflanzenphysiol 66:294–296

Revathi S, Venugopal S (2013) Physiological and biochemical mechanisms of heavy metal tolerance. Int J Environ Sci 3(5): doi: 10.6088/ijes.2013030500004

Roosens N, Verbruggen N, Meerts P, Ximenez-Embun P, Smith JAC (2003) Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ 26:1657–1672

Roosens NHCJ, Willems G, Saumitou-Laprade P (2008) Using Arabidopsis to explore zinc tolerance and hyperaccumulation. Trend Plant Sci 13:208–215

Rotkittikhun P, Chaiyaret R, Krutrachue M, Pokethitiyook P, Baker AJM (2007) Growth and lead accumulation by the grasses Vetiveria zizanioides and Thysanolaena maxima in lead-contaminated soil amended with pig manure and fertilizer: a green house study. Chemosphere 66:45–53

Rtiegsegger A, Brunold C (1992) Effect of cadmium on γ-glutamylcysteine synthesis in maize seedlings. Plant Physiol 99:428–433

Sahi SV, Bryant NL, Sharma NC, Singh SR (2002) Characterization of lead hyperaccumulator shrub, Sesbania drummondii. Environ Sci Technol 36:4676–4680

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Ann Rev Plant Biol 49:643–668

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Densely B, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Sánchez MJ, Sánchez CM, Lorenzo LF (2000) Cadmium and lead contents in suburban and urban soils from two medium-sized cities of Spain: influence of traffic intensity. Bullet Environ Contamin Toxicol 64:250–257

Santos FS, Hernández-Allica J, Becerril JM, Amaral- Sobrinho N, Mazur N, Garbisu C (2006) Chelate induced phytoextraction of metal polluted soils with Brachiaria decumbens. Chemosphere 65(1):43–50

Schnoor JL (2000) Phytostabilization of metals using hybrid poplar trees. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 133–150

Schnoor JL, McCutcheon SC (2003) Phytoremediation transformation and control of contaminants. Wiley-Interscience Inc, Hoboken, NJ, USA

Sebastiani L, Scebba F, Tognetti R (2004) Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides*×*maximowiczii*) and I-214 (*P.*×*euramericana*) exposed to industrial waste. Environ Exp Bot 52:79–88

Sekabira K, Oryem Origa H, Basamba TA, Mutumba G, Kakudidi E (2011) Int J Environ Sci Technol 8:115–128

Sekhar KC, Kamala CT, Chary NS, Sastry ARK, Rao TN, Vairamani M (2004) Removal of lead from aqueous solutions using an immobilized biomaterial derived from a plant biomass. J Hazard Mater 108:111–117

Sen S, Demirer GN (2003) Anaerobic treatment of real textile wastewater with a fluidized bed reactor. Water Res 37:1868–1878

Sharma S, Dietz KJ (2006) J Exp Bot 57:711

Shaw BP, Sahu SK, Mishra RK (2004) Heavy metal induced oxidative damage in terrestrial plants. In: Prasad MNV (ed) Heavy metal stress in plants – from biomolecules to ecosystems, 2nd edn. Springer, New York, pp 84–126

Shingu Y, Kudo T, Ohsato S, Kimura M, Ono Y, Yamaguchi I, Hamamoto H (2005) Characterization of genes encoding metal tolerance proteins isolated from Nicotiana glauca and Nicotiana tabacum. Biochem Biophys Res Commun 331:675–680

Singh OV, Labana S, Pandey G, Budhiraja R, Jain RK (2003) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61:405–412

Singh OV, Labana S, Pandley G, Budhiaraja R, Jain RK (2001) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61:405–412

Smith RAH, Bradshaw AD (1992) Stabilization of toxic mine wastes by the use of tolerant plant populations. Trans Inst Min Metall 81:A230–A237

Smits P (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39

Smolders E, Degryse F (2002) Fate and effect of zinc from tire debris in soil. Environ Sci Technol 36:3706–3710

Solhi M, Hajabbasi MA, Shareatmadari H (2005) Heavy metals extraction potential of sunflower (*Helianthus annuus*) and canola (*Brassica napus*). Isfahan Agricultural Research, Soil and Water Department, College of Isfahan, Isfahan University of Technology, Isfahan

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signaling: a love parade beneath our feet. Crit Rev Microbial 30:205–240

Sörensen J (1997) The rhizosphere as a habitat for soil microorganisms. In: van Elsas JD, Trevors JT, Wellington EMH (eds) Soil microbiology. Marcel Dekker, New York, pp 21–45

Sousa FW, Moreira SA, Oliveira AG, Cavalcante RM, Nascimento RF, Rosa MF (2007) The use of green coconut shells as absorbents in the toxic metals. Quimica Nova 30(5):1153–1157

Stoltz E, Greger M (2002) Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ Exper Bot 47(3):271–280

Suresh B, Ravishankar GA (2004) Phytoremediation – a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24:97–124

Suszcynsky EM, Shann JR (1995) Phytotoxicity and accumulation of mercury subjected to different exposure routes. Environ Toxicol Chem 14:61–67

Sutherland RA, Day JP, Bussen JO (2003) Lead concentrations, isotope ratios and source apportionment in road deposited sediments, Honolulu, Oahu, Hawaii. Water Air Soil Pollut 142:165–186

Taiz L, Zieger E (1999) Plant physiology. M.A. Sinauer, Sunderland, p 690

Talke IN, Kramer U, Hanikenne M (2006) Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator Arabidopsis halleri. Plant Physiol 142:148–167

Tandy S, Schulin R, Nowack B (2006) The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. Chemosphere 62(9):1454–1463

Tang C, Chen V (2002) Nanofiltration of textile wastewater for water reuse. Desalin 143:11–20

Tarley CRT, Arruda MAZ (2004) Biosorption of heavy metals using rice milling by-products characterization and application for removal of metals from aqueous effluents. Chemosphere 54:987–995

Terry N, Carlson C, Raab TK, Zayed A (1992) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344

Thangavel P, Subhuram CV (2004) Phytoextraction – role of hyper accumulators in metal contaminated soils. Proc Indian Natl Sci Acad B 70(1):109–130

Thurman DA, Rankin AJ (1982) The role of organic acids in zinc tolerance in *Deschampsia caespitosa*. New Phytol 91:629–634

Tordoff GM, Baker AJM, Willis AJ (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41(1–2):219–228

Turer D, Maynard JB, Sansalone JJ (2001) Heavy metal contamination in soils of urban highways: comparison between runoff and soil concentrations at Cincinnati, OH. Water Air Soil Pollut 132:293–314

Uroz S, Calvaruso C, Turpault MP, Frey Klett P (2009) Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol 17:378–387

Utsunamyia T (1980) Japanese patent application no. 55-72959

Vannela R, Verma SK (2006) $Co^2$+, $Cu^2$+, and $Zn^2$+ accumulation by cyanobacterium Spirulina platensis. Biotechnol Prog 22:1282–1293

Vatamaniuk OK, Mari S, Lu YP, Rea PA (1999) Proc Natl Acad Sci U S A 96:7110

Viklander M (1998) Particle size distribution and metal content in street sediments. J Environ Engine 124:761–766

Vinod KG, Jainb CK, Alib I, Sharmaa M, Saini VK (2003) Removal of cadmium and nickel from wastewater using bagasse fly ash – a sugar industry wastes. Water Res 37:4038–4044

Volesky B (1990) Removal and recovery of heavy metals by biosorption. In: Volesky B (ed) Biosorption of heavy metals. CRC Press, Boca Raton, pp 7–44

Volland S, Schaumlöffel D, Dobritzsch D, Krauss GJ, Lütz-Meindl U (2012) Identification of phytochelatins in the cadmium-stressed conjugating green alga Micrasterias denticulata. Chemosphere 91:448–454

Wafwoyo W, Chung WS, Marshall WE (1999) Utilization of peanut shells as adsorbents for selected metals. J Chem Technol Biotechnol 74:1117–1121

Wang QR, Cui YS, Liu XM, Dong YT, Christine P (2003) Soil contamination and plant uptake of heavy metals at polluted sites in China. J Environ Sci Health 38(5):823–838

Wang SW, Shan XQ, Zhang SZ (2002) Comparison between fractionation and bioavailability of trace elements in rhizosphere and bulk soils. Chemosphere 46:1163–1171

Wartelle LH, Marshall WE (2000) Citric acid modified agricultural byproducts as copper ion adsorbents. Adv Environ Res 4:1–7

Watanabe ME (1997) Phyto-remediation on the brink of commercialization. Environ Sci Technol 31:182–186
Welbaum GE, Sturz AV, Dong Z, Nowak J (2004) Managing soil microorganisms to improve productivity of agro-ecosystems. Crit Rev Plant Sci 23:175–193
Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation(phytoremediation) of soils. Plant Soil 321:385–408
Wenzel WW, Adriano DC, Salt D, Smith R (1999a) Phytoremediation: a plant-microbe-based remediation system. In: Adriano DC, Bollag J-M, Frankenberger WT Jr, Sims RC (eds) Agronomy monograph 37, Madison, USA, pp 457–508
Wenzel WW, Adriano DC, Salt D, Smith R (1999b) Phytoremediation: a plant-microbe-based remediation system. In: SSSA (ed) Bioremediation of contaminated soils, Agronomy monograph no. 37, SSSA, Madison, WI, USA, pp 457–508
Willems G, Dräger DB, Courbot M (2007) The genetic basis of zinc tolerance in the metallophyte Arabidopsis halleri ssp. Halleri (Brassicaceae) an analysis of quantitative trait loci. Genetics 176:659–674
Williamson A, Johnson MS (1981) Reclamation of metalliferous mine wastes. In: Lepp NW (ed) Effect of heavy metal pollution on plants, vol 2. Applied Science, Barking, Essex, UK, pp 185–212
Wilson JA, Demis J, Pulford LD, Thomas S (2001) Sorption of Cr (III) and Cr (VI) by natural (bone) charcoal. Environ Geochem Health 23:291–295
Wu J, Hsu FC, Cunningham SD (1999) Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. Environ Sci Technol 33:1898–1904
Xing JP, Jiang RF, Ueno D, Ma JF, Schat H, McGrath SP, Zhao FJ (2008) Variation in root-to-shoot translocation of cadmium and zinc among different accessions of the hyperaccumulators Thlaspi caerulescens and Thlaspi praecox. New Phytol 178:315–325
Xiong J, He Z, Liu D, Mahmood Q, Xiao'E Y (2008) The role of bacteria in the heavy metals removal and growth of Sedum alfredii Hance in an aqueous medium. Chemosphere 70:489–494
Yamane K, Rahman MS, Kawasaki M, Taniguchi M, Miyake H (2004) Pretreatment with a low concentration of methyl viologen decreases the effects of salt stress on chloroplast ultrastructure in rice leaves (*Oryza sativa* L.). Plant Prod Sci 7:435–441
Yang Q, Tu S, Wang G, Liao X, Yan X (2012) Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by Pteris vittata L. Int J Phytoremed 14:89–99
Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368(2–3):456–464
Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of Bacillus subtilis strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. Chemosphere 64:991–997
Zhang XH, Liu J, Huang HT, Chen J, Zhu YN, Wang DQ (2007) Chromium accumulation by the hyperaccumulator plant Leersia hexandra Swartz. Chemosphere 67:1138–1143
Zhang P, Ryan JA (1999) Formation of chloropyromorphite from galena (PbS) in the presence of hydroxyapatite. Environ Sci Technol 33:618–624
Zhou M, Liu YG, Zeng GM, Li X, Xu WH, Fan T (2007) Kinetic and equilibrium studies of Cr (VI) biosorption to dead Bacillus licheniformis biomass. World J Microbiol Biotechnol 23:43–48
Zhu YG, Rosen BP (2009) Perspectives for genetic engineering for the phytoremediation of arsenic-contaminated environments: from imagination to reality? Curr Opin Biotechnol 20:220–224
Zhu YG, Chen SB, Yang JC (2004) Effects of soil amendments on lead uptake by two vegetable crops from a lead-contaminated soil from Anhui, China. Environ Int 30:351–356
Zhu YL, Zayed AM, Qian JH, De-Souza M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J Environ Qual 28(1):339–344
Zvinowanda CM, Okonkwo JO, Shabalala PN, Agyei NM (2009) A novel adsorbent for heavy metal remediation in aqueous environments. Int J Environ Sci Technol 6(3):425–434
Zwonitzer JC, Pierzynski GM, Hettiarachchi GM (2003) Effects of phosphorus additions on lead, cadmium and zinc bioavailabilities in a metal-contaminated soil. Water Air Soil Pollut 143: 193–209

# Phytoremediation Using Algae and Macrophytes: II

# 23

Keshav Singh Jatav and Ram Pratap Singh

## 23.1 Introduction

Phytoremediation, an emerging green technology, is the use of certain plant species to remediate contaminated soil and groundwater. It is a biological treatment utilizing any type of plant either terrestrial or marine plant. Organic as well as inorganic contaminants can be remediated using this technology. Phytoremediation has received increasing attention after the discovery of hyperaccumulating plants which are capable to accumulate, translocate, and concentrate high amount of certain toxic elements in their aboveground/harvestable parts. Phytoremediation is an attractive alternative to current remediation methods that are energy intensive and very expensive.

## 23.2 Phytoremediation Processes

Phytoremediation is based on different biological mechanisms occurring in plants and their associated microorganisms. These mechanisms involve photosynthesis, transpiration, metabolism, and mineral nutrition. At present, the following processes of this green technology as applicable for the remediation of toxic compounds are:

1. Phytoextraction: In this process, pollutant uptakes by plants are translocated to and stored in the harvestable biomass or aboveground part of the plants. This process is usually observed in hyperaccumulating plants resistant to contaminants (Blaylock and Huang 2000).
2. Phytostabilization: In this plant reduces the mobility and phytoavailability of pollutants in the soil sediments and groundwater but does not remove them from contaminated sites. These contaminants then are rendered in the stable form (ITRC 2001).
3. Phytovolatilization: This process involves the uptake of pollutants from soil and water and releases them from aerial plant parts in the form of gas (Terry et al. 1992).
4. Phytodegradation (phytotransformation): It is a kind of plant defense mechanism against environmental contaminants. The hyperaccumulating plants modify, inactivate, degrade, or immobilize the pollutants through their metabolism (EPA 1999).
5. Rhizofiltration: This process is concerned with the remediation of contaminated groundwater rather than the polluted soil. Usually, aquatic plants performed this process. The hyperaccumulating plants absorb and adsorb pollutants from aquatic environment (EPA 1999).

## 23.3 Plants Having Phytoremediation Potential

Researchers have recognized various plant groups having potential to remediate different types of contaminants present in soil and water resources. Many aquatic floating macrophytes, grasses/legumes, forbes, trees, shrubs, and vines are found to be hyperaccumulators/accumulators of organic as well as inorganic contaminants in different polluted sites. Some plants are listed in Table 23.1.

### 23.3.1 Phytoremediation Mechanism of Organic Contaminants

Hyperaccumulator plants possess genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plants. These genes govern process that can increase the solubility of metals in the soil

K.S. Jatav, M.Sc., Ph.D. (✉)
Department of Botany, Govt. Chhatrasal College Pichhore, Shivpuri, MP, India
e-mail: ksingh_botany@rediffmail.com

R.P. Singh, Ph.D. (✉)
Department of Botany, Govt. P.G. College, Morena, MP, India
e-mail: rpsingh.aga@gmail.com

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**Table 23.1** Hyperaccumulator/accumulator plant species of organic and inorganic contaminants

| Scientific name | Uptake of trace element | References |
|---|---|---|
| Aquatic macrophytes | | |
| *Lemna gibba* | As, U, Zn | Fritioff and Greger (2003) |
| *Lemna minor* | As, Zn, Cu, Hg | Kara (2004), Fritioff and Greger (2003) |
| *Eichhornia crassipes* | As, Fe, Cu, Zn, Pb, Cd, Cr, Ni, Hg | Dixit and Dhote (2010) |
| *Salvinia rotundifolia* | Pb | Banerjee and Sarkar (1997), Dhir (2009) |
| *Spirodela polyrhiza* | Pb, Cu, Zn | Loveson et al. (2013) |
| *Salvinia cucullata* | Cd, Pb | Phetsombat et al. (2006) |
| *Azolla caroliniana* | Zn | Deval et al. (2006) |
| *Typha angustata* | Zn, Cu, Pb | Kumar et al. (2008) |
| *Ipomoea aquatica* | Zn, Cu, Pb | Kumar et al. (2008) |
| *Hydrilla verticillata* | As, Cd | Ghos (2010) |
| Forbes | | |
| *Brassica juncea* | Pb, Zn, Ni, Cr, Cd, Ur | McCutcheon and Schnoor (2003) |
| *Helianthus annuus* | Pb, Ur, Sr, Cr, Cd, Cu, Mn, Ni, Zn | McCutcheon and Schnoor (2003) |
| *Thlaspi caerulescens* | Cd, Zn, Ni | |
| *Digitalis purpurea* | Cd | McCutcheon and Schnoor (2003) |
| *Arabidopsis thaliana* | Zn, Cu, Pb | Reeves and Baker (2000) |
| *Arabidopsis halleri* | Zn | Reeves and Baker (2000) |
| *Chenopodium amaranticolor* | Uranium | Eapen et al. (2003) |
| Trees, shrubs, and vines | | |
| *Gleditsia triacanthos* | Pb | Gawronski et al. (2003) |
| *Ilex* spp. | Cd | IERE (2003) |
| *Liquidambar styraciflua* | Perchlorate | McCutcheon and Schnoor (2003) |
| *Populus* spp. | Chlorinated solvents, atrazine, DDT, carbon tetrachloride | McCutcheon and Schnoor (2003) |
| *Populus tremula* | Pb | McCutcheon and Schnoor (2003) |
| *Salix* spp. | Perchlorate | IERE (2003) |
| *Viola* spp. | Metals (Cd) | IERE (2003) |
| *Zea mays* | Cd, Pb | Mojiri (2011) |
| *Jatropha curcas* | Cd, Cr, Ni | Jyoti Luhach and Smita Chaudhary (2012) |
| *Cyperus rotundus* | Pb, Zn | Anh-Bui et al. (2011) |
| Algal species | | |
| *Chlorella sorokiniana* | Heavy metals Cd, Cu, Zn | Yoshida et al. (2006) |
| *Spirogyra hyalina* | Cd, Hg, Pb, As, Co | Kumar and Oommen (2012) |
| *Phormedium bohner* | Cr | Dwivedi et al. (2010) |
| *Ascophyllum nodosum* | Co, Ni, Pb | Holan and Volesky (1994) |
| *Caulerpa racemosa* | Boron (B) | Bursali et al. (2009) |
| *Daphnia magna* | As | Irgolic et al. (1977) |
| *Laminaria japonica* | Zn | Fourest and Volesky (1997) |
| *Platymonas subcordiformis* | Sr | Mei et al. (2006) |
| *Sargassum filipendula* | Cu | Davis et al. (2000) |
| *Sargassum natans* | Pb | Holan and Volesky (1994) |

surrounding the roots as well as the transport proteins that move metals into root cells. Plants contribute a number of processes to remove or stabilize pollutants from soil and water (Davies 1983). Basically there are two major mechanisms which plants utilize to remove contaminants:

1. Direct uptake of contaminants and subsequent accumulation of non-phytotoxic metabolites into plant tissues
2. Release of exudates and enzymes that stimulate microbial activity resulting in the enhancement of microbial transformations in the rhizosphere

### 23.3.2 Direct Uptake

Direct uptake of organics by plants is a surprisingly efficient removal mechanism for moderately hydrophobic organic compounds, but all organic compounds are not equally accessible to plant roots in the soil environment. Plants are known to take up many pollutants, in particular weak electrolytes and compounds with intermediate lipophilicity. The inherent ability of the roots to take up organic compound can be described by the hydrophobicity (or lipophilicity) of the target compounds (Suthersan 1999). This parameter is often expressed as the log of the octanol-water partitioning coefficient, $K_{ow}$. Very polar compounds have difficulties crossing biomembranes and therefore are subjected to limited uptake. Very lipophilic compounds quickly cross biomembranes, but then sorb to the roots. Therefore, compounds with intermediate lipophilicity are best translocated in upper plant parts, which explains the bell-shaped relation between log $K_{ow}$ and the transpiration stream concentration factor (TSCF, concentration ratio between xylem solution and external solution) (Briggs et al. 1982, Burken and Schnoor 1998, Trapp 2000). Different plant roots show some differences in different soil conditions, but generally the higher a compound's log $K_{ow}$, the greater the root uptake.

Once an organic chemical is taken up, a plant can store the chemical and its fragments in new plant structures via lignification, or it can volatilize, metabolize, or mineralize the chemical all the way to carbon dioxide, water, and chlorides. Different plants exhibit different metabolic capacities, e.g., during the herbicide application, the crop species are capable of metabolizing the pesticide to nontoxic compound, whereas the weed species either lack this capacity or metabolize it at a too slow rate which results to the death of weed species (Suthersan 1999).

### 23.3.3 Degradation in Root Zone

In the root zone of plants, indigenous microorganisms are found in mutual relationship. This microflora is distinctly different from the characteristic soil population because plant creates a unique subterranean habitat for microorganisms. Different plant species often established somewhat different subterranean floras. The roots of the plant exude a wide spectrum of compounds including sugars, amino acids, carbohydrates, and essential vitamins (Suthersan 1999). These exudates may also be acetates, esters, benzene derivatives, and enzymes that may act as growth- and energy-yielding substrates for the microbial population in the root zone. This process allows microbial population for enhanced degradation of organics by the provision of appropriate beneficial primary substrate for the metabolic transformation of contaminants. In addition to the plant exudates, the rapid decay of fine-root biomass can become an important addition of organic carbon to soil which in turn may increase microbial mineralization rates (Suthersan 1999).

### 23.3.4 Phytoremediation Mechanism of Heavy Metals

Most metals interact with the inorganic and organic matter that is present in root-soil environment. Heavy metals have so many chemical and physical forms in the soil environment. The chemistry of the metal and its mobility will inherently impact the toxicity in the environment. Phytoremediation of heavy metal-contaminated soil can be divided into phytostabilization and phytoextraction approaches (Suthersan 1999).

#### 23.3.4.1 Phytostabilization of Heavy Metals

This involves the reduction in the mobility of heavy metal by minimizing soil erodibility and decreasing the potential for wind-blown dust and reduction in contaminant solubility by the addition of soil amendments. The density of vegetation at the contaminated site will significantly hold the soil and provide a stable cover against erosion. Phytostabilization has made available a variety of matters like alkalizing agents, phosphates, organic matter, and biosolids to the system to render the metals insoluble and unavailable to leaching. Materials with a calcareous character or a high pH can be added to influence the acidity, e.g., lime and gypsum. Specific binding conditions can be influenced by adding concentrated Fe, Mn, or Al compounds (ITRC 2001, Suthersan 1999).

#### 23.3.4.2 Phytoextraction of Heavy Metals

The utilization of nonedible or unusual plants that have potential to accumulate very high concentration of metals from contaminated soils in their biomass provides the basis for this phytoremediation technique. These plants are called hyperaccumulator plants, and they have the ability to tolerate high concentration of toxic metals in aboveground plant tissues (Blaylock and Huang 2000). In these plants, metals are translocated to the shoot and tissue via the root. The success of phytoextraction depends on the use of an integrated approach to soil and plant management. Nowadays, disciplines of various fields like physiology, agronomy, soil chemistry, soil fertility, and plant genetic engineering are being used to increase both the rate and efficiency of heavy metal extraction (Suthersan 1999).

### 23.3.5 Algae in Phytoremediation

Algae play an important role in controlling metal concentration in lakes and oceans (SIGG 1985, 1987). The algae possess many features for the selective remediation of heavy metals which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression, and potential for genetic manipulation. The use of microalgae is more beneficial as they are able to serve a multiple role such as bioremediation as well as generating biomass for biofuel production simultaneously with carbon sequestration (Olguin 2003; Mulbry et al. 2008). Wastewater remediation using microalgae is also an eco-friendly process as it does not release any kind of secondary pollution (Munoz and Guieysse 2006; Pizarro et al. 2006; Mulbry et al. 2008).

Metal absorption ability of macroalgae has been recognized for many years throughout the world. Recently, several species of green algae *Enteromorpha* and *Cladophora* have been utilized to measure heavy metal levels in many parts of the world. Rawat et al. (2011) have recognized microalgae as the most promising organism for bioprocess due to multiplicity of reactions. Mitra et al. (2012) have reported four algal divisions, viz., chlorophyta, cyanophyta, euglenophyta, and heterokontophyta as accumulators of boron and arsenic. *Chara vulgaris* is observed for strong phytoextraction ability of Congo red dye from its aqueous solution. *Chlorella* sp. was observed in reducing harmful nutrients in the aquaculture wastewater (Ahmad et al. 2013). *Gracilaria edulis* was reported as a phytoremediation agent to improve shrimp pond water quality by Lavania et al. (2012). The brown algae (*Phaeophyta*) are particularly efficient accumulator of metals due to high levels of sulfated polysaccharides and alginates within their cell walls for which metals show a strong affinity (Davis et al. 2002, Nielsen et al. 2005) suggested that *Fucus serratus* often dominate the vegetation of heavy metal-contaminated habitats. Marine green microalgae *Platymonas subcordiformis* had a very high strontium uptake capacity; however high concentration of strontium causes oxidative damage (Mei et al 2006). The blue green alga *Phormidium* can successfully hyperaccumulate heavy metals like Cd, Zn, Pb, Ni, and Cu (Wang et al. 1995). *Caulerpa racemosa* var. *cylindracea* can be used for the removal of boron (B) from aqueous solution (Bursali et al. 2009). Green microalgae *Dunaliella salina* have high tendency to accumulate Zn, Cu, and Co. However, this alga shows lower accumulation tendency to Cu and Co than Zn (Magda A Shafik 2008). Two marine algae *Thalassiosira weissflogii* and *Thalassiosira pseudonana* produce phytochelatins in great amounts due to the higher activity of phytochelatin synthase, which has greater affinity for the glutathione substrate or metal ions (Ahner et at. 2002; Yoshida et al. 2006) reported *Chlorella sorokiniana* incapable of taking up the heavy metal ions $Cd^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ in dark laboratory conditions. *Chlorella vulgaris* studied by Mamun et al. (2012) was found to have a potential to reduce nutrient content, chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) of wastewater.

### 23.3.6 Heavy Metal Accumulation and Tolerance Mechanism in Algae

Organisms utilize different defense mechanisms in response to heavy metal stress. These include exclusion, compartmentalization, making complexes, and the synthesis of binding proteins such as metallothionein (MTs) or phytochelatins (PCs) and translocate them into vacuoles (Mejare and Bulow 2001). Some potential ligands for heavy metals like carboxylic and amino acids, such as citrate, malate, and oxalate, histidine (His) and nicotianamine (NA), and phosphate derivatives are found to play an important role in tolerance and detoxification Sharma and Dietz (2009), Singh and Chauhan (2011). The adsorption, phytoremediation, and affinity of algae for heavy metal cations in wastewater treatment because of its high negatively charged surface (cell wall components) have been acknowledged for a long time (Sekabira et al. 2011).

### 23.3.7 Aquatic Macrophytes in Phytoremediation

A number of aquatic plant species have been investigated for the remediation of toxic contaminants such as As, Zn, Cd, Pb, Cr, Hg, etc. Rahman and Hasegawa (2011). *Ceratophyllum demersum*, submerged aquatic macrophytes, were reported as accumulator of arsenic. The accumulation was highest at pH 5 and decreased as pH values increased. Toxic effect was evident by plant necrosis and negative biomass production (Khang et al. 2012). Phetsombat et al. (2006) studied *Salvinia cucullata* (an aquatic fern) and reported it as an accumulator of cadmium and lead. The accumulation study showed the significant increase of both metals when the exposure time and concentration of these metals were increased in medium. Roots of *S. cucullata* had higher Cd and Pb contents than leaves, suggesting that the metals were bound to the root cells and were partially transported to the leaves. The toxicity symptoms of Cd and Pb to *Salvinia cucullata* showed chlorosis on leaves. Ghosh (2010) investigated two macrophytes *Hydrilla verticillata* and *Ipomoea aquatica* as strong accumulators of cadmium. *Azolla caroliniana* was reported as an efficient accumulator of $Zn^{2+}$ through liquid medium by Deval et al. (2012).

Heavy metals (Pb, Ni, and Cd) from municipal waste leachate were efficiently remediated by *Typha domingensis* (Mojiri et al. 2013). Loveson and coworkers (2013) studied a floating macrophyte *Spirodela polyrhiza* in polluted wetland and found it an efficient tool in removing heavy metals from wetland water. *Azolla* (an aquatic pteridophyte) was found to be a bioremediation agent. It is an ideal choice because of its multiplication rate, global distribution, high biomass production, high protein content, and its growth habitats (Sarita Sachdeva and Anita Sharma 2012). It can uptake and accumulate nutrients directly from flood water and has high affinity for P, Fe, and K. It accumulates these nutrients several times more than its requirement and then slowly releases these nutrients as it decomposes. Based on its capacity, it has also been used for the treatment of wastewaters (Wagner 1997).

### 23.3.8 Terrestrial Plants in Phytoremediation

A number of grasses/legumes, herbs, shrubs, and trees have been investigated having potential to remediate various pollutants from soil. *Cerastium arvense*, *Claytonia perfoliata*, and *Stellaria calycantha* were found to uptake and accumulate cadmium. *Lupinus albus*, a nitrogen fixing legume, has been found capable to take up arsenic, primarily in the root structure. This legume is capable to grow in acidic soils with low nutrient availability (Esteban et al. 2003). Yan et al. (2013) have suggested two perennial grasses *Arrhenatherum elatius* and *Sonchus transcaspicus* suitable for the phytostabilization of metal-polluted soils. *Arrhenatherum elatius* accumulated metals like Ni, Cu, Cd, Co, Mn, Pb, Cr, and Zn in roots, while in *S. transcaspicus*, metals were preferentially accumulated in shoots. Anh-Bui et al. (2011) reported *Pteris vittata* L., *Pityrogramma calomelanos* L., *Cynodon dactylon* L., *Eleusine indica* L., *Cyperus rotundus* L., and *Equisetum ramosissimum* as hyperaccumulators of heavy metals. *Cyperus rotundus* L. and *Equisetum ramosissimum* accumulate very high Pb (0.15–0.65 %) and Zn (0.22–1.56 %).

A study carried out by Mojiri (2011) on *Zea mays* for phytoremediation indicates that corn is an effective accumulator plant for phytoremediation of cadmium- and lead-polluted soils. *Brassica napus* cv. Westar (canola), *Hibiscus cannabinus* cv. Indian (canaf), and *Festuca arundinacea* Schreb. cv. Alta (tall fescue) were found to reduce total soil selenium (Gary et al. 1996). A bush/small tree *Jatropha curcas*, belonging to the family Euphorbiaceae, was found effective in removing cadmium, chromium, and nickel present in oily sludge of petroleum refinery (Jyoti Luhach and Smita Chaudhary 2012).

## 23.4 Conclusion

It is clear that phytoremediation is an eco-friendly and economic technology which is quite significant and helpful for minimizing the pollutants such as heavy metals, toxic elements, and other harmful chemicals from soil and water resources. It can be useful to sustain environment cleanliness.

## References


Ahmad J, Fathurrahman L, Siti Hajar AH (2013) Batch phytoremediation of aquaculture wastewater of silver Barramundi (*Lates calcarifer*) utilizing green microalgae; *Chlorella* sp. J Fish Aquat Sci 8(4):516–525

Ahner BA, Wei L, Oleson JR, Ogura N (2002) Glutathione and other low molecular weight thiols in marine phytoplankton under metal stress. Mar Ecol Prog Ser 232:93–103

Amin M, Aziz HA, Zahed MA, Aziz SQ, Selamat M, Razip B (2013) Phytoremediation of heavy metals from urban waste leachate by southern cattail (Typha domingensis). Int J Sci Res Environ Sci 1(4):63–70

Anh-Bui TK, Kim-Dang D, Tua TV, Kien NT, Anh DT (2011) Phytoremediation potential of indigenous plants from Thai Nguyen province, Vietnam. J Environ Biol 32:257–262

Banerjee G, Sarkar S (1997) The role of *Salvinia rotundifolia* in scavenging aquatic Pb (II) pollution: a case study. Bioprocess Biosyst Eng 17:295–300

Blaylock M, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals using plants to clean up the environment. Wiley, New York, NY, pp 55–70

Briggs G, Bromilow R, Evans A (1982) Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic Sci 13:495–504

Burken JS, Schnoor JL (1998) Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environ Sci Technol 32:3379–3385

Bursali EA, Cavas L, Seki Y, Bozkurt SS, Yurdakoc M (2009) Sorption of boron by invasive marine seaweed: Caulerpa racemosa var. cylindracea. Chem Eng J 150

Davies BE (1983) Heavy metal contamination from base metal mining and smelting: implication for man and his environment. In: Thornton I (ed) Applied environmental geochemistry. Academic, London

Davis TA, Volesky B, Mucci A (2002) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311

Deval CG, Mane AV, Joshi NP, Saratale GD (2012) Phytoremediation potential of aquatic macrophyte *Azolla caroliniana* with references to zinc plating effluent. EJFA 24(3):208–223

Dhir B (2009) *Salvinia*: an aquatic fern with potential use in phytoremediation. Environ Int J Sci Technol 4:23–27

Dixit S, Dhote S (2010) Evaluation of uptake rate of heavy metals by *Eichhornia crassipes* and *Hydrilla verticillata*. Environ Monit Assess 169:367–374

Dwivedi S, Srivastava S, Mishra S, Kumar A, Tripathi RD, Rai UN, Dave R, Tripathi P, Chakrabarty D, Trivedi PK (2010) J Hazard Mater 173:95–98

Eapen S, Saseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003) Potential for rhizofiltration of Uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environ Res 91(2):127–133

Environmental Protection Agency (EPA) (1999) Phytoremediation resource guide. U.S. Office of Solid Waste and Emergency Response Technology Innovation Office Washington, DC 20460

Esteban E, Vazquez S, Carpena R (2003) White lupin response to arsenate. University of Madrid, Spain

Fourest E, Volesky B (1997) Alginate properties and heavy metal biosorption by marine algae. Appl Biochem Biotechnol 67:33

Fritioff A, Greger M (2003) Aquatic and terrestrial plant species with potential to remove heavy metals from stormwater. Int J Phytoremediation 5:211–224

Gary B, Husein A, Bruce M, Wu L,-Davis UC, Charles C, Akohoue S, Stella Z (1996) Evaluation of different plant species used for phytoremediation of high soil selenium. J Environ Qual 26(3). doi: 10.2134/jeq1997.00472425002600030008x

Gawronski SW, Raczka M, Trampczynska A (2003) Ornamental trees and shrubs as phytoremediants. In *COST* Action 837 "Workshop on Phytoremediation of toxic metals". Stockholm, Sweden, June 12–15

Ghosh S (2010) Wetland Macrophytes as toxic metal accumulators. Int J Environ Sci 1:4

Holan ZR, Volesky B (1994) Biosorption of lead and nickel by biomass of marine algae. Biotechnol Bioeng 43:1001

Institute for Environmental Research and Education (IERE) (2003) Vashon heavy metal phytoremediation Study sampling and analysis strategy (DRAFT)

Interstate Technology and Regulatory Council (ITRC) (2001) Phytotechnology technical and regulatory guidance document. Available from www.itrcweb.org/Documents/PHYTO-2.pdf

Irgolic KJ, Woolsont EA, Stockton RA, Newman RD, Bottino NR, Zingaro RA, Kearney PC, Pyles RA, Maeda S, McShane WJ, Cox ER (1977) Environ Health Perspect 19:6166

Kara Y (2004) Bioaccumulation of copper from contaminated wastewater by using *Lemna minor*. Bull Environ Contam Toxicol 72: 467–471

Kumar JI, Oommen CJ (2012) Removal of heavy metals by biosorption using freshwater alga Spirogyra hyalina. Environ Biol.;33(1):27–31

Kumar JI, Hiren S, Kumar RN, Bhatt I (2008) Macrophytes in phytoremediation of heavy metal contaminated water and sediments in Pariyej Reserve, Gujrat, India. Turk J Fish Aquat Sci 8:193–200

Kuyucak N, Volesky B (1988) Biosorbents for recovery of metals from industrial solutions. Biotechnol Lett 10:137

Lavania B, Akira K, Shamila A (2012) *Gracilaria edulis* as phytoremediation agent to improve shrimp pond water quality. J Sustain Sci Manag 7(2):254–256

Loveson A, Sivalingam R, Syamkumar R (2013) Aquatic macrophyte *Spirodela polyrrhiza* as a phytoremediation tool in polluted wetland water from Eloor, Ernakulam District, Kerala. J Environ Anal Toxicol 3:5

Luhach J, Chaudhary S (2012) Phytoremediation potential of *Jatropha curcas* for removal of heavy metals from refinery sludge. Int J Sci Eng Res 3(10)

Mamun AA, Amid A, Rashid SS (2012) Phytoremediation of partially treated wastewater by *Chlorella vulgaris*. International Conference on Chemical Processes and Environmental Issues (ICCEEI) Singapore

McCutcheon SC, Schnoor JL (eds) (2003) Phytoremediation: transformation and control of contaminants. Wiley-Interscience, Inc., Hoboken, NJ

Mei L, Xitao X, Renhao X, Zhili L (2006) Effects of strontium-induced stress on marine microalgae Platymonas subcordiformis (Chlorophyta: Volvocales). Chin J Oceanol Limnol 24:154

Mejare M, Bulow L (2001a) Metal-binding proteins and peptides in bior- emediation and phytoremediation of heavy metals. Trends Biotechnol 19:67

Mejare M, Bulow L (2001b) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19:67–73

Mitra N, Rezvan Z, Ahmad MS, Hosein MGM (2012) Studies of water arsenic and boron pollutants and algae phytoremediation in three springs, Iran. Int J Ecosys 2(3):32–37

Mojiri A (2011) The potential of corn (*Zea mays*) for phytoremediation of soil contaminated with cadmium and lead. J Bio Environ Sci 5(13):17–22

Mulbry W, Kondrad S, Pizarro C, Kebede-Westhead E (2008) Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Bioresour Technol 99:8137–9142

Munoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815

Nielsen HD, Burridge TR, Brownlee C, Brown MT (2005) Prior exposure to Cu contamination influences the outcome of toxicological testing of Fucus serratus embryos. Mar Poll Bull 50:1675–80

Olguiin EJ (2003) Phytoremediation: key issues for cost effective nutrient removal processes. Biotech Adv 22:81–91

Phetsombat S, Kruatrachue M, Pokethitiyook P, Upatham S (2006) Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. J Environ Biol 27(4):645–652

Pizarro C, Mulbry W, Blersch D, Kangas P (2006) An economic assessment of algal turf rubber technology for treatment of dairy manure effluent. Ecol Eng 26:321–327

Rahman MA, Hasegawa H (2011) Aquatic Arsenic: phytoremediation using floating macrophytes. Chemosphere 83:633–646

Rawat I, Kumar RR, Mutanda T, Bux F (2011) Dual role of microalgae: phytoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energ 88:3411–3424

Reeves RD, Baker AJM (2000) Phytoremediation of toxic metals. Wiley, New York, NY, pp 193–229

Sarita Sachdeva, Anita Sharma (2012) Azolla: role in phytoremediation of heavy metals. Proceeding of the national conference “Science in Media 2012” organized by YMCA University of Science and Technology, Faridabad, Haryana. IJMRS’s International Journal of Engineering Sciences

Sekabira K, Oryem Origa H, Basamba TA, Mutumba G, Kakudidi E (2011) Application of algae in biomonitoring and phytoextraction of heavy metals contamination in urban stream water. Int J Environ Sci Tech 8:115–128

Shafik MA (2008) Phytoremediation of some heavy metals by *Dunaliella salina*. Global J Environ Res 2(1):01–11

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50

Sigg L (1985) Metal transfer mechanisms in lakes: role of settling particles. In: Stumm W (ed) Chemical processes in lakes. Wiley Interscience, New York, NY, pp 283–310

Sigg L (1987) Surface chemical aspects of the distribution and fate of metal ions in lakes. In: Stumm W (ed) Aquatic surface chemistry. Wiley Interscience, New York, NY, pp 319–348

Singh D, Chauhan SK (2011) Organic acids of crop plants in alumium detoxification. Curr Sci 100:1509–1515

Siriwan P, Kruatrachue M, Pokethitiyook P, Upatham S (2006) Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. J Environ Biol 27(4):645–652

Suthersan SS (1999) “Phytoremediation” remediation engineering: design concepts. CRC, Boca Raton

Terry N, Carlson C, Raab TK, Zayed AM (1992) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344

Trapp S (2000) Modeling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. Pest Manag Sci 56:767–778

Vinh KH, Hatayama M, Inoue C (2012) Arsenic accumulation by aquatic macrophyte coontail (Ceratophyllum demersum L.) exposed to arsenic, and the effect on iron on the uptake of arsenite and arsenate. Environ Exp Bot 83:47–52

Wagner GM (1997) Azolla: a review of its biology and utilization. Bot Rev 63:1–26

Wang TC, Weissman JS, Ramesh G, Varadarajan R, Benemann JR (1995). In: Bioremediation of inorganics. 33

Yan L, Li X, He M, Zeng F (2013) Behavior of native species *Arrhenatherum elatius* (Poaceae) and *Sonchus Transcaspicus* (Asteraceae) exposed to a heavy metal polluted field: plant metal concentration, phytotoxicity and detoxification responses. Int J Phytoremediation 15(10):924–937

Yoshida N, Ryuichiro I, Tomoko O (2006) Identification and characterization of heavy metal-resistant unicellular alga isolated from soil and its potential for phytoremediation. Bioresour Technol 97:18–25

# Phytoremediation to Remove Metals/Metalloids from Soils

# 24

Jian Chen, Yi Chen, Zhi-Qi Shi, Yi Su, and Fengxiang X. Han

## 24.1 Introduction

Plants not only take up nutrients but also absorb metal and metalloid elements from the rhizosphere. Phytoremediation refers to the utilization of green plants to remove, transform, or stabilize contaminants, including toxic metals and organic pollutants in water, sediments, or soils (Cherian and Oliveira 2005). Phytoremediation has been accepted and utilized widely because of its cost-effectiveness, permanently removing the pollutants and protecting the nature (Chen et al. 2009a). Compared to physical approaches (e.g., excavation and landfill), phytoremediation can reduce costs by 50–65 % for remediating one acre of lead (Pb)-contaminated soil (Ensley 1997). The major disadvantage of chemical approach (e.g., soil washing) is secondary pollution for the remediative sites. Phytoremediation is an environment-friendly approach since vegetation is beneficial for ecosystem restoration by improving green coverage (Pilon-Smits and Freeman 2006). However, phytoremediation does have a few disadvantages and limitations. Firstly, it required a long time to grow plants and clean up hazardous metal-polluted soils. Generally, it takes approximately 13–16 years to completely phytoremediate a hazard waste site (Salt et al. 1995; Boyd 1996). Secondly, the use of invasive or nonnative plant species may affect biodiversity. Thirdly, the toxic plant biomass harvested from the phytoremediation process needs proper handling and disposal. Therefore, the field application of phytoremediation needs to be regulatory concerned (Henry 2000; Ghosh and Singh 2005).

Generally, the technique for phytoremediating a metal-contaminated soil mainly includes phytoextraction, phytostabilization, and phytovolatilization. Phytoextraction refers to the use of the so-called hyperaccumulator plants to remove toxic metals/metalloids from soil by concentrating and extracting metals/metalloids in harvestable tissues such as shoots. The roots of natural hyperaccumulator plants can absorb, concentrate, and transport large amounts of metals/metalloids from soil into aboveground biomass. These plant species can usually accumulate 100 times more metal/metalloid than typically measured in shoots of common non-accumulating plants (Reeves and Baker 2000; Lasat 2002). Phytostabilization refers to the use of plants to immobilize metals or reduce the bioavailability of metals in soil. The plants used for phytostabilization prefer to stabilize metals by accumulation in roots or precipitation within the rhizosphere rather than removing them from contaminated soil (Pulford and Watson 2003; Mench et al. 2006). Phytovolatilization refers to the use of plants to take up metals from the soil, transforming them into a volatile form and then transpiring them into the atmosphere. This approach can be used for the removal of volatile heavy metals, such as mercury (Hg) and selenium (Se) (McGrath et al. 2002). With the uncovering of the mechanism of metal metabolism in plants, the goal-directed genetic modification of plants provides a powerful tool to improve the efficiency of phytoremediation by enhancing the accumulation and transformation of heavy metals in plants (Kotrba 2013).

J. Chen, Ph.D. • Z.-Q. Shi, Ph.D.
Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, 50 Zhongling Street, Nanjing 210014, China

Y. Chen, M.S.
Institute of Food Quality and Safety, Jiangsu Academy of Agricultural Sciences, 50 Zhongling Street, Nanjing 210014, China

College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu Province, China

Y. Su, Ph.D.
College of Science, Technology, Engineering, Mathematics and Nursing, Texas A&M University-Texarkana's, Texarkana, TX, USA

F.X. Han, Ph.D. (✉)
Department of Chemistry and Biochemistry, Jackson State University, 1400 J. R., Lynch Street, Jackson, MS 39217, USA
e-mail: fengxiang.han@jsums.edu

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## 24.2 Phytoremediation of Metal-/ Metalloid-Contaminated Soils Using Natural Plants

### 24.2.1 Phytoextraction

The key of using phytoextraction successfully is to find hyperaccumulator plants. The ideal hyperaccumulator plant should have the following characteristics: (1) rapid growth rate with high biomass yield; (2) the ability to accumulate, translocate, and tolerate high concentrations of metal/metalloid in harvestable tissue; and (3) availability and habitat preference (Nanda Kumar et al. 1995; Garbisu and Alkorta 2001; Sarma 2011). Hyperaccumulator plant species are usually identified from metal-/metalloid-polluted areas or naturally mineralized areas where the high concentrations of heavy metals stimulate the evolutionary adaptation of plants in sites. Metal transfer factors (TFs) from soil, the ratio of metals between soil and plant parts, is an important criterion for the selection of plant species for phytoremediation, and TFs > 1 means higher accumulation of metals in plants than soil (Barman et al. 2000). With the increasing reports of novel hyperaccumulators during the last decade, the threshold criteria for the identification of a typical hyperaccumulator plant remain controversial. Ent et al. (2013) analyze the literature of hyperaccumulator plants and recommend that the dried foliage of a hyperaccumulator plant should concentrate at least more than 100 μg/g for cadmium (Cd), Se, and thallium (Tl); 300 μg/g for cobalt (Co), copper (Cu), and chromium (Cr); 1,000 μg/g for nickel (Ni), lead (Pb), and arsenic (As); 3,000 μg/g for zinc (Zn); and 10,000 μg/g for manganese (Mn), with plants growing naturally. According to these criteria, more than 500 plant species can be considered as hyperaccumulators of one or more metal elements (Ent et al. 2013). Generally, several plant genera and families (e.g., Fabaceae, Asteraceae, Rubiaceae, Brassicaceae, Scrophulariaceae, *Pteris*, *Thlaspi*, and Chenopodiaceae) show great potential to hyperaccumulate one or more heavy metal species. The updated metal hyperaccumulators have been well summarized by Rascio and Navari-Izzo (2011) and Ent et al. (2013). Chinese brake fern (*P. vittata*) was identified as As hyperaccumulator plants and has been successfully applied in phytoremediating many industrial contaminated fields (Ma et al. 2001). Notably, several metals can be synergetically accumulated by the same plant. *Thlaspi caerulescens* and *Arabis paniculata* can accumulate both Zn and Cd (Brown et al. 1994; Tang et al. 2009). Some metals show antagonistic effects in plant uptake. For instance, Se decreases Cd uptake by maize plants (Shanker et al. 1996). However, in other plants (e.g., *Triticum aestivum* and *Pisum sativum*), Se seems to enhance the uptake of Cd (Landberg and Greger 1994). Therefore, the phytoaccumulators should be selected carefully before the field application of phytoextraction.

Phytoextraction depends on the absorption, translocation, and metabolism of toxic metals in plants. The uptake of heavy metals or metalloids from soil by plant roots is the first step of phytoextraction. The efficiency of metal/metalloid uptake into plant roots relies on the bioavailability of metals/metalloids in soil. Because of the complicated interaction between metal/metalloid and the plant–soil system, the mechanisms of metal/metalloid bioavailability in soil are not completely understood yet. Some metals in cationic forms, such as $Cd^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Ni^{2+}$, and $Cu^{2+}$, are soluble and readily bioavailable for root uptake. These metal cations are supposed to enter into root cells from soil solutions through specific transporters, which are proteins located in plant cell membrane (Hall and Williams 2003; Verbruggen et al. 2009; Krämer 2010). Other metals, such as Pb, prefer to exist in precipitated compounds as phosphates or carbonates, which are insoluble and unavailable for root uptake. Binding with the soil matrix can significantly restrict the bioavailability of metals (Han et al. 2006; Han 2007). Soil pH seems to be the predominant factor for solubility of metals in soils (Chuan et al. 1996). Plant roots are able to cause acidification of the rhizosphere by excreting $H^+$, which stimulates the release of metal ions from soil particles into solution (Lasat 2002). In addition, plant roots can exude a class of organic compounds (e.g., malate, acetate, and deoxymugineic acid), which combine with metals to improve the bioavailability of metals in soils (Lasat 2002). Therefore, a set of synthetic chelates (e.g., EDTA, HEDTA, DTPA, EGTA, NTA, EDDS, and EDDHA) with high affinity for metals have been utilized to stimulate metal uptake by plant roots, which is called chelate-assisted phytoextraction (Han et al. 2004; Evangelou et al. 2007; Chen et al. 2009b). However, the addition of synthetic chelates or the acidic modification of rhizosphere may result in the increased release of metals into groundwater, which should be regulatory concerned (Hsiao et al. 2007; Quartacci et al. 2007; Leštan et al. 2008; Neugschwandtner et al. 2008). In addition, recent studies suggest that plant-associated microbes (e.g., plant growth-promoting bacteria, mycorrhizae) can promote plant root uptake of heavy metals by producing various metabolites (e.g., 1-aminocyclopropane-1-carboxylic acid deaminase, indole-3-acetic acid, siderophores, organic acids, etc.). The microbe-assisted phytoextraction has been well reviewed by Rajkumar et al. (2012).

Phytoextraction requires high-efficiency translocation of metals/metalloids from roots to harvestable shoots. Once entering into plant roots, metal ions are transported to shoots through the vascular system in xylem (Mendoza-Cózatl et al. 2008). Compared to non-hyperaccumulating plants, hyperaccumulators show higher metal concentrations in xylem sap (Lasat et al. 1998). The process of enhanced active loading of metals into xylem is mediated by several transporters, such as P-type ATPase, heavy metal transporting ATPases (HMAs), multidrug and toxic compound extrusion (MATE) (or efflux) membrane proteins, and oligopeptide transporters

(OPTs) (Verbruggen et al. 2009). The status of metals can be variable in xylem sap. Most Zn and Cd present as free ionic form in hyperaccumulators *T. caerulescens* and *Arabis halleri*, respectively (Salt et al. 1999; Ueno et al. 2008). In Ni hyperaccumulator *Alyssum montanum*, histidine can chelate $Ni^{2+}$ to form histidine–$Ni^{2+}$ compounds for the transportation into xylem, which is also supported by the fact that several Ni hyperaccumulators respond to $Ni^{2+}$ exposure by a large dose-dependent increase in histidine concentrations in xylem (Clemens et al. 2002; Ghosh and Singh 2005).

After transporting to shoots along xylem sap, metals are redistributed and relocated in leaf cells. Hyperaccumulators detoxify excessive metals by metabolizing or capturing them in different tissues or organelles to maintain concentrations within the safe range in plants. There are various locations for heavy metals in different hyperaccumulators. For example, about 96 % of total As was found in pinnae in As hyperaccumulator *Pteris vittata*. The X-ray absorption near edge structure (XANES) analyses showed that approximately 75 % of the As in fronds was present in the As(III) oxidation state and the rest as As(V) (Lombi et al. 2002). In Cd hyperaccumulator *T. caerulescens*, the majority of Cd is located in cells on the way of water migration from the vascular cylinder to epidermal cells (Wójcik et al. 2005). In the leaf of Zn hyperaccumulator *Sedum alfredii*, 91–94 % of Zn was found in cell walls and the soluble fraction, while only 6–9 % of Zn was distributed in the cell organelle fraction (Li et al. 2006). Vacuoles are suggested to be the target organelle to sequester phytochelatin (PC)-conjugated metals. The transportation of PC–metal complexes is mediated by different transporters, such as cation diffusion facilitators (CDF), HMA, $Ca^{2+}$/cation antiporter (CaCA), and ATP-binding cassette (ABC) transporters, located in vacuole membrane (Verbruggen et al. 2009; Rascio and Navari-Izzo 2011).

### 24.2.2 Phytostabilization

The successful phytostabilization aims to stabilize the vegetation cover and limit metal uptake by crops and to immobilize heavy metals in plant roots or rhizosphere in order to prevent metal migration to groundwater or to surrounding areas (Mench et al. 2000; Mukhopadhyay and Maiti 2010). Unlike phytoextraction, phytostabilization tends to stabilize metals by accumulation in roots or precipitation within the rhizosphere rather than removing them from contaminated soil. Therefore, phytostabilization is relatively easier to implement than other phytoremediative techniques. However, mandatory monitoring is required because heavy metals still remain in soil (Ghosh and Singh 2005; Padmavathiamma and Li 2007).

The major mechanism of phytostabilization is that plant accumulates and captures large amounts of metals/metalloids in root cells with little translocation to shoots. The mechanisms of metal uptake by plant roots are similar to those of hyperaccumulators mentioned above. The major difference with hyperaccumulators is that metals/metalloids are sequestered in root cells by forming insoluble complexes to limit their translocation to shoots. Therefore, the plants used in phytostabilization should have high-density and fast-growing root system. Some tree species have been documented as potential candidates for the phytostabilization of heavy metals from soil. EXAFS (extended X-ray absorption fine structure) spectroscopy analysis suggests that Pb can be bound within the ligno-cellulosic structure in the roots of *Juglans regia* (Marmiroli et al. 2005). In a field test, two willow species (*Salix fragilis* and *Salix triandra*) showed great potential for the phytostabilization of heavy metal-contaminated soils under the optimal growth conditions (Tack et al. 2005). Another manipulating mechanism of phytostabilization is that root exudates (e.g., organics) cause metals to precipitate in the rhizosphere (Yang et al. 2005; Alkorta et al. 2010). Therefore, soil amendments by the addition of organic matter in phytostabilization are not only beneficial for plant growth but also promote the formation of insoluble metal complexes that reduce metal bioavailability (Clemente et al. 2003; Adriano et al. 2004). Soil liming can also stimulate the precipitation of metal ions by elevating soil pH. Therefore, the combination of several amendments is beneficial for phytostabilization. For instance, the simultaneous application of compost, cyclonic ashes, and steel shots was effective for amendment-assisted phytostabilization of metal-contaminated soil (Ruttens et al. 2006).

The original vegetation on metal-contaminated sites may be damaged due to the phytotoxicity of heavy metals, which causes soil erosion leading to metal leaching to groundwater and sediment (Quinton and Catt 2007). The restoration of the ecosystem on sites during the process of phytostabilization can limit metal leaching and spread by improving soil structure (Lei and Duan 2008). In this kind of case, plants are metal-tolerant species with the ability of excluding heavy metals rather than accumulating them. Some excluder plants have been identified, such as *Silene vulgaris* (Ni excluder) (Wenzel et al. 2003), *Hyparrhenia hirta* (Cu excluder) (Poschenrieder et al. 2001), *Armeria maritima* (Co excluder) (Brewin et al. 2003), *Oenothera biennis* and *Commelina communis* (Cd excluders) (Wei et al. 2005), and *Taraxacum mongolicum* (Zn excluder) (Wei et al. 2005).

Hydraulic control is an important physiological mechanism of metal phytostabilization as well. During the process of plant transpiration, fast-growing deep-rooted trees (e.g., poplar, cottonwood, willow) can draw as much as 200 gallons of water per day (EPA 2000; Quinn et al. 2001). The rapid water uptake by roots is able to minimize the migrating rate of metals to groundwater by lowering an aquifer level (EPA 2003).

### 24.2.3 Phytovolatilization

Several metal species, such as Se, As, and Hg, can exist as gaseous forms in the environment. Volatile Se compounds, such as dimethylselenide, are 1/600 to 1/500 as toxic as inorganic forms of Se (Padmavathiamma and Li 2007). *Typha latifolia* and some members of the *Brassicaceae* are capable of metabolizing various inorganic or organic species of Se (e.g., selenate, selenite, and Se-methionine [Met]) into gaseous Se forms (e.g., dimethylselenide), which can be volatilized and released into the atmosphere (Pilon-Smits et al. 1999; Terry et al. 1999; Bañuelos 2000). *S*-Adenosyl-L-Met:L-Met *S*-methyltransferase (MMT) is the key enzyme responsible for the process of Se phytovolatilization (Tagmount et al. 2002). As a hyperaccumulator, *P. vittata* is a plant species that is effective at volatilizing As. When *P. vittata* grows in As-contaminated soil, vapor samples from chambers covering the shoots contain significantly volatilized As (37 % for arsenite and 63 % for arsenate) (Sakakibara et al. 2007). Phytovolatilization has been successfully implemented in tritium ($^3H$) which is a radioactive isotope of hydrogen (Dushenkov 2003). Phytovolatilization of Hg mainly comes from the test of transgenic plants, which will be discussed in detail in the following section.

Phytovolatilization seems to be an easy approach to remove some metals/metalloids from soil; it is limited due to the loss of control over the migration of the volatilized elements from plants. The volatilized elements would not only pose a risk to human health nearby the contaminated site but also deposit on the ground again. Therefore, it has been suggested that phytovolatilization may be not an ideal tool for the remediation of metal-/metalloid-contaminated sites (Chen et al. 2009b).

## 24.3 The Potential of Using Transgenic Plants for the Phytoremediation of Metal-/Metalloid-Contaminated Soils

The extensive application of natural hyperaccumulators is always limited due to their relatively low biomass yield or in adaptation to various metal-/metalloid-contaminated sites. Many genes involved in metal uptake, translocation, and sequestration have been identified from hyperaccumulators. Transferring these genes into candidate plants provides promising strategy for genetic engineering of plants to improve phytoremediation traits (Eapen and D'Souza 2005).

Metal transporters are indispensable in metal uptake and homeostasis in plants. The transporters responsible for metal accumulation in plants have been well summarized by Kotrba et al. (2009). For example, overexpression of *NtCBP4* coding for a calmodulin-binding protein results in the increased uptake and translocation of $Pb^{2+}$ to shoots (Arazi et al. 1999). Transferring Zn transporter gene *ZAT* from *Thlaspi goesingense* to *Arabidopsis thaliana* leads to twofold higher Zn accumulation in roots (van der Zaal et al. 1999). The increased Fe tolerance has been obtained by overexpressing metal transporter AtNramp1 (Curie et al. 2000). The cross kingdom gene transfer may also help plants enhance metal accumulation or tolerance. The *znt* gene codes for $Zn^{2+}$, $Cd^{2+}$, and $Pb^{2+}$ P1-ATPase responsible for the metalloresistance of *E. coli* based on metal efflux from the cell.*A. thaliana* expressing *zntA* showed enhanced tolerance to $Cd^{2+}$ and $Pb^{2+}$ (Lee et al. 2003). The yeast protein YCF1 is a member of ABC transporter family involved in the transfer of Cd into vacuoles by conjugation with glutathione (Li et al. 1997). The introduction of YCF1 into *A. thaliana* resulted in the increased tolerance of transgenic plants to Cd and Pb (Song et al. 2003). In addition, in transgenic tobacco expressing yeast *FRE1* and *FRE2* genes coding for ferric reductase, Fe accumulation enhanced 1.5 times than wild type (Samuelsen et al. 1998). The overexpression of a mammalian *hMRP1* gene coding for ABC-type multidrug resistance-associated transporter in tobacco showed improved $Cd^{2+}$ tolerance (Yazaki et al. 2006).

Phytochelatins (PCs), metallothioneins (MTs), and metal-binding proteins are the main players which are vital in the detoxification of metals in plants. It has been extensively reported that the overexpression of PC synthase gene homologues improved metal accumulation and tolerance in different plant species (Gisbert et al. 2003; Li et al. 2004; Martínez et al. 2006; Peterson and Oliver 2006; Mendoza-Cózatl et al. 2008). Transferring mammalian Methyltransferase gene in plants resulted in the enhanced metal tolerance without an increase in metal accumulation in plant tissues (Cherian and Oliveira 2005; Eapen and D'Souza 2005). These reports suggest that overexpression of MT may be suitable for improving phytostabilization rather than phytoextraction.

The genetically modified phytovolatilization has been mainly focused on Hg and Se contamination. One of the best biological systems for detoxifying $Hg^{2+}$ or MeHg compounds involves two enzymes referred to as mercuric ion reductase (MerA) and organomercurial lyase (MerB). In some Hg-resistant microbes, MerB catalyzes the reaction of MeHg to $Hg^{2+}$, and then MerA transforms $Hg^{2+}$ to elemental Hg that can volatilize out of cells (Nascimento and Chartone-Souza 2003). Therefore, transgenic plants containing *merA* and *merB* might be capable of completing the process of $Hg^0$ volatilization. Because elemental Hg has much lower toxicity than $Hg^{2+}$ or MeHg, these transgenic plants probably have the ability to remediate mercury-contaminated soil by taking up MeHg or $Hg^{2+}$ and transforming them to $Hg^0$ that can be released into the air. The research group of Richard Meagher at the University of Georgia has confirmed the feasibility of this technique. They successfully developed a transgenic poplar (*Liriodendron tulipifera*) species that contains *merA* and *merB*. The transgenic poplar grows rapidly and has the ability to take up $Hg^{2+}$ and MeHg effectively and volatilize $Hg^0$ to the atmosphere. The rate of mercury volatilization by this transgenic plant was ten times higher than that of control plant (Bizily et al. 2000). Selenocysteine methyltransferase (SMT) catalyzes the biosynthesis of MetSeCys, which can be further converted to volatile dimethyldiselenide (DMDSe) in hyperaccumulating plant species (Zhu et al. 2009). Transgenic plants overexpressing *SMT* showed enhanced Se accumulation and efficient volatile forms from leaves (LeDuc et al. 2004; Bañuelos et al. 2006). Cystathionine γ-synthase (CGS) is responsible for the formation of another volatile Se form dimethylselenide (DMSe) (Zhu et al. 2009). Transgenic Indian mustard expressing *CGS1* from *A. thaliana* showed increased DMSe formation and evaporation (Huysen et al. 2003).

## 24.4 Regulatory Concerns for the Application of Phytoremediation

Although phytoremediation is a novel, cost-effective, and environment-friendly technique to clean up heavy metal-contaminated soils, regulatory standards have not yet been developed specifically for phytoremediation applications. The regulatory concerns should be conducted throughout the whole process of phytoremediation in order to optimize its application.

Before starting the application of phytoremediation, the geostatistical assessment of metal-contaminated soils should be performed. The first step is soil sampling for the determination of metal concentrations. It is recommended that samples should be collected in a way that takes into account of sites representing the most relevant characteristics of the soil environment. The sampling spots are defined in terms of the different environmental conditions, i.e., vegetation, soil type, altitude, parent material, etc. It is better to collect soil samples from different soil layers in a spot rather than from only one layer in order to determine the metal distribution in the soil (Chen et al. 2010). After getting the metal concentrations in soil samples, a set of statistical analyses are needed to make the assessment of metal contamination in site. Principal component analysis (PCA) and cluster analysis (CA) are the most appropriate statistical methods to identify the origin of heavy metals and the correlations between heavy metals at a contaminated site, respectively (Micó et al. 2006; Idris 2008; Franco-Uría et al. 2009; Li et al. 2009). The data from geostatistical analysis can be integrated to produce spatial distribution maps of metal contaminants by using geographic information system (GIS) techniques. These maps provide valuable information for hazard assessment and for decision support (Korre et al. 2002). The degree of total metal pollution can be evaluated from the soil pollution index (SPI) distribution maps (Lee et al. 2006). Then the appropriate phytoremediation techniques can be selected according to the site assessment based on geostatistical analyses.

Several aspects of regulatory concerns should be noted during the application process of phytoremediation because each application will be site specific and must be evaluated on a case-by-case basis by a regulator. In order to finish the installation and operation of phytoremediation systems successfully, compliance with applicable regulations is mandatory (Chen et al. 2010). Firstly, native plant species with potential for specific metal extraction are preferentially considered when selecting remediating plants. However, the safety issues with regard to releasing exotic or transgenic plants into the environment should be evaluated. In the absence of sufficient literature and/or local experience, it is prudent to conduct small-scale tests to determine optimum plant species before choosing a plant species for a site (Henry 2000; Ghosh and Singh 2005). Secondly, according to the progress of phytoremediation, incorporation with other remedial techniques (e.g., soil amendment and intercropping systems with multiple plant species) can improve the efficiency of phytoremediation. Thirdly, remote sensing technology can be utilized for the long-term monitoring of phytoremediation. Accumulated metals can significantly change plant physiological status (Shiyab et al. 2009). The traditional biochemical assay is time/labor consumptive and needs large amounts of plant materials. Spectral reflectance is a nonintrusive technique that has been used to monitor plant physiological status under heavy metal stress (Merzlyak et al. 2003; Sridhar et al. 2007a, b; Su et al. 2007). For example, spectral reflectance around 680 nm and 550 nm can reflect chlorophyll content in Cd-treated plants (Sridhar et al. 2007b). The spectral reflectance in the 700–1,300 nm region is well corrected with the number of mesophyll cells in the leaves of Cr-treated *P. vittata* (Sridhar et al. 2007a). Normalized difference vegetation index (NDVI) defined as $(R_{810}-R_{680})/(R_{810}+R_{680})$ can be used to detect the changes of biomass and leaf internal structure of barley due to the phytoextraction of Zn (Sridhar et al. 2007b). Water index (WI) defined as $R_{900}/R_{970}$ had been reported to be highly correlated with relative water content of the plant under heavy metal stress (Sridhar et al. 2007b). A spectral index $R_{1110}/R_{810}$ can be used to monitor internal leaf structural changes caused by the accumulation of certain heavy metal species (Sridhar 2004; Sridhar et al. 2007b). The detailed manipulating mechanism of remote sensing technology in monitoring the phytoremediation of metal-contaminated soils has been described in detail by Chen et al. (2010).

After finishing the process of phytoremediation, the handling of plant biomass is the most important thing. For phytostabilization, conventional farming operation is indispensable to maintain the normal growth of plants in site because they will not be harvested. But for phytoextraction, the handling and disposal of a huge quantity of metal-accumulating biomass is a big challenge, which needs great concern. Volume reduction of plant materials is supposed to be the first step of postharvest biomass treatment (Blaylock and Huang 2000). According to the literature, compaction and pyrolysis are feasible approaches to recycle metals from harvested biomass (Bridgwater et al. 1999; Sas-Nowosielska et al. 2004). In addition, incineration is an ideal alternative technique to dispose the biomass harvested after phytoextraction. It may be possible to recycle the metal residue from the ash. But the recycling of different metals from ash needs to be studied further (Sas-Nowosielska et al. 2004). Gasification is a new technology, in which harvested biomass can be subjected to a series of chemical reactions to produce clean and combustive gas for generating thermal and electrical energy (Lyer et al. 2002). Although these techniques are theoretically feasible in the disposal of harvested biomass after phytoextraction, the analysis of cost/benefit and environmental acceptability should be conducted before proceeding.

## 24.5 Conclusions

Based on the current reports, phytoremediation is emerging as a potential approach for remediating metal-/metalloid-contaminated soils. Public acceptance of a phytoremediation project on a site can be very high because it is beneficial to the ecosystem on-site. However, the selection of different styles of phytoremediation should be site by site according to the site assessment. More field tests and mechanism studies of phytoremediation are much needed. First, the identification of plant species with the ability of hyperaccumulating multiple toxic metals will be desired. Second, the further understanding of the detailed mechanisms for the accumulation/tolerance of metals in plants is essential for mining valuable genes to improve transgenic-assisted phytoremediation. Third, more quantitative data analyses about the field trial are useful for constructing models of phytoremediation. Finally, more studies on regulatory requirements, site assessment, and risk evaluation are essential to improve the establishment of regulatory standards for phytoremediation.

## References


Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural remediation in environmental cleanup. Geoderma 122:121–142

Alkorta I, Becerril JM, Garbisu C (2010) Phytostabilization of metal contaminated soils. Rev Environ Health 25:135–146

Arazi T, Sunkar R, Kaplan B, Fromm H (1999) A tobacco plasma membrane calmodulin-binding transporter confers $Ni^{2+}$ tolerance and $Pb^{2+}$ hypersensitivity in transgenic plants. Plant J 20:171–182

Bañuelos GS (2000) Phytoextraction of selenium from soils irrigated with selenium-laden effluent. Plant Soil 224:251–258

Bañuelos G, LeDuc DL, Pilon-Smits EAH, Terry N (2006) Transgenic Indian Mustard overexpressing selenocysteine lyase or selenocysteine methyltransferase exhibit enhanced potential for selenium phytoremediation under field conditions. Environ Sci Technol 41:599–605

Barman SC, Sahu RK, Bhargava SK, Chaterjee C (2000) Distribution of heavy metals in wheat, mustard, and weed grown in field irrigated with industrial effluents. Bull Environ Contam Toxicol 64:489–496

Bizily SP, Rugh CL, Meagher RB (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nat Biotechnol 18:213–217

Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, USA, pp 53–70

Boyd V (1996) Pint-sized plants pack a punch in fight against heavy metals. Environ Prot 7:38–39

Brewin LE, Mehra A, Lynch PT, Farago ME (2003) Mechanisms of copper tolerance by *Armeria maritima* in Dolfrwyong Bog, North Wales—initial studies. Environ Geochem Health 25:147–156

Bridgwater AV, Meier D, Radlein D (1999) An overview of fast pyrolysis of biomass. Org Geochem 30:1479–1493

Brown SL, Chaney RL, Angle JS, Baker AJM (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soil. J Environ Qual 23:1151–1157

Chen J, Shiyab S, Han FX, Monts DL, Waggoner CA, Yang Z, Su Y (2009a) Bioaccumulation and physiological effects of mercury in *Pteris vittata* and *Nephrolepis exaltata*. Ecotoxicology 18:110–121

Chen J, Yang ZM, Su Y, Han FX, Monts DL (2009b) Phytoremediation of heavy metal/metalloid-contaminated soils. In: Steinberg RV (ed) Contaminated soils: environmental impact, disposal and treatment. Nova Science Publishers, NY, USA, pp 181–206

Chen J, Shi ZQ, Hu LB, Su Y, Han FX (2010) Site assessment, long-term monitoring and regulatory concerns for application of phytoremediation technology for remediation of heavy metal/metalloid-contaminated soils. In: Faerber T, Herzog J (eds) Solid waste management and environmental remediation. Nova Science Publishers, Inc., NY, USA, pp 407–422

Cherian S, Oliveira MM (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390

Chuan MC, Shu GY, Liu JC (1996) Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. Water Air Soil Pollut 90:543–556

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315

Clemente R, Walker JD, Roig A, Berna PM (2003) Heavy metal bioavailability in a soil affected by mineral sulphides contamination following the mine spillage at Aznalc´ollar (Spain). Biodegradation 14:199–205

Curie C, Alonso JM, Jean MLE, Ecker JR, Briat J-F (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. Biochem J 347:749–755

Dushenkov D (2003) Trends in phytoremediation of radionuclides. Plant Soil 249:167–175

Eapen S, D'Souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23:97–114

Ensley B (1997) Phytoremediation is the most cost effective approach for many sites. IBC phytoremediation conference, Seattle, WA, USA, 18–19 June

Ent A, Baker AM, Reeves R, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362:319–334

EPA (2000) United States Protection Agency Reports: Introduction to Phytoremediation. EPA 600/R-99/107

EPA (2003) Phytoremediation of Groundwater at Air Force Plant 4, Carswell, Texas, Innovative Technology Evaluation Report. EPA/540/R-03/506 103

Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68:989–1003

Franco-Uría A, López-Mateo C, Roca E, Fernández-Marcos ML (2009) Source identification of heavy metals in pastureland by multivariate analysis in NW Spain. J Hazard Mater 165:1008–1015

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77:229–236

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18

Gisbert C, Ros R, De Haro A, Walker DJ, Pilar Bernal M, Serrano R, Navarro-Aviñó J (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem Biophys Res Commun 303:440–445

Hall JL, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613

Han FX (2007) Biogeochemistry of trace elements in arid environments. Springer, Germany, p 380

Han FX, Sridhar BBM, Monts DL, Su Y (2004) Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea* l Czern. New Phytol 162:489–499

Han FX, Su Y, Monts DL, Waggoner CA, Plodinec MJ (2006) Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. Sci Total Environ 368:753–768

Henry JR (2000) An overview of the phytoremediation of lead and mercury. National Network of Environmental Management Studies (NNEMS), U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response Technology Innovation office, Washington, D.C. http://clu-in.org

Hsiao KH, Kao PH, Hseu ZY (2007) Effects of chelators on chromium and nickel uptake by Brassica juncea on serpentine-mine tailings for phytoextraction. J Hazard Mater 148:366–376

Huysen T, Abdel-Ghany S, Hale K, LeDuc D, Terry N, Pilon-Smits EH (2003) Overexpression of cystathionine-γ-synthase enhances selenium volatilization in *Brassica juncea*. Planta 218:71–78

Idris AM (2008) Combining multivariate analysis and geochemical approaches for assessing heavy metal level in sediments from Sudanese harbors along the Red Sea coast. Microchem J 90:159–163

Korre A, Durucan S, Koutroumani A (2002) Quantitative-spatial assessment of the risks associated with high Pb loads in soils around Lavrio, Greece. Appl Geochem 17:1029–1045

Kotrba P (2013) Transgenic approaches to enhance phytoremediation of heavy metal-polluted soils. In: Gupta DK (ed) Plant-based remediation processes. Springer, Berlin Heidelberg, pp 239–271

Kotrba P, Najmanova J, Macek T, Ruml T, Mackova M (2009) Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnol Adv 27:799–810

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Landberg T, Greger M (1994) Influence of selenium on uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Triticum aestivum*). Physiol Plant 90:637–644

Lasat MM (2002) Phytoextraction of toxic metals. J Environ Qual 31:109–120

Lasat MM, Baker AJ, Kochian LV (1998) Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. Plant Physiol 118:875–883

LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP, AbdelSamie M, Chiang C-Y, Tagmount A, deSouza M, Neuhierl B, Böck A, Caruso J, Terry N (2004) Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian Mustard increases selenium tolerance and accumulation. Plant Physiol 135:377–383

Lee S, Moon JS, Ko T-S, Petros D, Goldsbrough PB, Korban SS (2003) Overexpression of Arabidopsis phytochelatin synthase paradoxically leads to hypersensitivity to cadmium Stress. Plant Physiol 131:656–663

Lee CS, Li X, Shi W, Cheung SC, Thornton I (2006) Metal contamination in urban, suburban, and country park soils of Hong Kong: a study based on GIS and multivariate statistics. Sci Total Environ 356:45–61

Lei D, Duan C (2008) Restoration potential of pioneer plants growing on lead-zinc mine tailings in Lanping, southwest China. J Environ Sci 20:1202–1209

Leštan D, Luo C, Li X (2008) The use of chelating agents in the remediation of metal-contaminated soils: a review. Environ Pollut 153:3–13

Li Z-S, Lu Y-P, Zhen R-G, Szczypka M, Thiele DJ, Rea PA (1997) A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium. Proc Natl Acad Sci 94:42–47

Li Y, Dhankher OP, Carreira l, Lee D, Chen A, Schroeder JI, Balish RS, Meagher RB (2004) Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiol 45:1787–1797

Li TQ, Yang XE, Yang JY, He ZL (2006) Zn accumulation and subcellular distribution in the Zn hyperaccumulator *Sedum alfredii* Hance. Pedosphere 16:616–623

Li J, He M, Han W, Gu F (2009) Analysis and assessment on heavy metal sources in the coastal soils developed from alluvial deposits using multivariate statistical methods. J Hazard Mater 164:976–981

Lombi E, Zhao FJ, Fuhrmann M, Ma LQ, McGrath SP (2002) Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. New Phytol 156:195–203

Iyer PVR, Rao TR, Grover PD (2002) Biomass: Thermochemical characterization. 3rd ed.Indian Institute of Technology, Delhi, India, 1–42

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579

Marmiroli M, Antonioli G, Maestri E, Marmiroli N (2005) Evidence of the involvement of plant ligno-cellulosic structure in the sequestration of Pb: an X-ray spectroscopy-based analysis. Environ Pollut 134:217–227

Martínez M, Bernal P, Almela C, Vélez D, García-Agustín P, Serrano R, Navarro-Aviñó J (2006) An engineered plant that accumulates higher levels of heavy metals than *Thlaspi caerulescens*, with yields of 100 times more biomass in mine soils. Chemosphere 64:478–485

McGrath SP, Zhao J, Lombi E (2002) Phytoremediation of metals, metalloids, and radionuclides. Adv Agron 75:1–56

Mench M, Vangronsveld J, Clijsters H, Lepp NW, Edwards R (2000) *In situ* metal immobilization and phytostabilization of contaminated soils. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water. CRC Press LLC, Boca Raton, Florida, USA

Mench M, Vangronsveld J, Bleeker P, Ruttens A, Geebelen W, Lepp N (2006) Phytostabilisation of metal-contaminated sites. In: Echevarria G, Goncharova N, Morel J-l (eds) Phytoremediation of metal-contaminated soils. Springer, Netherlands, pp 109–190

Mendoza-Cózatl DG, Butko E, Springer F, Torpey JW, Komives EA, Kehr J, Schroeder JI (2008) Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. Plant J 54:249–259

Merzlyak MN, Gitelson AA, Chivkunova OB, Solovchenko AE (2003) Application of reflectance spectroscopy for analysis of higher plant pigments. Russ J Plant Physiol 50:704–710

Micó C, Recatalá l, Peris M, Sánchez J (2006) Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere 65:863–872

Mukhopadhyay S, Maiti SK (2010) Phytoremediation of metal enriched mine waste: a review. Global J Environ Res 4:135–150

Nanda Kumar PBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Nascimento AMA, Chartone-Souza E (2003) Operon *mer*: bacterial resistance to mercury and potential for bioremediation of contaminated environments. Genet Mol Res 2:92–101

Neugschwandtner RW, Tlustoš P, Komárek M, Száková J (2008) Phytoextraction of Pb and Cd from a contaminated agricultural soil

using different EDTA application regimes: laboratory versus field scale measures of efficiency. Geoderma 144:446–454
Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut 184:105–126
Peterson AG, Oliver DJ (2006) Leaf-targeted phytochelatin synthase in *Arabidopsis thaliana*. Plant Physiol Biochem 44:885–892
Pilon-Smits EAH, Freeman JL (2006) Environmental cleanup using plants: biotechnological advances and ecological considerations. Front Ecol Environ 4:203–210
Pilon-Smits EAH, Desouza MP, Hong G, Amini A, Bravo RC, Payabyab ST, Terry N (1999) Selenium volatilization and accumulation by twenty aquatic plant species. J Environ Qual 28:1011–1017
Poschenrieder C, Bech J, Llugany M, Pace A, Fenes E, Barcelo J (2001) Copper in plant species in a copper gradient in Catalonia (North East Spain) and their potential for phytoremediation. Plant Soil 230:247–256
Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540
Quartacci MF, Irtelli B, Baker AJM, Navari-Izzo F (2007) The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica carinata*. Chemosphere 68:1920–1928
Quinn JJ, Negri CM, Hinchman RR, Moos LP, Wozniak JB, Gatliff E (2001) Predicting the effect of deep-rooted hybrid poplars on the groundwater flow system at a large-scale phytoremediation site. Int J Phytoremediat 3:41–60
Quinton JN, Catt JA (2007) Enrichment of heavy metals in sediment resulting from soil erosion on agricultural fields. Environ Sci Technol 41:3495–3500
Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30:1562–1574
Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181
Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 193–229
Ruttens A, Mench M, Colpaert JV, Biosson J, Carleer R, Vangronsveld J (2006) Phytostabilization of a metal contaminated sandy soil. I: influence of compost and/or inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of metals. Environ Pollut 144:524–532
Sakakibara M, Watanabe A, Sano S, Inoue M, Kaise T (2007) Phytoextraction and phytovolatilization of arsenic from as-contaminated soils by *Pteris vittata*. Proceedings of the annual international conference on soils, sediments, water and energy 12: Article 26
Salt DE, Blaylock M, Nanda Kumar PBA, Dushenkov V, Ensley BD, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol 13:468–474
Salt DE, Prince RC, Baker AJM, Raskin I, Pickering IJ (1999) Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. Environ Sci Technol 33:713–717
Samuelsen AI, Martin RC, Mok DWS, Mok MC (1998) Expression of the yeast *FRE* Genes in transgenic tobacco. Plant Physiol 118:51–58
Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138
Sas-Nowosielska A, Kucharski R, Małkowski E, Pogrzeba M, Kuperberg JM, Kryński K (2004) Phytoextraction crop disposal—an unsolved problem. Environ Pollut 128:373–379
Shanker K, Mishra S, Srivastava S, Srivastava R, Dass S, Prakash S, Srivastava MM (1996) Effect of selenite and selenate on plant uptake of cadmium by maize (*Zea mays*). Bull Environ Contam Toxicol 56:419–424
Shiyab S, Chen J, Han FX, Monts DL, Matta FB, Gu M, Su Y (2009) Phytotoxicity of mercury in Indian mustard (Brassica juncea l.). Ecotoxicol Environ Saf 72:619–625
Song W-Y, Ju Sohn E, Martinoia E, Jik Lee Y, Yang Y-Y, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat Biotechnol 21:914–919
Sridhar BBM (2004) Monitoring plant spectral reflectance and internal structure changes during the phytoremediation processes for selected heavy metals. Mississippi State University, Starkville, Mississippi, USA
Sridhar BBM, Han FX, Diehl SV, Monts DL, Su Y (2007a) Monitoring the effects of arsenic and chromium accumulation in Chinese brake fern (*Pteris vittata*). Int J Remote Sens 28:1055–1067
Sridhar BBM, Han FX, Diehl SV, Monts DL, Su Y (2007b) Spectral reflectance and leaf internal structure changes of barley plants due to phytoextraction of zinc and cadmium. Int J Remote Sens 28:1041–1054
Su Y, Sridhar BBM, Han FX, Diehl SV, Monts DL (2007) Effect of bioaccumulation of Cs and Sr natural isotopes on foliar structure and plant spectral reflectance of Indian mustard (*Brassica juncea*). Water Air Soil Pollut 180:65–74
Tack FMG, Vervaeke P, Meers E, Vandecasteele B (2005) Phytoremediation/stabilisation of dredged sediment derived soils with willow. Geol Res Abst 7:08101
Tagmount A, Berken A, Terry N (2002) An essential role of *S-Adenosyl-l-*Methionine:l-Methionine *S*-Methyltransferase in selenium volatilization by plants. Methylation of selenomethionine to selenium-methyl-l-selenium-methionine, the precursor of volatile selenium. Plant Physiol 130:847–856
Tang Y-T, Qiu R-l, Zeng X-W, Ying R-R, Yu F-M, Zhou X-Y (2009) Lead, zinc, cadmium hyperaccumulation and growth stimulation in *Arabis paniculata* Franch. Environ Exp Bot 66:126–134
Terry N, Carlson C, Raab TK, Zayed A (1999) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344
Ueno D, Iwahita T, Zhao FJ, Ma JF (2008) Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. Plant Cell Physiol 49:540–548
van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC, Hooykaas PJJ (1999) Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol 119:1047–1056
Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776
Wei S, Zhou Q, Wang X (2005) Identification of weed plants excluding the uptake of heavy metals. Environ Int 31:829–834
Wenzel WW, Bunkowski M, Pushenererter M, Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyper-accumulator and excluder plants on serpentine soil. Environ Pollut 123:131–138
Wójcik M, vangronsveld J, D'Haen J, Tukiendorf A (2005) Cadmium tolerance in *Thlaspi caerulescens* II. Localization of cadmium in *Thlaspi caerulescens*. Environ Exp Bot 53:163–171
Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353
Yazaki K, Yamanaka N, Masuno T, Konagai S, Kaneko S, Ueda K, Sato F (2006) Heterologous expression of a mammalian ABC transporter in plant and its application to phytoremediation. Plant Mol Biol 61:491–503
Zhu YG, Pilon-Smits EA, Zhao FJ, Williams PN, Meharg AA (2009) Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. Trends Plant Sci 14:436–442

# Phytoremediation Using Terrestrial Plants

# 25

Enrique Javier Peña Salamanca, Carlos Arturo Madera-Parra, Carlos Andres Avila-Williams, Ana Lucia Rengifo-Gallego, and Daniel Ascúntar Ríos

## 25.1 Introduction

Terms like *phytoextraction*, usually defined as the utilization of plants to transport and concentrate pollutants from the soil into the harvestable parts of roots and aboveground shoots (Kumar et al. 1995; quoted by Hernández-Allica et al. 2008), *ecoremediation* (ERM) which comprises methods of protection or restoration of the environment by means of natural processes existing in ecosystems (Bulk and Slak 2009), and *phytoremediation* have gained an important place in the modern environmental dynamics for ecosystem management and resource usage.

Phytoremediation is a term applied to a group of technologies that use plants to reduce, remove, degrade, transform, or immobilize environmental pollutants, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition suitable for private or public applications of ecosystem services (Peer et al. 2005).

Interest in phytoremediation as a method to reduce contamination has been growing rapidly in recent years. Several technologies have been utilized to remove heavy metals and other toxic organic compounds from soil and water in many countries like the United States, Russia, and most of European countries (Uera et al. 2007). This set of technologies became a promising alternative for the decontamination of petroleum-polluted soils, especially in the tropics where climatic conditions enhance plant growth and microbial activity and where financial resources can be limited (Merkl et al. 2005).

Phytoremediation using tropical terrestrial plants offers a suitable alternative for pollution cleanup, considering its wide diversity and specific characteristic of adaptation to adequate or harsh conditions throughout its life cycle. This includes acclimation to receive high or low levels of solar radiation; longer photoperiods along entire year (12 h); overcoming extended drought, high precipitation, and/or distinct wet and dry seasons; and rich and higher microorganism diversity. Additionally, such conditions promote high respiration rates of plants, resulting in comparably lower net photosynthesis rates.

Sun et al. (2004) underline in their study how tropical plants were selected for their ability to tolerate high salinity and remove specific hydrocarbons in coastal topsoil prior to further investigation of the phytoremediation feasibility in deep contaminated soils.

This chapter on *Phytoremediation Using Terrestrial Plants* includes the main features regarding tropical native plants and its uses for this type of technology, emphasizing on their potential, diversity, experiences with different species, and other characteristics that allow an improved approach to understanding the prospective of using tropical terrestrial plants to reduce pollution of different environmental components.

Moreover, constructed wetlands have become an efficient phytoremediation technology for pollutant removal, taking advantage that plants in a natural wetland provide a substrate (roots, stems, and leaves) upon which microorganisms can grow as they break down organic materials and uptake heavy metals. Engineered wetland phytoremediation is an aesthetically pleasing, solar-driven, passive technique useful for cleaning up wastes including metals, pesticides, crude oil, polyaromatic hydrocarbons, endocrine disruptors, and landfill

E.J.P. Salamanca, Ph.D. (✉)
C.A. Avila-Williams, B.Sc., Biologist.
Department of Biology, Universidad del Valle,
Calle 13 N°100 – 00, Valle del Cauca, Cali, Colombia
e-mail: Enrique.pena@correounivalle.edu.co

A.L. Rengifo-Gallego, B.Sc., Biological. Sciences. Ph.D. Student.
Departament of Biology, Biology of Plants and Microorganism
Research Group, Universidad del Valle, Cali,
Valle del Cauca, Colombia

Biology Department, Biology of Plants and Microorganism
Research Group, Universidad del Valle, Bogota,
Cundinamarca, Colombia

C.A. Madera-Parra, Ph.D. • D.A. Ríos, B.Sc., M.Sc.
EIDENAR School, Engineering Faculty, Universidad del Valle,
Valle del Cauca, Cali, Colombia

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leachates, becoming an increasingly recognized pathway to advance the treatment capacity of wetland systems, especially in tropical conditions, where this treatment capacity can be enhanced (Zhang et al. 2010). This chapter will provide an overview of constructed wetlands' main applications for phytoremediation and discuss some experiences using tropical native plants, specifically in Colombia.

## 25.2 Phytoremediation Using Native Plants in the Tropics

### 25.2.1 Inventory of Tropical Plants Suitable for Phytoremediation

Phytoremediation involves more effort than planting vegetation and awaits for the contaminant to disappear. It requires an understanding of the processes that need to occur, the plants selected, and what needs to be done to ensure plant growth (USEPA 2001). Selection of appropriate technology is based on the environmental chemistry of the contaminant, along with the uses and type of soil or aqueous streams contaminated as they might influence to a great extent on biomass production (Cunningham and Ow 1996; Dushenkov 2003).

Several species of plants can stimulate microbial growth and facilitate biodegradation of hydrocarbons, particularly those that cannot be taken up because of their hydrophobicity (Paquin et al. 2002). Others have been reported as efficient absorbing different metals with unknown biological function such as cadmium, chromium, and lead inter alia (Tangahu et al. 2011), principally those from tropical environments where long plant-growing seasons and increased soil temperature can accelerate degradation (Paquin et al. 2002). Plants can counteract, absorb, or stabilize different contaminants, making them unavailable for other organisms (Merkl et al. 2004). Therefore, if the contaminant is not phytotoxic, phytoremediation mechanisms become a suitable tool of sustainable management.

#### 25.2.1.1 Commonly Used Plants for Phytoremediation

Plant selection criteria for most phytoremediation prospects are evapotranspiration potential, enzymes produced, growth and survival rate, biomass production, root system, and their ability to tolerate the contaminant (Paquin et al. 2002; Prasad 2003). Mechanisms and efficiency of this technology, called phytoremediation, depend on the type of contaminant, bioavailability, and substrate properties (Cunningham and Ow 1996).

More than 400 taxa were reported hyperaccumulating heavy metals (Suresh and Ravishankar 2004) and other contaminants, ranging from annual herbs to perennial shrubs and trees. Owing to their multiple ramified root systems with abundant room for microbial activity, Poaceae family is considered to be particularly suitable for phytoremediation (Aprill and Sims 1989). In addition, the deep roots in Fabaceae family members and their ability to grow in poor-nutrient soils thanks to the independency on nitrogen because of the symbiosis with nitrogen-fixing *Rhizobium* spp. make them suitable too (Merkl et al. 2004). Other families such as Brassicaceae, Asteraceae, and Solanaceae have gained popularity in phytoremediation as a result of their cosmopolitan status or helpful enzymes produced. A recompilation of some recurrent selected plants for different investigations in phytoremediation is given in Table 25.1.

#### 25.2.1.2 Tropical Plants

Bioremediation, in general, is considered a promising technology for the tropics because climatic conditions favor microbial growth and activity. Also, plant biomass production is high in the tropics, provided that adequate nutrients are available. The screening of plant species for their ability to grow and establish in contaminated soil is one of the first steps in the selection of species for phytoremediation in the tropics (Merkl et al. 2004) as they have evolved in a very large number due to the abundant taxa. Moreover, there are still many parts of the tropics in which no plant collections have focused on metalliferous soils (Reeves 2003).

This highly selective process has revealed more tropical plant species with potential importance for both phytoremediation and phytomining; it is certain that others remain to be discovered through further field exploration and detailed soil and plant analysis as little or no analytical work has been done (Reeves 2003). A list of some selected plants for phytoremediation in tropics is given in Table 25.2.

### 25.2.2 Potential of Tropical Plants for Phytoremediation

Numerous factors influence phytoremediation, including the type and amount of contaminant, soil characteristics, water content, nutrient availability, species and plant growth, of which none should be ignored (Merkl et al. 2005). The interplay of these factors has to be further studied in the tropics, where climatic and edaphic conditions vary from those where phytoremediation studies have concentrated so far. In tropical regions, evapotranspiration is a very important factor to take into account, since volatile contaminants are mainly removed through this pathway (Olguin and Sanchez-Galvan 2010). Furthermore, the minimal differences present in tropics, among growth seasonality and nutrient translocation as compared to temperate and colder regions, increase the potential in these areas to find more plants suitable for remediation.

Phytodecontamination of organic pollutants involves several processes leading to volatilization, degradation, or accumulation in aerial parts that have to be burned once harvested

**Table 25.1** Common plants used in phytoremediation

| Classification | Binomial name |
|---|---|
| PTERIDOPHYTA | |
| Pteridopsida | |
| Polypodiales | |
| Dryopteridaceae | *Dryopteris filix-mas* (L.) Schott, 1834 |
| Pteridales | |
| Pteridaceae | *Pteris vittata* (L.) |
| Salviniales | |
| Azollaceae | *Azolla pinnata* (R. Br.) |
| MAGNOLIOPHYTA | |
| Liliopsida | |
| Commelinales | |
| Pontederiaceae | *Eichhornia crassipes* (Mart.) Solms, 1883 |
| Acorales | |
| Acoraceae | *Acorus calamus* (L.) |
| Alismatales | |
| Araceae | *Colocasia esculenta* (L.) Schott, 1832 |
| | *Lemna minor* (L.) Griff, 1851 |
| Magnoliopsida | |
| Asterales | |
| Asteraceae | *Ageratum conyzoides* (L.) |
| | *Berkheya coddii* |
| | *Blumea lacera* (Burm.f) D.C. |
| | *Mikania cordata* (Burm.f) B.L.Rob., 1934 |
| Brassicales | |
| Brassicaceae | *Alyssum murale* (Waldst. and Kit.) |
| | *Arabidopsis thaliana* (L.) Heynh. |
| | *Brassica chinensis* (L.) |
| | *Brassica oleracea* (L.) |
| | *Cochlearia pyrenaica* (Bab.) Dalby |
| | *Thlaspi caerulescens* (J. Presl and C. Presl) |
| Caryophyllales | |
| Aizoaceae | *Mesembryanthemum crystallinum* (L.) |
| | *Sesuvium portulacastrum* (L.) |
| Amaranthaceae | *Amaranthus cruentus* (L.) |
| | *Beta vulgaris* (L.) |
| Chenopodiaceae | *Atriplex halimus* (L.) |
| | *Atriplex nummularia* (Lindl.) |
| Tamaricaceae | *Tamarix smyrnensis* (Bunge) |
| Fabales | |
| Fabaceae | *Kummerowia striata* (Thunb.) Schindl |
| | *Medicago sativa* (L.) |
| Lamiales | |
| Bignoniaceae | *Jacaranda mimosifolia* (D. Don) |
| Lamiaceae | *Clerodendrum trichotomum* (Thunb.) |
| | *Aeollanthus biformifolius* (De Wild.) |
| Malpighiales | |
| Euphorbiaceae | *Ricinus communis* (L.) |
| Salicaceae | *Populus* spp. (L.) |
| | *Salix* spp. (L.) |
| Malvales | |
| Malvaceae | *Hibiscus tiliaceus* (L.) |
| Myrtales | |
| Myrtaceae | *Eucalyptus* spp. (L'Her.) |
| Poales | |
| Cyperaceae | *Cyperus papyrus* (L.) |
| Poaceae | *Brachypodium sylvaticum* (Huds.) Beauv. |
| | *Chloris barbata* (L.) Sw. |
| | *Chrysopogon zizanioides* (L.) Roberty |
| | *Cynodon dactylon* (L.) Pers. |
| | *Festuca arundinacea* (Schreb.) |
| | *Polypogon monspeliensis* (L.) Desf. |
| | *Sorghum sudanense* (Piper) Stapf |
| | *Zea mays* (L.) |
| Rosales | |
| Urticaceae | *Urtica dioica* (L.) |
| Solanales | |
| Solanaceae | *Lycopersicon esculentum* (Mill.) |
| | *Nicotiana glauca* (Graham) |
| | *Solanum nigrum* (L.) |

(Mougin 2002), but the impact of each process has not been elucidated. In Venezuela, planting *Brachiaria* is already a commonly used strategy to treat petroleum-contaminated soil, yet the ability to enhance the degradation of hydrocarbons is to be investigated (Merkl et al. 2004).

As stated before, there are still several issues unsolved or partially understood about phytoremediation, which is why it is recommended to set up long-term studies in order to gain full insight of the diverse mechanisms occurring, allowing to draw recommendations on the convenience and frequency of harvesting and on the advantages of using specific species. The huge biodiversity that is commonly found in tropical and subtropical regions represents a challenge for finding new species with outstanding characteristics for tolerance to toxic and recalcitrant pollutants or to extreme environmental conditions, such as high temperature or salinity (Olguin and Sanchez-Galvan 2010).

### 25.2.3 Uses of Tropical Plants in Phytoremediation

#### 25.2.3.1 Main Features

Phytoremediation for environmental cleanup is being recognized as a suitable alternative solution. Plants function in phytoremediation in two ways, the major one being facilitation of favorable conditions for microbial degradation, specifically root-colonizing microbes, and the second aspect is the root itself, providing an inexpensive mean to access contaminants in subsurface soil and water (Suresh and Ravishankar 2004). In general terms, that would be the same for tropical and non-tropical areas, with the difference of more suitable conditions for greater microbial activity and biomass production in tropical regions owing the warmth and humidity among other environmental features promoting plant and microbial growth.

**Table 25.2** Tropical plants used in phytoremediation

| Classification | Binomial name | |
|---|---|---|
| PTERIDOPHYTA | | Role in phytoremediation |
| Pteridopsida | | |
| Pteridales | | |
| Pteridaceae | *Pteris vittata* (L.) | Phytoextraction and accumulates Mg |
| Salviniales | | |
| Azollaceae | *Azolla pinnata* (R. Br.) | Accumulates Pb, Cu, Cd, Fe |
| MAGNOLIOPHYTA | | |
| Liliopsida | | |
| Commelinales | | |
| Commelinaceae | *Tradescantia spathacea* (Sw.) | Wastewater treatment |
| Pontederiaceae | *Eichhornia crassipes* (Mart.) Solms, 1883 | Accumulates Cr, Pb, Cu, Cd, Fe |
| Zingiberales | | |
| Heliconiaceae | *Heliconia psittacorum* (L.f.) | Wastewater treatment. Accumulates metals. Transforms N and eliminates ionic charge |
| Alismatales | | |
| Araceae | *Colocasia esculenta* (L.) Schott, 1832 | Wastewater treatment. Accumulates metals |
| | *Lemna minor* (L.) Griff., 1851 | Accumulates metals |
| Magnoliopsida | | |
| Apiales | | |
| Araliaceae | *Hydrocotyle umbellata* (L.) | Accumulates Pb, Cu, Cd, Fe |
| Asterales | | |
| Asteraceae | *Helianthus annuus* (L.) | Accumulates Pb and U. Removes $^{137}$Cs and $^{90}$Sr in hydroponic reactors |
| Brassicales | | |
| Brassicaceae | *Brassica napus* (L.) | Remediation of soils contaminated with $^{137}$Cs |
| | *Brassica juncea* (L.) | Hyperaccumulates metals |
| Ceratophyllales | | |
| Ceratophyllaceae | *Ceratophyllum demersum* (L.) | Accumulates metals. Removes TNT |
| Fabales | | |
| Fabaceae | *Centrosema brasilianum* (L.) Benth. | Phytoremediation of crude oil |
| | *Calopogonium mucunoides* (Desv.) | Phytoremediation of crude oil |
| | *Medicago sativa* (L.) | Tolerance toward B |
| | *Phaseolus acutifolius* (A. Gray) | Accumulates $^{137}$Cs |
| | *Vicia faba* (L.) | Remediation of petroleum hydrocarbons |
| Lamiales | | |
| Bignoniaceae | *Jacaranda mimosifolia* (D. Don) | Tolerance toward B |
| Malpighiales | | |
| Salicaceae | *Salix* spp. (L.) | Phytoextraction of heavy metals. Wastewater and runoff treatment |
| Malvales | | |
| Malvaceae | *Hibiscus tiliaceus* (L.) | Phytoremediation of PAHs |
| Myrtales | | |
| Myrtaceae | *Eucalyptus* spp. (L'Her.) | Removes Na and As |
| Poales | | |
| Poaceae | *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf | Phytoremediation of crude oil |
| | *Chrysopogon zizanioides* (L.) Roberty | Phytoremediation of PAHs. Wastewater treatment |
| | *Cyperus haspan* (L.) | Wastewater treatment |
| | *Gynerium sagittatum* (Aubl.) P. Beauv. | Wastewater treatment. Hyperaccumulates metals |
| Solanales | | |
| Solanaceae | *Datura innoxia* (Mill.) | Accumulates Ba |
| | *Nicotiana glauca* (Graham) | Tolerance toward B |
| | *Solanum nigrum* (L.) | Hairy cell cultures detoxify PCBs |
| Urticales | | |
| Cannabaceae | *Cannabis sativa* (L.) | Hyperaccumulates metals |

#### 25.2.3.2 Advantages

Phytoremediation techniques tend to be more publicly acceptable, aesthetical, and less disruptive than the current physical and chemical counterparts (Tangahu et al. 2011). Advantages of this technology are its effectiveness in contaminant reduction, low cost as it generally does not need specialized equipment or key personal for its application (Macek et al. 2000), and the fact of being applicable for a wide range of contaminants including organic and inorganic, and overall it is an environmental friendly method (Prasad 2003; Mougin 2002). Phytoremediation is probably the cleanest and cheapest technology effectively employed in the remediation of hazardous sites and even contributes to the improvement of poor soils such as those with high metal or high salt levels (Tangahu et al. 2011), and the treated soil can be reused if the target pollutant levels are reached.

When bioavailable, and in relatively low concentrations, the most common contaminants can be degraded by microorganisms which have developed numerous degradation pathways. On the contrary, aging of the pollutant seems to limit biodegradation and the availability is reduced (Mougin 2002). Therefore, consortia between microorganisms and plants might be an asset given that where one's ability is impaired, the other could help.

#### 25.2.3.3 Disadvantages

Currently, commercial applications of phytotechnologies are being hindered by the perception that it might require an excessive amount of time to be effective (Paquin et al. 2002; Prasad 2003). However, this can be countered if being demonstrated the minimal risk to the environment during the operation contrary to the use of conventional technologies where the risk of contamination by leaching can be high (Robinson et al. 2003).

The limitations of phytoremediation are that the contaminants below the rooting depth cannot be extracted by the plant root system (Suresh and Ravishankar 2004), plants cannot grow at high toxic levels of contaminants (Prasad 2003; Suresh and Ravishankar 2004), and in some cases the long-term application of the process, as it could take years to regulate the contamination levels (Mougin 2002) because of the limit of contaminant each plant can process (Tangahu et al. 2011).

It is clear that there is still a lot of development to be done for the phytoremediation technologies, especially in tropical areas (Dushenkov 2003), but the field is a fast-developing one, and in the near future of phytoremediation, it can become an integral part of environmental management and risk reduction at a world scale (Prasad 2003; Reeves 2003; Dushenkov 2003).

#### 25.2.3.4 Biomass Disposal

When plants containing heavy metals are harvested, they can either be eliminated or treated to recycle the metal compounds. In such plants where a substantial translocation to the aerial parts occurs, a great part of the biomass produced is considered contaminated according to the nature of the element being absorbed, and therefore, it should be eliminated as a dangerous or radioactive material carrying out extra costs and affecting the overall efficiency (Prasad 2003). Furthermore, as it might be needed, several growth cycles before the results of the remediation process can be evident; a lot of biomass might be obtained, boosting the importance of planning in advance the use of such matter. The most interesting alternative is the biodiesel production as plant oil would be generated available to produce thermal energy while the affected soils are being remediated (Tangahu et al. 2011).

## 25.3 Phytoremediation Applications: Constructed Wetlands

### 25.3.1 Constructed Wetlands

Wetlands are defined as a portion of land where the water table is at/or above the ground surface long enough to maintain saturated soil conditions and the growth of related vegetation (Crites et al. 2006). Wetland's capability for changing wastewater characteristics has been verified in a number of studies in a variety of geographical settings, by using preexisting natural marshes, swamps, strands, bogs, peatlands, cypress domes, and systems specially constructed for wastewater treatment; therefore, the term constructed wetlands (CWs), which Vymazal (2010) describes as engineered systems that have been planned and built to benefit from natural interaction between vegetation, soil, and microorganisms for pollutant removal, is designed specifically to take advantage of many of the same processes that occur in nature, but to do so within a more controlled environment.

#### 25.3.1.1 Constructed Wetland Classifications

CWs for wastewater treatment may be classified according to the life form of the dominating plant (Fig. 25.1) into systems with free-floating, floating-leaved, rooted emergent and submerged macrophytes (Vymazal 2010), conditions that can be combined with different flow settings, which according to WSP (2008) are distinguished into three types of configurations, that also differ from one another in system layout, removal efficiency of certain pollutants, area requirements, technical complexity, applications, and costs:

(a) *Surface flow or free-water surface* (*FWS*) *constructed wetland*: large, shallow lagoons that may contain *submerged*, *emergent, or floating plant species*. The microorganisms responsible for biological treatment of the wastewater form biofilms on the stems and leaves of the plants. These systems can be used for secondary treatment of wastewater, but they are most commonly used as

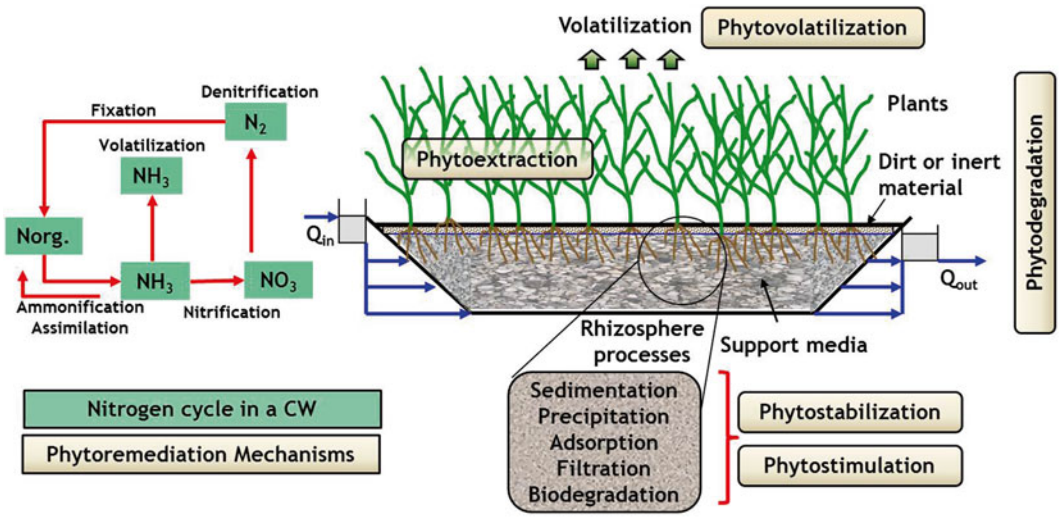


**Fig. 25.1** Phytoremediation processes and nitrogen flows on a constructed wetland

tertiary treatment, in order to remove nutrients like nitrogen and phosphorous.

(b) *Horizontal subsurface flow* (*HSSF*) *constructed wetland*: also known as vegetated submerged bed systems, it consists of shallow basins filled with coarse sand or gravel as filter media. Native or foreign wetland plants are grown on the surface of the filter bed, and pretreated wastewater flows through the bed horizontally below the surface. Several investigations have revealed the great prospective of these treatment systems and the potential of reusing their effluent for irrigation or other purposes.

(c) *Vertical flow* (*VF*) *constructed wetland*: shallow sand filter beds with a distribution system on the surface that allows the wastewater to percolate vertically through the unsaturated media as plants support the vertical drainage process. An important feature of this type is the intermittent hydraulic loading with resting intervals between discharges, which provides an effective aeration mechanism because pores of the filter bed refill with oxygen during the intervals. As a result, high nitrification rates can be achieved in the filters, and denitrification can be carried out by recirculating the effluent into the primary treatment unit.

#### 25.3.1.2 Removal Mechanisms in Constructed Wetlands

CWs have been found to be effective in treating organic matter (OM), suspended solids (SS), nitrogen (N), and phosphorous (P), as well as for metal and pathogen reduction. The diversity of treatment mechanisms in CWs includes biological processes (microbial metabolic activity and plant uptake) and physicochemical processes (sedimentation, adsorption, and precipitation), processes that when combined effectively remove pollutants in wastewater. Biodegradation occurs when dissolved organic matter is carried into biofilms attached on submerged plant stems, root systems, and filtering media by diffusion and dispersion process. SS are removed by filtration and gravitational settlement (Wetlands International 2003; WSP 2008). Each different wastewater component has its own removal pathway, some mechanisms being more suitable depending on the pollutant to be removed, transformed, or retained. The main mechanisms and its associated removed contaminant are resumed on Table 25.3.

**Table 25.3** Removal mechanisms for determined pollutants in CW

| Removal mechanism | Removed contaminant |
|---|---|
| Bioconversion | OM, SS, N, P |
| Predation | Pathogens |
| Adsorption | OM, SS, N, P |
| Sedimentation | OM, SS, N, P, pathogens |
| Filtration | OM, SS, N, P, pathogens |
| Plant uptake | N, P |
| Volatilization | N |
| UV radiation | Pathogens |
| Excretion of antibiotics by plants | Pathogens |
| Ammonification, nitrification/ denitrification (limited) | N |

For example, nitrogen removal is achieved by two major processes, which includes physicochemical and biological methods. In CWs, denitrification process may remove 60–70 % of the overall removed nitrogen, and 20–30 % may be derived from plant uptake or assimilation and adsorption (Lee et al. 2009).

#### 25.3.1.3 Performance of CWs for Phytoremediation

Performance criteria for phytoremediation in CWs may be based on the contaminant concentration in the system outflow or on the total percent mass removal of it. Either way, it is important that the selected criteria truthfully reflect the actual performance of the CW relative to the objectives and intended

**Table 25.4** Treatment efficiency of various types of CWs

| CW type | Average removal efficiencies (%) BOD | TSS | TP | TN | NH4–N |
|---|---|---|---|---|---|
| Free-water surface (FWS) CW | 72–74 | 68–77 | 34–50 | 41–58 | 39–53 |
| Horizontal subsurface flow (HSSF) CW | ~75 | ~75 | ~50 | 33–43 | 30–39 |
| Vertical flow (VF) CW | ~90 | ~89 | ~56 | ~43 | 30–73 |

uses of the wetland treatment system and potential use of its effluent (Dipu et al. 2010).

In general, CWs require little operation and maintenance (O&M) when compared with technical treatment systems (WSP 2008). Vymazal (2010) made a thorough review of a great number of CWs, resulting in the average removal efficiencies presented on Table 25.4 for different types of CWs and depending on the pollutant. Treatment efficiency for OM (biological oxygen demand, BOD) and particulates (total suspended solids, TSS) is high in all types of CWs. Normally, CWs are not designed to remove P (total phosphorous, TP), which is why Vymazal's review (2010) shows low P retention in all types of CWs. Most studies on P cycling in wetlands have shown that soil/peat accumulation is the major long-term P sink.

Removal of N is usually low given that nitrification is scarce or does not take place in HSSF CWs or FWS. Volatilization may be a significant route for N removal in CWs with open water surface where algal assemblages can create high pH values during the day through their photosynthetic activity. VF CW systems consist of almost entirely aerobic conditions, which promote high nitrification, but no denitrification takes place. In order to achieve effective removal of total N, hybrid systems could be implemented, combining VF CWs followed by HSSF CWs which could provide suitable conditions for reduction of nitrate formed during nitrification. If plants are harvested, plant uptake can be considered as a viable mechanism for nutrient removal, but it may only achieve around a small percentage of the inflow nutrient load (Vymazal 2010).

CWs may need to be designed to meet higher level of performance in order to address local environmental objectives or other pollutant control issues. The integrity of good design may be jeopardized during construction, leading to reduced performance and impacts on the long-term sustainability of the system. Also, even though it requires minimum O&M attention, unawareness of minimal activities required on-site can reduce its performance (Melbourne Water 2010).

### 25.3.2 Subsurface Flow Constructed Wetlands for Phytoremediation

The most relevant technology using phytoremediation strategy is CWs (Dipu et al. 2010). Horizontal subsurface flow constructed wetlands (HSSF CWs) are the predominant wetland concept in Europe (Vymazal 2008; GTZ 2010). Progressively, this technology has become an important low-impact alternative to be implemented rather than conventional wastewater treatment processes during the last few years and has also demonstrated a consistent capacity to remove organic carbon and particulate matter efficiently from sewage in several regions around the world, with interesting results on tropical conditions, given that those type of environments and its associated biodiversity can potentially enhance CW performance for pollutant removal and transformation (Ascúntar et al. 2009). HSSF CWs showed an increase rate of contaminant uptake in warmer climates; therefore, this treatment has been expected to operate more efficiently in tropical regions (Chek Rani et al. 2011).

In HSSF CWs, rhizosphere processes have been successfully used to treat industrial and domestic effluents using specific aquatic macrophytes. One of the integrated components of this process is the need of adaptive and efficient plants, which are fed by absorbing nutrients from wastewater at a faster rate, turning this vegetative material to a desirable subproduct. The plants hold themselves in the inter-porous particles of the support media through their roots and rhizomes creating a complex network of underground stem. Roots grow rapidly and provide air pockets through the support media, providing a host area for many biological communities to colonize, develop, and mineralize wastewater components and by-products (Chavan and Dhulap 2012).

#### 25.3.2.1 Support Media

There are basically two different concepts of filling material for HSSF CWs, the sand-based and gravel-based beds. Gravel-bed systems are widely used in America, North Africa, South Africa, Asia, Australia, and New Zealand. The sand-bed systems have their origin in Europe but nowadays are used all over the world (GTZ 2010). The media depth and the water depth in these wetlands have ranged from 0.3 to 0.9 m in operational systems in the United States (Crites et al. 2006). Systems that use sand instead of gravel can increase the P retention capacity, but selecting this media would reduce the hydraulic conductivity through the porous media (Chek Rani et al. 2011).

The HSSF CW average bed typically contains up to 0.6 m of support media. This is sometimes overlain with a thin layer of fine gravel that is 76 to 150 mm deep or other materials can be used like inert ash (Ascúntar et al. 2009). This top

**Table 25.5** Constructed wetland support media characteristics

| Media type | Effective size, D10 (mm) | Porosity ($n$) | Hydraulic conductivity (ks, m/s) | Suggestion |
|---|---|---|---|---|
| Coarse sand | 2 | 0.32 | $1.2 \times 10^{-2}$ | Low-TSS wastewater |
| Gravelly sand | 8 | 0.35 | $5.8 \times 10^{-2}$ | Combined in different bed layers from top to bottom |
| Fine gravel | 16 | 0.38 | $8.7 \times 10^{-2}$ | |
| Medium gravels | 32 | 0.40 | $11.6 \times 10^{-2}$ | |
| Coarse rock | 128 | 0.45 | $115.7 \times 10^{-2}$ | For inlet and outlet structures |

**Table 25.6** Major roles of macrophytes in CWs

| CW plants | Role | Common species |
|---|---|---|
| Aerial plant tissues | • Enhancing wildlife and aesthetic values<br>• Influence on microclimate (insulation during harsh climate conditions)<br>• Aesthetic appearance<br>• Nutrient and other pollutant storages (e.g., heavy metals) | *Heliconia psittacorum* (L.f.) |
| Plan tissues on support media | • Producing litter as a source of organic carbon for denitrification and other microbial processes<br>• Surface area for attached microorganisms | *Cyperus papyrus* (L.)<br>*Colocasia esculenta* (L.) Schott, 1832 |
| Roots and rhizomes | • Promoting the settling and retention of suspended solids<br>• Dispersing flow to minimize short-circuiting<br>• Providing surfaces for the development of microbial biofilms<br>• Transporting into their root zone by excretion of photosynthetic oxygen to enhance bioconversion<br>• Assimilating pollutants<br>• Release of nutrients in slowly available organic forms<br>• Release of antibiotics | *Chrysopogon zizanioides* (L.) Roberty<br>*Cyperus haspan* (L.)<br>*Gynerium sagittatum* (Aubl.) P. Beauv. |

material facilitates initial rooting medium for the vegetation and is kept under dry condition during normal operations. Table 25.5 shows the commonly used materials for HSSF CW.

Natural soil may not be suitable as a substrate for wastewater treatment in CWs. Several studies with this type of support media have reported clogging problems, causing overflows, erosion, and deficient plant growth. Effective volume can be reduced in a soil-based CW by obstruction of its interstices, which could rapidly decrease the hydraulic retention time (HRT) of the treatment unit and increase flow velocities, generating short-circuiting and overflows (Sundaravadivel and Vigneswaran 2009). Thus, a combination of gravel and coarse sand are preferred as support media and, currently, in several CWs, only gravel is used as support media.

#### 25.3.2.2 Plant Role and Selection

It is difficult to predict when and under what circumstances the plant contributions will be more relevant, and this may be the reason for the controversy surrounding their actual roles. Most studies relate to the overall effect of plants on CW systems, with much less focus on the specific plant species or mechanisms and their involvement on CW efficiency. It is important to consider that such studies are difficult to conduct under field conditions because of the complexity of the outdoor facility, with multiple variables affecting its performance, such as inlet wastewater quality, media composition, climate, fauna and flora, etc. (Shelef et al. 2013). Tanner et al. (2006) and Sundaravadivel and Vigneswaran (2009) agree that the roles provided by CW plants can be assigned to determine part of the plant as seen on Table 25.6.

The choice of plants is an important issue in CWs, as they must survive the potential toxic effects of the wastewater and its variability. The most widely used CW design in Europe is the horizontal subsurface flow system vegetated with the common reed (*Phragmites australis*), although other plant species, such as cattails (*Typha* spp.), bulrushes (*Scirpus* spp.), and reed canary grass (*Phalaris arundinacea*), have been used for both domestic and industrial wastewater treatments (Calheiros et al. 2007).

In tropical countries, locally available species of *Phragmites*, *Cyperus*, bulrush, and *Typha* have been the most common choice to date. Most recently, Konnerup et al. (2009) successfully used *Heliconia psittacorum* and *Canna generalis* in order to increase the aesthetic value of wetlands and to increase the local people's awareness of wastewater

**Table 25.7** Advantages and disadvantages of CWs

| Advantages | Limitations |
|---|---|
| • Less expensive to build than other treatment options<br>• Utilization of natural processes<br>• Simple construction, O&M<br>• Cost-effectiveness<br>• Process stability<br>• They require little or no energy to operate<br>• They can provide additional wildlife habitat<br>• They can be aesthetically pleasing additions to homes and neighborhoods | • Large area requirement<br>• May be economical relative to other options only where land is available and affordable<br>• Design criteria have yet to be developed for different types of wastewater and climates<br>• Not appropriate for treating some wastewater with high concentrations of certain pollutants<br>• There may be a prolonged initial start-up period before vegetation is adequately established |

treatment in Thailand (Chek Rani et al. 2011). Ascúntar et al. (2009) also used *Heliconia psittacorum* in Colombia on a pilot-scale CW for secondary treatment of domestic wastewater with high efficiency for removal of solids (>90 %) and organic matter (>60 %). Furthermore in Colombia, Madera et al. (2015) assessed three native species (*Gynerium sagittatum*, *Colocasia esculenta,* and *Heliconia psittacorum*) for landfill leachate treatment at bench scale under tropical conditions obtaining high removal efficiencies (>80 %) for heavy metals like Cd(II), Pb(II), Hg(II), and Cr(VI).

#### 25.3.2.3 Design Considerations for HSSF CWs

HSSF CWs are designed based on HRT and average design flow. The shortest detention times are usually necessary for BOD, nitrate N, and TSS removal from domestic wastewater, while ammonia and metal removal usually requires longer detention times (Crites et al. 2006). The design of HSSF CWs has evolved from early empirical rules to advanced models, which try to explain the complexity of hydrodynamics in a porous medium combined with many physical and biochemical processes involved in pollution reduction (Marsili-Libelli and Checchi 2005; Langergraber et al. 2009).

HSSF CWs have been designed using either simple "rule of thumb" set at 5 $m^2$ $PE^{-1}$ or plug-flow first-order models (Kadlec and Wallace 2008). Recently, more complex dynamic, compartmental models have been developed (Langergraber et al. 2009). Dimensioning HSSF CWs is usually based either on volume or area (Ewemoje and Sangodoyin 2011).

Many texts and design guidelines for HSSF CWs have been published such as USEPA (2000), Cooper (1990), WPCF (1990), Reed et al. (1995), Kadlec and Knight (1996), Campbell and Ogden (1999), Ellis et al. (2003), and DNR (2007); nonetheless, there are few guidelines recorded for tropical climates, like Melbourne Water (2005), UN-Habitat (2008), and Melbourne Water (2010). Consequently, there are still voids regarding the application, design and performance of this technology, especially in the design phase, being oxygen availability and nitrogen removal the most common setbacks in tropical regions (Chek Rani et al. 2011).

#### 25.3.2.4 Advantages and Limitations of CWs

Even though the potential for application of wetland technology worldwide is enormous, the rate of adoption of it for wastewater treatment in tropical countries has been slow. It has been identified that the current limitations in these regions are due to the fact that there is limited knowledge and experience with CW design and management. Table 25.7 shows some advantages and disadvantages of implementing CWs for wastewater treatment.

## 25.4 Experiences Using CW for Phytoremediation

### 25.4.1 Worldwide Phytoremediation Experiences with CWs

Nepal has widely implemented CWs for wastewater treatment. Reed bed treatment systems (RBTS), as they call it, have set a valuable precedent for other larger systems in other parts of the country as well as systems envisaged under national urban development projects, becoming an important small-scale decentralized wastewater treatment solution (WATERAID 2008).

Another clear example of FWS CW implementation is the Putrajaya Wetlands, which were the first man-made wetland in Malaysia, with an area of 197 ha and 12.3 million Wetland plants, it became one of the largest fully constructed freshwater wetlands in the tropics. The wetlands are strategically located to act as buffer to the Putrajaya Lake which drains a catchment area of 50.9 $km^2$. This facility is also used for urban runoff treatment (Chai Huat 2002). North America has been using CWs for many purposes, such as the case of the FWS CW in Monastery Run, Pennsylvania, USA, where this technology was implemented to treat alkaline mine drainage waters generated upstream, with positive results for Fe, Al, and Mn reduction (USEPA 2005).

Large-scale CW systems have been applied with excellent results regarding biological performance, cultural acceptance and aesthetic benefits. Such is the case of the wastewater treatment plant of Liedekerke in Belgium, used for 70,000 PE, which has an FWS CW of 1.3 ha as a final

**Fig. 25.2** Implemented HSSF CWs in La Voragine, Cali, Colombia

process for effluent refining. Since its implementation, 12,834 birds were spotted during 2 years of operation; 132 species, 39 families, and 29 Red List species were also sighted (WATERAID 2008).

Another example is the system located in Can Cabanyes, Granollers, near Barcelona, Spain, which consists of a 1.0 ha HSSF CW and is one of the restoration measures for a degraded zone near the river Congost. The system currently serves three main purposes: (1) effluent polishing before discharge, (2) landscape restoration, and (3) habitat function. Other restoration measures include the construction of a nature education center and some walkways along the wetland and the river. So this technology, aside from being a wastewater management system, becomes a center for education, for recreation (walking, fishing), and even for art activities like photography (Rousseau and Hooijmans 2010). As stated by Vymazal (2010), HSSF CWs have regularly been used to treat domestic and municipal wastewaters around the world. However, currently, HSSF CWs are used to treat many other types of wastewaters including industrial and agricultural, landfill leachate, and runoff waters (Fig. 25.2).

### 25.4.2 Experiences with CWs for Phytoremediation of Specific Pollutants

When CWs are used for phytoremediation, different mechanisms regarding plant development interact at the same time; this enhances the vegetation restorative capacity acting as a cleaning agent of pollutants (Fig. 25.3). Using these natural mechanisms, it has been possible to engineer CW treatment systems to remove specific contaminants from wastewater by taking advantage of the plant's metabolism.

For instance, Aziz et al. (2011) stated that reed beds using gravel as a support media proved an excellent tolerance to a month treatment of leachate irrigation, which resulted in a Zn removal that reached 70.0 %, while $Cr^{+6}$ only achieved 31.2 %.

Lizama et al. (2011) carried out a review on the potential of arsenic (As) removal in CWs, stating that this technology is highly suitable for this purpose and that the main environmental factors influencing this process include pH, alkalinity, dissolved oxygen, the presence of iron and sulfate, competing chemicals, organic carbon, and the support media; they also identified the major removal mechanisms that contribute to As removal, such as sorption, precipitation, and coprecipitation, but bacteria can mediate these processes and can play a significant role under favorable environmental conditions.

Ganjo and Khwakaram (2010) used multiple cells of CWs with different plants obtaining results that showed how removal efficiency of all studied heavy metals (Fe, Mn, Zn, and Cu) was much higher in *T. angustifolia* followed by *Ph. australis*, *B. maritimus,* and *A. donax* in triple experiment sand pots. This implies that the combination of different vegetal species can enhance the treatment performance and, perhaps, in a specific sequential order.

Stottmeister et al. (2006) affirmed that the removal capacities for As and Zn are larger in soil-based (gravel) wetlands than in hydroponic systems or in algae ponds. Although gravel and plants each separately has low-binding capacities

| Phytoremediation mechanism | Definition |
|---|---|
| Phytoextraction/ Phytoaccumulation | The removal of inorganic contaminants from the soil through plant uptake and subsequent harvest and removal of biomass. Phytoextraction is typically used to remove metals from the soil. |
| Phytodegradation (Phytotransformation) | The breakdown of contaminants by the metabolic processes in a plant. Also includes the breakdown of contaminants in the soil by enzymes or other products produced by the plant. Primarily used for organic contaminants. |
| Rhizofiltration/ Rhizodegradation | The breakdown or degradation of organic contaminants in the soil. The contaminants are either adsorbed onto the root surface or absorbed by the plant roots. The contaminants are broken down by enhanced microbial activity in the rhizosphere (the zone of soil influenced by the roots). |
| Phytovolatilization | The uptake of contaminants by plants and release into the atmosphere as the plants transpire water. Contaminant is removed from the soil and may be degraded as it moves through the plant's vascular system before final removal from the system. |
| Phytosequestration/ Phytostabilization | This process sequesters or reduces contaminant bioavailability through precipitation or immobilization of contaminants in the soil, on the root surface, or within the root tissues. |
| Phytostabilization | Plants are used to stabilize contaminated soils or sediments, thus protecting them from transport by wind or water erosion. The main function is to contain the contaminated material. |

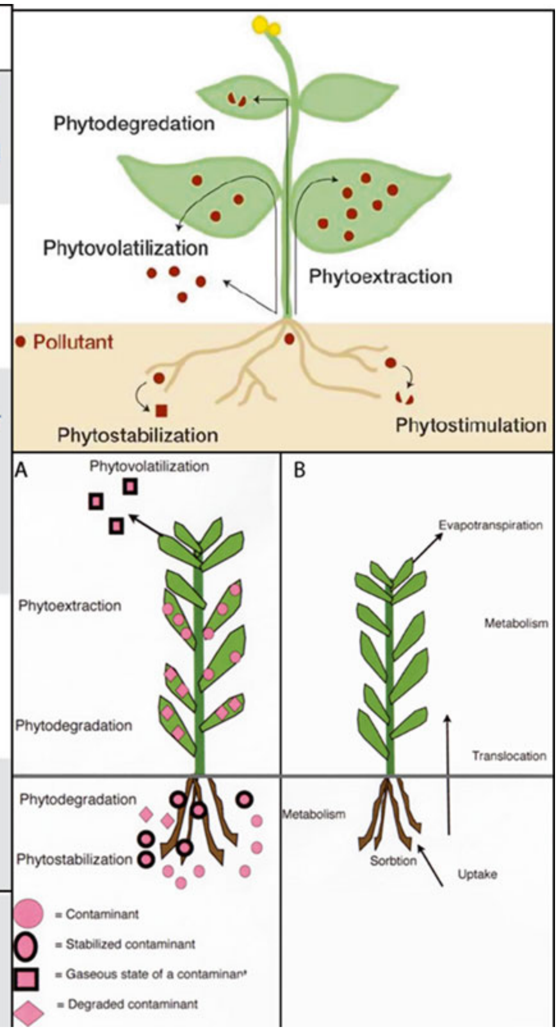


**Fig. 25.3** Phytoremediation mechanisms in CWs

for As, it was found that the combination of both enhances coprecipitation of As, particularly in the oxic zones of the rhizosphere. Also, they found that the combination of gravel/soil matrix and plants has a better treatment performance than systems with only soil or only plants without soil, which encourage the implementation of CWs for remediation of wastewater from mines and industries which emit acidity, As, Zn, and other heavy metals.

Liao and Chang (2004) found water hyacinth to be a promising candidate for phytoremediation of wastewater polluted with Cu, Pb, Zn, and Cd, while assessing the root system from the Erh-Chung wetlands located south of Taipei City. This 320 ha wetland had an average depth of 0.88 m. The concentrations in the root tissue were found in the order of Cu > Zn > Ni > Pb > Cd. The absorption capacity for water hyacinth was estimated at 0.24 kg/ha for Cd, 5.42 kg/ha for Pb, 21.62 kg/ha for Cu, 26.17 kg/ha for Zn, and 13.46 kg/ha for Ni.

### 25.4.3 Experiences of Phytoremediation in Colombia

In Colombia, universities have lead the development of CW technology, starting with small-scale laboratory investigations, pilot-scale studies, and, gradually, large-scale facility implementation. Arias and Brix (2003) highlighted the development and research for CW technology by acknowledging several Colombian experiences documented since 1998 with worldwide availability. For instance, a laboratory-scale research evaluated the capacity of organic and inorganic compound reduction in an experimental vertical flow CW fed with water from the Bogota River in order to assess the capability of this technology for pollution control on this water stream. It was possible to obtain COD reductions of 37 %, BOD 10 %, total coliforms 49 %, TSS 27 %, $NO_2$ 83 %, and $NO_3$ 30 %. From these results, it was concluded that, on laboratory scale, this technology was able to improve

**Fig. 25.4** Bench-scale CW treatment system for synthetic landfill leachate treatment

the water quality of Bogota River (Rodríguez and Ospina 2005). Other researches like the one carried out by Arias et al. (2010) consisted of the design and implementation of a small and simple CW system to treat wastewater from a pig production unit at the Center Renewable Natural Resources "La Salada," based on a pilot essay of phytoremediation using native plants. The objective was to assess the efficiency of CW to reduce the pollutant load, as economic systems of treatment for pig farmers in Colombia. The results showed that the selected combination of different support media (gravel, sand, and rice husk) and native plants was able to reach a treatment efficiency superior of 80 % for organic matter and solid removal, in compliance with the Colombian environmental regulation for wastewater discharges.

Pilot-scale studies, with synthetic wastewater, assessed organic matter removal in terms of COD and $BOD_5$ in six HSSF CWs, planted with three different macrophytes: *Canna limbata*, *Heliconia psittacorum,* and *Phragmites* sp.; the average removals of COD were 97.31 and 95.94 % for *Canna limbata*, 94.49 and 93.50 % for *Heliconia psittacorum*, and 97.39 and 97.13 % for *Phragmites* sp. In $BOD_5$ efficiency was 100 and 99.36 % for *Canna limbata*, 99.09 and 97.49 % for *Heliconia psittacorum,* and 100 and 99.45 % for *Phragmites* sp. It was concluded that there were significant differences in COD removal between different plants ($P < 0.05$), but not for $BOD_5$ (Montoya et al. 2010).

Bench-scale study, with synthetic landfill leachate, assessed organic matter, nitrogen, and heavy metal removal in terms of COD, $NH_4^+$–N, TKN, $NO_3^-$–N, Pb(II), Cd(II), Hg(II), and Cr(VI) in 22 HSSF CWs, planted with three different macrophytes: *Colocasia esculenta* (*Ce*), *Gynerium sagittatum* (*Gs*), *and Heliconia psittacorum* (*He*) (Fig. 25.4); average removal efficiencies of COD, TKN, and $NH_4^+$–N were 66, 67, and 72 %, respectively, and heavy metal removal ranged from 92 to 98 % in all units. Cr(VI) was not detected in any effluent sample. The bioconcentration factors (BCFs) were $10^0$–$10^2$. The BCF of Cr(VI) was the lowest, 0.59 and 2.5 (L $kg^{-1}$) for *Gs* and *He*, respectively, while Cd(II) had the highest (130–135 L $kg^{-1}$) for *Gs*. Roots showed a higher metal content than shoots. Translocation factors (TFs) were lower; *He* was the plant exhibiting TFs >1 for Pb(II), Cr(T), and Hg(II) and 0.4–0.9 for Cd(II) and Cr(VI). The evaluated plants demonstrate their suitability for phytoremediation of landfill leachate, and all of them can be categorized as metal accumulators (Madera et al. 2015).

Ascúntar et al. (2009) assessed a pilot-scale HSSF CW planted with *Phragmites australis* and *Heliconia psittacorum* (Fig. 25.5), used as a secondary treatment for the primary effluent of an anaerobic pond that served the Ginebra municipality in Valle del Cauca, Colombia. Even though they were evaluating the system's hydrodynamic performance, they also monitored its efficiency for organic matter removal, obtaining 68.5 % for BOD5, 63.9 % for unfiltered COD, 49.4 % for filtered COD, and 90.4 % for TSS on the CW planted with *Phragmites australis*. Similar results were obtained for the one planted with *Heliconia psittacorum*.

On the east of Colombia, a prototype of CW simple and efficient configuration called HUMEDAR-I was implemented. Its configuration involves an anaerobic reactor of parallel compartments plug flow (RACFP), followed by a high-rate CW, using native and common macrophytes, supported on a recycled plastic support media of special design with approximately 300 $m^2/m^3$ of specific surface (Otálora 2011). This type of system was built in an oil extraction facility for domestic wastewater treatment planted with *Bidens laevis* (bur-marigold or smooth beggar-ticks), registering

**Fig. 25.5** Pilot-scale HSSF CW units. *Left*, *Phragmites australis*; *center*, unplanted; *right*, *Heliconia psittacorum*

overall removal efficiency for BOD, COD, and TSS higher than 80 %, in compliance with the Colombian environmental regulation for wastewater discharges.

A successful experience was acknowledged by WSP (2008) at the Universidad Técnica de Pereira (UTP), Pereira's technical university, where CWs were implemented at the existing wastewater treatment plant (La Florida) as part of a sanitation project to mitigate the negative environmental impacts on the Otún river basin.

La Florida system originally consisted of a pretreatment unit and a combination of a septic tank and anaerobic filter, treating wastewater arising from a nearby small community. But the efficiency of the system was low, possibly because groundwater infiltration of the sewerage system diluted the wastewater, resulting in low influent concentrations of several contaminants. The effluent of the system did not comply with the Colombian legislation, which calls for more than 80 % $BOD_5$ load removal. As a solution, several HSSF CWs were added as tertiary treatment units and operated in parallel to resolve the problem.

Different filter media and local plant types like *Typha* sp., *Juncus* sp., and *Renealmia alpinia* (Villegas et al. 2006) also were selected for the CWs, which then were operated under relatively high organic loading rates and short retention times (less than 1 day). Careful monitoring revealed that CWs with fine sand (0.3 mm) as filter media initially performed well in removing organic pollutants (50–70 %) and bacteria (up to 2 log units of fecal coliforms). However, they quickly clogged up, resulting in surface flow. The CWs with gravel as filter media had lower removal performance in terms of organic contamination, but did not present operational problems.

A member of the local community is in charge of the O&M of the system. The investigation revealed that the overall treatment system, including the constructed wetland units, was meeting the requirements of the Colombian legislation, and thus, the combination of septic tank, anaerobic filter, and CWs was, in principle, adequate for polishing of effluents from anaerobic treatment stages in tropical conditions, a condition that was also registered by Villegas et al. (2006).

A 157 $m^2$ HSSF CW system was constructed for \$14,000 in 2006 in Pasto ($L = 17.5$, $W = 9.0$ m), a municipality in southern Colombia for a 112 $m^3$/d flow. The CW comprises various pretreatment and primary treatment units, followed by a single relatively small constructed wetland designed for a population of 1,000. The treatment system is designed to receive the wastewater of about 1,000 inhabitants of a nearby community. The filter material is composed of fine gravel and organic soil. The local community is using the treated effluent for crop production. An NGO was in charge of implementing such system and also carried out important social work with the population. Activities included participatory analysis of priorities to achieve better health and environmental protection, consultation with community leaders, hygiene promotion, and environmental education WSP (2008).

More recently, Madera et al. (2015) assessed the removal efficiencies of organic matter, Cd(II), Hg(II), Cr(VI), and Pb(II) in four HSSF CW systems planted with polyculture of *Gynerium sagittatum* (*Gs*), *Colocasia esculenta* (*Ce*), and *Heliconia psittacorum* (*He*) treating landfill leachate at pilot scale, with good removal efficiencies for COD, DTOC, and $BOD_5$. The removal efficiencies were relatively good with higher performances for all parameters (>50 %). Regarding

Cr(VI), the concentrations found in the inflow were always higher than the Colombian standard for water reuse in agriculture (100 μg $L^{-1}$). The removal efficiency of this metal was similar in all HSSF CWs with values ranging between 50 and 80 %, and effluent from CWs 3 and 4 exhibited concentrations lower than the Colombian standard; meanwhile, the other two effluents were 20 % higher.

## References


Aprill W, Sims RC (1990). Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. 20(1-2):253–265

Arias CA, Brix H (2003) Humedales artificiales para el tratamiento de aguas residuales. Revista Ciencia e Ingeniería Neogranadina 13: 17–24. Red de Revistas de América Latina y el Caribe, España y Portugal

Arias SA, Betancur FM, Gómez G, Salazar JP, Hernández ML (2010) Fitorremediación con humedales artificiales para el tratamiento de aguas residuales porcinas. Informador Técnico (Colombia) 74:12–22

Ascúntar D, Toro AF, Peña MR, Madera CA (2009) Changes of flow patterns in a horizontal subsurface flow constructed wetland treating domestic wastewater in tropical regions. Ecol Eng 35:274–280

Aziz RA, Kamarudzaman AN, Kamaruddin NA, Salleh MN (2011) Study on phytoremediation performance and heavy metals uptake in leachate by reed beds plant. International conference on environmental, biomedical and biotechnology, Singapore. IPCBEE 16: 47–51

Calheiros CSC, Rangel A, Castro PML (2007) Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water Res 41:1790–1798

Campbell CS, Ogden MH (1999) Constructed wetlands in the sustainable landscape. Wiley, New York

Chai Huat K (2002) The Putrajaya wetland project. Angkasa Consulting Services Sdn Bhd (ACS), Malaysia

Chavan BL, Dhulap VP (2012) Optimization of pollutant concentration in sewage treatment using constructed wetland through phytoremediation. Int J Adv Res Eng Appl Sci 1(6):1–16

Chek Rani SH, Md Din MF, Mohd Yusof MB, Chelliapan S (2011) Overview of subsurface constructed wetlands application in tropical climates. Univ J Environ Res Technol 1(2):103–114

Cooper PF (1990) European design and operation guidelines for Reed Bed treatment systems. Report prepared for the European Community/European Water Pollution Control Association emergent hydrophyte treatment system expert contact group. WRc Report UI 17

Crites RW, Middlebrooks J, Reed SC (2006) Natural wastewater treatment systems. CRC, Boca Raton, FL

Dipu S, Anju A, Kumar V, Thanga SG (2010) Phytoremediation of dairy effluent by constructed wetland technology using wetland macrophytes. Global J Environ Res 4(2):90–100

DNR (2007) Constructed wetlands technology assessment and design guidance. Iowa Department of Natural Resources (DNR). Publication funded through a grant from the U.S. Environmental Protection Agency under the Federal Nonpoint Source Management Program. USA

Dushenkov S (2003) Trends in phytoremediation of radionuclides. Plant and Soil 249:167–175

Ellis JB, Shutes RBE, Revitt DM (2003) Guidance manual for constructed wetlands. Urban Pollution Research Centre. R&D technical report P2-159/TR2. Middlesex University, London

Ewemoje OE, Sangodoyin AY (2011) Developing a pilot scale horizontal sub surface flow constructed wetlands for phytoremediation of primary lagoon effluents. 11th edition of the World wide workshop for young environmental scientists (WWWYES-2011). Urban Waters: resource or risks? Arcueil, France

Ganjo DGA, Khwakaram AI (2010) Phytoremediation of wastewater using some of aquatic macrophytes as biological purifiers for irrigation purposes removal efficiency and heavy metals Fe, Mn, Zn and Cu. World Acad Sci Eng Tech 42: 2010

GTZ (2010) Constructed wetlands for grey water and domestic wastewater treatment in developing countries. Technology review "Constructed Wetlands". Sustainable sanitation and ecosan program of Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH

Kadlec RH, Knight RL (1996) Treatment wetlands. Lewis, London

Kadlec RH, Wallace SD (2008) Treatment wetlands, 2nd edn. CRC, Boca Raton, FL

Konnerup D, Koottatep T, Brix H (2009) Treatment of domestic wastewater in tropical subsurface flow constructed wetlands planted with Canna and Heliconia. Ecol Eng 35:248–257

Langergraber G, Giraldi D, Mena J, Meyer D, Pena M, Toscaco A, Brovelli A, Korkusuz EA (2009) Recent developments in numerical modelling of subsurface flow constructed wetlands. Sci Total Environ 407:3931–3943

Lee CG, Fletcher TD, Sun G (2009) Nitrogen removal in constructed wetland systems. Eng Life Sci 9(1):11–22

Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J Aquat Plant Manage 42:60–68

Lizama K, Fletcher TD, Sun G (2011) Review: removal processes for arsenic in constructed wetlands. Chemosphere 84:1032–1043

Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. In: Suresh B, Ravishankar GA (2004) Phytoremediation—a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24(2–3): 97–124

Madera-Parra CA, Peña-Salamanca EJ, Peña MR, Rousseau DPL, Lens PNL (2015). Phytoremediation of landfill leachate with *Colocasia esculenta*, *Gynerum sagittatum* and *Heliconia psittacorum* in constructed wetlands. Int J Phytoremediation 17:16–24

Marsili-Libelli S, Checchi N (2005) Identification of dynamic models for horizontal subsurface constructed wetlands. Ecol Model 187:201–218

Melbourne Water (2005) Constructed wetland systems design guidelines for developers version 3. Melbourne Water Corporation, Victorian Government, Australia

Melbourne Water (2010) Constructed wetlands guidelines. Healthy waterways. Melbourne Water Corporation, Victorian Government, Australia

Merkl N, Schultze-Kraft R, Infante C (2004) Phytoremediation in the tropics—the effect of crude oil on the growth of tropical plants. Bioremediation J 8(3–4):177–184

Merkl N, Schultze-Kraft R, Infante C (2005) Phytoremediation of petroleum-contaminated soils. Water Air Soil Pollut 165:195–209

Montoya JI, Ceballos L, Casas JC, Morató J (2010) Estudio comparativo de la remoción de materia orgánica en humedales construidos de flujo horizontal subsuperficial usando tres especies de macrófitas. Revista EIA 14: 75-84. Escuela de Ingeniería de Antioquia, Medellín (Colombia)

Mougin C (2002) Bioremediation and phytoremediation of industrial PAH-polluted soils. Polycycl Aromat Comp 22:1011–1043

NDWRCDP (2004) Constructed wetlands: a critical review of wetland treatment processes. National Decentralized Water Resources Capacity Development Project (NDWRCDP). Department of Civil and Environmental Engineering, University of Washington, USA

Olguin EJ, Sanchez-Galvan G (2010) Aquatic phytoremediation: novel insights in tropical and subtropical regions. Pure Appl Chem 82(1): 27–38

Otálora AP (2011) Evaluación del sistema de tratamiento de aguas residuales domésticas mediante humedales artificiales de alta tasa en la locación petrolera de caño Gandúl. MSc thesis. Departamento de Ingeniería Química. Facultad de ingeniería. Universidad Nacional de Colombia

Paquin D, Ogoshi R, Campbell S, Li QX (2002) Bench-scale phytoremediation of polycyclic aromatic hydrocarbon-contaminated marine sediment with tropical plants. Int J Phytoremediat 4(4):297–313

Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS (2005) Phytoremediation and hyperaccumulator plants. In: Tamas M, Martinoia E (eds) Molecular biology of metal homeostasis and detoxification, vol 14, Topics in current genetics. Springer, Berlin, pp 299–340

Prasad MNV (2003) La ecofisiología vegetal: una ciencia de síntesis; Capitulo 36 Fitorremediación. Aplicaciones, ventajas e inconvenientes. Paraninfo SA

Reed SC, Crites RW, Middlebrooks EJ (1995) Natural systems for waste management and treatment. McGraw-Hill, New York

Reeves RD (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. Plant and Soil 249:57–65

Robinson B, Fernández JE, Madejón P, Marañón T, Murillo JM, Green S, Clothier B (2003) Phytoextraction: an assessment of biogeochemical and economic viability. Plant and Soil 249:117–125

Rodríguez T, Ospina IM (2005) Constructed wetland of vertical flow to improve Bogotá river water quality. Ciencia e Ingeniería Neogranadina 15:74–84, Facultad de Ingeniería. Universidad Militar Nueva Granada, Bogotá D.C Colombia

Rousseau D, Hooijmans T (2010) Sistemas naturales (alternativas) para el tratamiento de aguas (residuales). Version 3. SWITCH – Taller de Capacitación. San José, Costa Rica

Shelef O, Gross A, Rachmilevitch S (2013) Role of plants in a constructed wetland: current and new perspectives. Water 5:405–419

Stottmeister U, Buddhawong S, Kuschk P, Wiessner A Mattusch J (2006) Constructed wetlands and their performance for treatment of water contaminated with arsenic and heavy metals. Constructed wetlands and their performance for treatment. Soil and water pollution monitoring, protection and remediation. Springer, pp 3–23

Sun WH, Lo JB, Robert FM, Ray C, Tang CS (2004) Phytoremediation of petroleum hydrocarbons in tropical coastal soils. I. Selection of promising woody plants. Environ Sci Pollut Res Int 11(4):260–266

Sundaravadivel M, Vigneswaran S (2009). Constructed wetlands for wastewater treatment. Wastewater recycle and reclamation, vol 1. UNESCO EOLSS – Encyclopedia of Life Support Systems

Suresh B, Ravishankar GA (2004) Phytoremediation—a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24(2–3):97–124

Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 2011: Article ID 939161

Tanner CC, Champion PD, Kloosterman V (2006) New Zealand constructed wetland planting guidelines. National Institute of Water and Atmospheric Research report. New Zealand Water and Wastes Association

Uera RB, Paz-Alberto AM, Sigua GC (2007) Phytoremediation potentials of selected tropical plants for ethidium bromide. Enviorn Sci Pollut Res 14(7):505–509

UN-HABITAT (2008) Constructed wetlands manual. UN-HABITAT water for Asian cities programme Nepal, Kathmandu. United Nations Human Settlements Programme. United Nations, Nairobi, Kenya

USEPA (2000) Constructed wetlands treatment of municipal wastewaters. U.S. EPA Office of Research and Development, Washington, DC

USEPA (2001) Phytoremediation of contaminated soil and ground water at hazardous waste sites, Ground water issue. Office of Solid Waste and Emergency Response, Washington, DC

USEPA (2005) Decision rationale total maximum daily loads watershed for acid mine drainage affected segments, Westmoreland county. United States Environmental Protection Agency, Pennsylvania, USA

UTP (2002) Humedales artificiales para el tratamiento de aguas residuales en el corregimiento de la Florida, Municipio de Pereira – Risaralda. Grupo de investigación en agua y saneamiento. Facultad de ciencias ambientales, Universidad Tecnológica de Pereira (UTP), Colombia

Villegas JD, Guerrero J, Castaño JM, Paredes D (2006) Septic tank (ST)-up flow anaerobic filter (UFAF)-subsurface flow constructed wetland (SSF-CW) systems aimed at wastewater treatment in small localities in Colombia. Rev Téc Ing Univ Zulia 29(3)

Vymazal J (2008) Constructed wetlands for wastewater treatment: a review. The 12th World Lake conference. Taal 2007, India

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549

WATERAID (2008) Decentralised wastewater management using constructed wetlands in Nepal. Environment and Public Health Organisation (ENPHO), WaterAid in Nepal Kupondole, Lalitpur, Nepal

Wetlands international (2003). The use of constructed wetlands for wastewater treatment. Wetlands International—Malaysia Office. Conservation and environmental grants by Ford Motors Company

WPCF (1990) Manual of practice, natural systems, wetlands chapter. February 1990, MOP FD-16 WPCF, USA

WSP (2008). Constructed wetlands: a promising wastewater treatment system for small localities. Experiences from Latin America. Water and Sanitation Program

Zhang BY, Zheng JS, Sharp RG (2010) Phytoremediation in engineered wetlands: mechanisms and applications. Procedia Environmental Sciences 2: 1315–1325. International Society for Environmental Information Sciences 2010 annual conference (ISEIS)

# Phytoremediation and Necrophytoremediation of Petrogenic Hydrocarbon-Contaminated Soils

26

Esmaeil Shahsavari, Eric M. Adetutu, and Andrew S. Ball

## 26.1 Introduction

As a consequence of increasing human demand for a range of petrogenic products including natural gas, diesel, gasoline, and asphalts, and the increase in activities associated with the exploration, and processing of petroleum, contamination of terrestrial and marine environments with petrogenic hydrocarbons is a relatively common occurrence. For example worldwide by 2005, on average approximately nine incidents involving the release of petroleum into the environment were reported every year (Stroud et al. 2007). The threat that these accidental or deliberate oil spills have on human and environmental health is illustrated by the fact that many common petrogenic products such as benzene, toluene, ethylbenzene, xylenes, and naphthalene are categorised as hazardous chemicals (Sarkar et al. 2005). There is therefore an urgent need to remediate hydrocarbon-contaminated sites worldwide. However, remediation of contaminated sites is costly; in the USA alone, over US $1 trillion was expected to be spent to clean up the environment; 90 % of these sites were contaminated by petrogenic hydrocarbons (Stroud et al. 2007).

A broad range of in situ and ex situ remediation methods including chemical, physical, and biological approaches have been widely used to remediate petrogenic hydrocarbon-contaminated site (Table 26.1). The use of biological methods to treat the contamination is becoming not only increasingly accepted but also preferred. This is because biological methods tend to be more environmentally friendly, cost-effective and unlike physical and chemical methods, biological methods are not very prone to secondary contamination (Table 26.1). In this chapter, these biological methods are examined in detail, with emphasis on two particularly promising techniques: phytoremediation and necrophytoremediation.

## 26.2 Petrogenic Hydrocarbons

Petrogenic hydrocarbons consist of various amounts of short, medium, and long chain aliphatic (i.e. alkanes, alkenes), aromatic (e.g. benzene, toluene, ethyl benzene, and xylene), and polycyclic aromatic hydrocarbons (known as PAHs; such as naphthalene, phenanthrene, and pyrene). Based on their general structure, however, hydrocarbons can be divided into two main groups: aliphatic and aromatic. Aliphatic hydrocarbons (e.g. alkanes) contain both saturated and unsaturated linear or branched open-chain structures (Table 26.2) (Stroud et al. 2007), while aromatic hydrocarbons contain one or more aromatic rings (e.g. benzene ring). In terms of the aromatic fraction, PAHs are important pollutants which contain two or more fused phenyl and/or pentacyclic rings (Table 26.2) (Haritash and Kaushik 2009).

Sixteen PAHs have been listed as priority pollutants by the US Environmental Protection Agency (US-EPA) (Table 26.3), (Mougin 2002). These recalcitrant chemicals are characterised by thermodynamic stability, very low aqueous solubility, an effective tendency to absorb to particle surfaces (e.g. soil particles) in the environment, and low sensitivity to volatilisation and photolysis (Mougin 2002).

As a result, PAHs are regarded as recalcitrant and hence the disposal rate of PAHs is higher than their rate of degradation in contaminated environments (Mougin 2002). In addition to their recalcitrance, PAHs also exhibit toxic, mutagenic, and carcinogenic properties, thereby representing a significant threat to the health of living organisms including humans (Bamforth and Singleton 2005; Lors et al. 2010; Samanta et al. 2002). Because of these properties, significant attention has been paid to the degradation of PAHs rather than aliphatic hydrocarbons in the past (Stroud et al. 2007). However, aliphatic hydrocarbons represent the major component of

E. Shahsavari, Ph.D. (✉) • E.M. Adetutu, Ph.D. • A.S. Ball, Ph.D.
School of Applied Sciences, RMIT University,
Bundoora, Melbourne, VIC 3001, Australia
e-mail: esmaeil.shahsavari@rmit.edu.au

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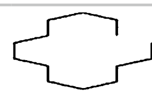
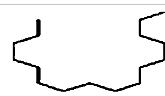
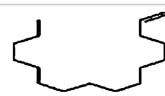
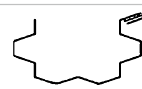
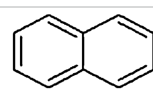
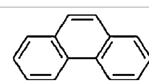
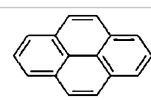
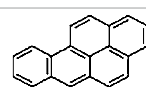
**Table 26.1** A summary of the methods used for the removal of hydrocarbons from contaminated environments

| Methods | Example of method | Advantages | Disadvantages |
|---|---|---|---|
| • Physical | Excavation | Fast<br>Removing contaminants permanently<br>Ideal for high levels of contamination | Expensive<br>Destructive<br>Prone to secondary contamination |
| • Chemical | Direct injection of chemical oxidants or surfactants into contaminated soil and groundwater | Fast<br>Not generating large volumes of waste material<br>Ideal for high level of contamination | Expensive<br>Destructive<br>Prone to second contamination |
| • Biological (bioremediation) | Using microbiological processes (living organisms such as bacteria, fungi, and plant) | Environmental friendly<br>Cost-effective<br>Minimum site disruption<br>Useful for low level of contaminants | Requires longer time<br>Low predictability<br>Dependent on climatic factors |

**Table 26.2** Physicochemical properties of selected aliphatic and aromatic hydrocarbons

| Type | Group | Name | Formula | Structure | Solubility (mg $L^{-1}$) | Melt point (°C) | Boil point (°C) | Log $k_{ow}$ |
|---|---|---|---|---|---|---|---|---|
| Aliphatic | Alkane | Tetradecane | $C_{14}H_{30}$ |  | 0.000282 | 5.5 | 253 | 7.2 |
| | Model Alkane | Hexadecane | $C_{16}H_{34}$ |  | 0.0009 | 18 | 287 | 9.1 |
| | Alkene | Hexadecene | $C_{16}H_{32}$ |  | N/A | 3–5 | 274 | NA |
| | Alkyne | Hexadecyne | $C_{16}H_{30}$ |  | N/A | 15 | 148 | NA |
| Aromatic | PAH | Naphthalene | $C_{10}H_8$ |  | 30 | 79–83 | 217.9 | 3.36 |
| | Model PAH | Phenanthrene | $C_{14}H_{10}$ |  | 1.1 | 97–101 | 340 | 4.16 |
| | PAH | Pyrene | $C_{16}H_{10}$ |  | 0.135 | 156 | 404 | 5.19 |
| | PAH | Benzo[a]Pyrene | $C_{20}H_{12}$ |  | 0.0038 | 175–179 | 495 | 6.06 |

Stroud et al. 2007. Used with permission from John Wiley and Sons

crude oil and petrogenic products. The physicochemical properties of mid-length aliphatic hydrocarbons (Table 26.2) showed that they are non-polar and water insoluble. For example, hexadecane (i.e. model alkane) has a water solubility of 0.0009 mg $L^{-1}$ and it exists as a liquid at room temperature (Table 26.2). Consequently, they are not easily volatilised or leached from soil and tend to be adsorbed to soil particles and organic matter. In addition, the physiochemical properties of aliphatic hydrocarbons may result in these types of hydrocarbons, in some instances being more persistent than PAHs in the soil (Table 26.2). For example, the hydrophobicity of hexadecane is much higher than phenanthrene (by three orders of magnitude); hexadecane also has a higher octanol–water partition coefficient ($\log_{ow} = 9.1$) than phenanthrene ($\log_{ow} = 4.16$) (Table 26.2). This hydrophobicity plays an important role in hydrocarbon behaviour in soil, affecting sequestration and both chemical and biological availability (Stroud et al. 2007). The presence of long length aliphatic hydrocarbons results in the production of oil films and slicks which limit nutrient and oxygen exchange in the soil (Wasmund et al. 2009), resulting in a significant decline in soil structure and important changes in microbial population (Militon et al. 2010).

In addition, like other hydrocarbons, aliphatic hydrocarbon pollution of local groundwater can lead to immense economic loss, and ecological disaster, while also disrupting agricultural or aquaculture production (Tang et al. 2010). In the recent British Petroleum Deepwater Horizon oil spill in the Gulf of Mexico, 26.5 million litres of petroleum went into the surrounding environment (Simons et al. 2012); this led not only to an ecological disaster affecting marine animal and bird species in the Gulf of Mexico but also to significant damage to the tourism industry as well as the fishing industry (Bozeman 2011). In addition, as a consequence of the negative aspects of aliphatic hydrocarbons, the remediation of these types of hydrocarbons has also been widely studied in recent years (Adetutu et al. 2012; Gaskin et al. 2008; Gaskin and Bentham 2010; Militon et al. 2010; Shahsavari et al. 2013b).

**Table 26.3** List of 16 PAH priority pollutants defined by US-EPA

| Two ring | Three ring | Four ring |
|---|---|---|
| Naphthalene | Fluoranthene | Chrysene |
| Fluorene | Phenanthrene | Pyrene |
| Acenaphthene | Anthracene | Benzo[a]anthracene |
| Acenaphthylene | | Benzo[b]fluoranthene |
| | | Benzo[k]fluoranthene |
| Five ring | | Six ring |
| Benzo[a]pyrene | | Benzo[g,h,i]perylene |
| Indeno[1,2,3-c,d] pyrene | | |
| Dibenzo[a,h] anthracene | | |

Perelo 2010. Used with permission of Elsevier

## 26.3 Bioremediation of Hydrocarbon-Contaminated Soils

Bioremediation is defined as the use of living organisms (especially microorganisms) to remove or breakdown contaminants present in the environment (Iwamoto and Nasu 2001; Sarkar et al. 2005; Wenzel 2009). The main advantages of bioremediation are its cost-effectiveness and non-invasive approach (green technology) which keeps the ecosystem intact (Table 26.1) (Alcalde et al. 2006; Perelo 2010). Bioremediation can be useful in contaminated environments where cleanup by physical or chemical methods cannot be used because of the low level of contaminations (Perelo 2010). However, bioremediation has a number of limitations (Table 26.1). The biodegradation processes occurring during bioremediation are affected by a number of factors including hydrocarbon physicochemistry, environmental conditions, bioavailability, and the presence of hydrocarbon-utilising microorganisms (Stroud et al. 2007). These factors and their effects on the bioremediation of petrogenic hydrocarbons are summarised in Table 26.4.

All of these factors (Table 26.4) need to be considered before selecting and applying any bioremediation method. In addition, each contaminated site is different and therefore specific remediation action plans must be developed for each site; however, there are four generic technologies which are outlined in Table 26.5 and discussed in more detail below.

### 26.3.1 Natural Attenuation Strategy

Natural attenuation is the simplest bioremediation method; the only requirement is to monitor the natural degradation process. This approach can be applied in specific circumstances; for example, it can be used for remote areas or when levels of contamination are relatively low (Pilon-Smits 2005). It is estimated that approximately 25 % of all petroleum-contaminated land has been remediated using natural attenuation (Stroud et al. 2007). Very recently, Aleer

**Table 26.4** Important factors and their impact on the degradation of petrogenic hydrocarbons in the contaminated environments

| Factors | Impact |
|---|---|
| • Hydrocarbon physicochemistry | Affects the bioavailability of contaminants (complex structure and less soluble = less hydrocarbon degradation) |
| • Environmental conditions (e.g. nutrient, oxygen, pH, and temperature) | Affect microbial activity (optimal environmental conditions = higher hydrocarbon-utilising microbial activity) |
| • Bioavailability | Determines the rate of degradation (less bioavailability = less hydrocarbon degradation) |
| • Presence of hydrocarbon-utilising microorganisms | Determines the rate of degradation (higher hydrocarbon-utilising microbial activity = higher hydrocarbon degradation) |

**Table 26.5** Bioremediation technologies used for hydrocarbon-contaminated environments

| Technology | Key point | Advantages | Disadvantages |
|---|---|---|---|
| • Natural attenuation | Using indigenous microorganisms and natural condition | Cheapest technology | Requires extensive long-term monitoring |
| | | | Not always successful |
| • Bioaugmentation | Addition of hydrocarbon-degrading microorganisms | Using high biomass of hydrocarbonoclastic microorganisms | Changes the natural microbial structure |
| | | | Poor adaptation of hydrocarbonoclastic microorganisms to the contaminated site |
| • Biostimulation | Addition of nutrient | More efficient than natural attenuation | Not always successful |
| • Phytoremediation | Using plants and their associated microorganisms | Supports hydrocarbonoclastic microorganisms within plant root | Toxicity of contaminants to the plant |

et al. (2011) and Makadia et al. (2011) reported on the use of previously bioremediated soil (reused soil) and compared its efficacy to biostimulation and bioaugmentation methods. They found that the results from the natural attenuation using previously bioremediated soil were similar to other bioremediation methods. The authors concluded that natural attenuation with reused soil represents a promising strategy for the bioremediation of petrogenic hydrocarbons. In this respect, Erkelens et al. (2012) also reported that previously bioremediated hydrocarbon-contaminated soil led to a 70 % increase in the remediation of TNT compared with the control.

## 26.4 Bioaugmentation Strategy

If natural attenuation is unsuitable as a remediation technology, perhaps due to low bioremediation potential, another technology will be required. One of these alternate methods is termed bioaugmentation: in this case the addition of hydrocarbon degraders (mostly bacteria and to a lesser extent fungi) which are generally isolated or enriched in the laboratory from samples taken from contaminated sites (Perelo 2010; Sarkar et al. 2005). Although the application of bioaugmentation to environments contaminated with petrogenic hydrocarbons has been extensively studied in both marine and terrestrial systems (Kadali et al. 2012; Li et al. 2012; Makadia et al. 2011; Sheppard et al. 2011; Simons et al. 2012; Tang et al. 2010), there exists potential question or concern relating to the introduction of exogenous organisms and the potential negative impacts of this introduction on the diversity and functionality of the natural ecosystem (Iwamoto and Nasu 2001).

### 26.4.1 Biostimulation Strategy

Biostimulation, the addition of nutrients to promote the activity of indigenous microorganisms (Sarkar et al. 2005), represents another widely applied bioremediation technology for the degradation of petrogenic hydrocarbons in contaminated soils. A broad range of organic and inorganic substances such as bulking agents (e.g. straw), nutrients, manure, sewage sludge (fresh and composted), and surfactants (e.g. Tween 80) have been used as biostimulators during the bioremediation of hydrocarbons (Adetutu et al. 2012; Liu et al. 2010; Ros et al. 2010; Shahsavari et al. 2013b).

It is important to note that the application of biostimulation as a remediation technology has resulted in a range of diverse outcomes. For example, Wellman et al. (2001) indicated that the degradation of diesel/motor oil (5,000 mg $kg^{-1}$) in a loamy soil amended with 20 % manure was greater (81 % degradation) than in contaminated soil amended with ammonium sulphate (54 % degradation) and unamended contaminated soil (32 % degradation). Liu et al. (2010) reported that the degradation of hydrocarbons reached 56 % in soil amended with manure (5 % v/v) compared with only 15.6 % in the plot control. In contrast, some researchers have reported no positive impact in terms of the degradation of pollutants as a result of the application of bioremediation technology. Palmroth et al. (2002) reported that a variety of soil amendments, including NPK fertiliser, a compost extract, and a microbial enrichment culture, did not significantly enhance the rate of degradation of diesel fuel. Also Schaefer and Juliane (2007) showed that the addition of coffee grains or horticultural waste to a soil contaminated with TPH (Total Petroleum Hydrocarbon) resulted in no significant increase in the rate of degradation compared to the control (unamended) soil. They concluded that hydrocarbon-utilising microorganisms preferred to use the more readily available amendments rather than crude oil (Schaefer and Juliane 2007). In some instances, biostimulation through the addition of surfactants has been investigated (Adetutu et al. 2012; Hultgren et al. 2009). Adetutu et al. (2012) showed that the presence of Tween 80 (1 % w/w) in weathered hydrocarbon-contaminated soil resulted in an increase in $^{14}C$-hexadecane mineralisation from 1.2 % in unamended control and 8.5 % in amended soil with nitrogen and phosphorus to 28.9 % after 98 days of incubation. The addition of surfactant may,

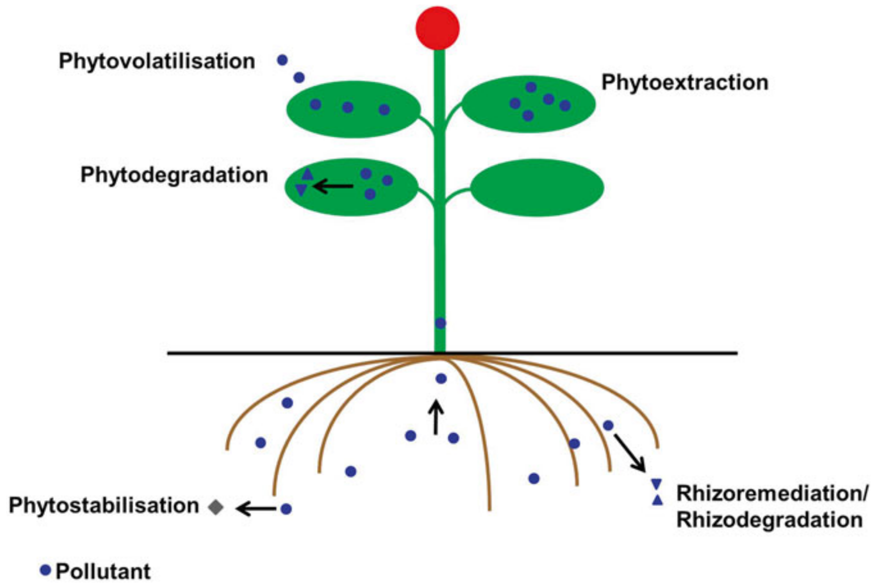



**Fig. 26.1** A schematic figure showing the different mechanisms involved in the phytoremediation of organic and inorganic pollutants (adapted from Pilon-Smits 2005)

however, lead to reduced rates of contaminant biodegradation as surfactant may separate the microbes from the contaminant–water interface, resulting in the preferential consumption of an alternative more readily degradable substrate rather than hydrocarbons. In addition, the surfactant may have a toxicological impact on the hydrocarbon-degrading microflora (Perelo 2010). As a consequence of these variable outcomes, it is clear that in order to achieve a reliable and safe in situ bioremediation it is necessary to fully characterise the contaminated site, environmental conditions, and the natural microbial community (Ros et al. 2010).

It is important to note that depending on the conditions, bioremediation strategies may be used in combination (e.g. phytoremediation and biostimulation). For example, Yousaf et al. (2010) reported that addition of 10 % compost enhanced plant tolerance towards crude oil in alfalfa and Bird's-foot trefoil plants; in alfalfa, the amount of plant biomass in clean and contaminated soils amended with compost was 1.77 and 2.07 g, respectively, compared to clean soil without compost with an average of 0.94 g after 3 months.

### 26.4.2 Phytoremediation Strategy

Phytoremediation is defined as the use of plants and their associated microbes for environmental cleanup from organic or inorganic contaminants (Salt et al. 1995). Plants and their rhizosphere microflora deal with pollutants through a range of mechanisms including phytodegradation, phytovolatilisation, phytodegradation, phytoextraction, phytostabilisation, and rhizodegradation (Fig. 26.1) (Pilon-Smits 2005).

Phytodegradation refers to the use of degradative enzymes produced by plant tissue to degrade organic pollutants (Fig. 26.1). Phytodegradation is useful for organics which are able to move within the plants. Among these compounds are herbicides and TNT (Pilon-Smits 2005). In phytovolatilisation, plant tissue takes up certain pollutants, then releases them in a volatile form into the air (Fig. 26.1). Phytovolatilisation can be used for the removal from soil of volatile organic compounds such as TCE (trichloroethylene) and MTBE (methyl tertiary butyl ether) and also for Se and Hg, metals that can exist in a volatile form (Fig. 26.1) (Pilon-Smits 2005). Phytoextraction is defined as the use of plants to take up the contaminants (especially metals) from the soil and accumulate them in harvestable plant tissues (Macek et al. 2000). After harvesting, the plant material can subsequently be used for wood, cardboard, and ash or if the metal is highly valuable, recycling of the accumulated element can be carried out; this is termed phytomining (Pilon-Smits 2005). Phytostabilisation refers to the elimination or reduction of the bioavailability of pollutants (e.g. metals) by plant roots in the environment, resulting in the prevention of erosion, leaching, or runoff pollutants (Fig. 26.1) (Pilon-Smits 2005). Rhizoremediation or rhizodegradation (phytostimulation) is defined as the use of microorganisms in the plant rhizosphere to remediate organic pollutants as a result of interactions between plants and microbes (Fig. 26.1). It is used for the remediation of soils contaminated with hydrophobic organics that cannot be taken up by plants but which can be degraded by microbes; it is believed that rhizodegradation is a main route for the degradation of petrogenic by-products (Hall et al. 2011).

## 26.5 Effect of Microflora in the Rhizosphere of Plants on the Degradation of Petrogenic Hydrocarbons

In regard to rhizoremediation, the degradation is mediated by the microbial biomass and associated activity (Pilon-Smits 2005). It is known that the plants and their associated rhizosphere microflora often show a mutualistic relationship with each other. While hydrocarbon-utilising bacteria are supported by plant roots through the release of nutrients and oxygen into the soil (Macek et al. 2000; Yousaf et al. 2010), in the same way, these bacteria may help plants to decrease the phytotoxicity of contaminants to an acceptable level which allows plants growth under adverse soil conditions (Germida et al. 2002; Yousaf et al. 2010). This mutualistic relationship is responsible for the increased degradation of contaminants in soils (Macek et al. 2000).

The rhizosphere is classically defined as the area around the root and it includes approximately 1 mm around the root (Pilon-Smits 2005); this area is under the immediate influence of the plant root. Plant roots release a number of exudates such as sugars, amino acids, organic acids, vitamins, tannins, alkaloids, sterols, enzymes, and growth factors which can be used as a source of nutrients by the microflora in the rhizosphere. It is known that 20 % of photosynthesis-derived organic compounds are released into the soil through the plant root system (Pilon-Smits 2005). As a result, the microbial populations are 5- to 100-fold higher in the rhizosphere than in the bulk soil (Pilon-Smits 2005). This enhancement in the microbial population and associated activities in the rhizosphere is defined as the 'rhizosphere effect' (Germida et al. 2002). Extensive plant root systems bring this large microbial population (including hydrocarbon degraders) as well as nutrients in contact with the contaminant (Germida et al. 2002). Petrogenic hydrocarbons exhibit strong hydrophobicity and tend to tightly adsorb to organic matter and become potentially unavailable for biodegradation. However, plant roots penetration into soil micropores results in exposure of the contaminant to increased microbial activity (Hutchinson et al. 2004).

Plant roots also can enhance the degradation rate of petrogenic hydrocarbons as a result of increasing of bioavailability of the contaminants by producing biosurfactants (i.e. a surfactant is synthetised by living organisms). Zhou et al. (2011) showed that solubilisation of polycyclic aromatic hydrocarbons by saponin (a plant-derived non-ionic biosurfactant) was enhanced compared to selected, synthetic non-ionic surfactants (e.g. Tween-20); the molar solubilisation ratio of saponin for phenanthrene showed a three- to sixfold increase when compared with synthetic non-ionic surfactants.

Plant roots also release hydrocarbon analogues as part of plant exudates (e.g. phenolic compounds that can play a role as PAH analogues) which help to stimulate the growth of hydrocarbon-utilising microorganisms as they are used as primary substrates for the degradation of petroleum hydrocarbons (Germida et al. 2002; Wenzel 2009). This is called cometabolic activity and defined as a process by which a compound that cannot be consumed for the growth and development of microorganisms can be modified or degraded when another growth-supporting substrate is present (Germida et al. 2002). Another activity of plants which may play a role in enhancing the rates of contaminant degradation is the release of degradative enzymes such as dehalogenases, nitroreductases, peroxidases, laccases, and nitrilases in root zones (Wenzel 2009). These enzymes can degrade specific contaminants; Boyajian and Carreira (1997) concluded that plant nitroreductases and laccase enzymes contributed to the degradation of nitroaromatic contaminants (e.g. trinitrotoluene) in soil. In terms of hydrocarbon degradation, fungal peroxidases and laccases are known to be capable of degrading PAHs in contaminated soils (Haritash and Kaushik 2009; Mougin 2002). In the same way, these enzymes, released by plant roots into soil, may also contribute to PAH degradation. However, our knowledge about the contribution of plant enzymes to the degradation of petrogenic hydrocarbons is limited.

In addition to these potential benefits, plant roots also improve the structure and aeration of contaminated soils by penetrating into soil micropores, reducing soil compaction and producing channels for air and water (Hutchinson et al. 2004). Roots not only transfer oxygen from above ground into the root zone but also oxygen may diffuse through old root channels, close by existing roots, leading to more extensive diffusion of oxygen in to the soils (Issoufi et al. 2006).

As well as these positive effects associated with the use of plants in remediation petrogenic hydrocarbons, there are a number of potential disadvantages. The presence of petrogenic hydrocarbons may have a direct, adverse impact on the growth and development of plants, including those that prevent or delay seed germination, destroy photosynthetic pigments, decrease the length of the roots and shoots, and alter the plant root architecture (Peng et al. 2009). As a result of toxicity, phytoremediation of contaminants including hydrocarbons will often only be feasible when the soil is pretreated to reduce phytotoxicity or a resistant plant species is selected (Frick et al. 1999). Not all plants therefore have the potential for use in the degradation of petrogenic hydrocarbons. In fact the number of plants reported in the literature to be capable of phytoremediation is limited (Table 26.6). Among the plants, grasses (e.g. Italian ryegrass) and legumes (e.g. alfalfa) are suitable candidates for the rhizoremediation of petrogenic hydrocarbons. While grasses have an intensive and fibrous root system, legumes can fix the nitrogen as nitrogen is an

**Table 26.6** Plant species that have been identified as potential agents for the phytoremediation of petrogenic hydrocarbons

| Plant name | Plant name |
|---|---|
| Alfalfa (*Medicago sativa*) | Meyer zoysiagrass (*Zoysia japonica*) |
| Arctared red fescue (*Festuca rubra*) | 4 O'clock (*Mirabilis jalapa*) |
| Bell rhodesgrass (*Chloris gayana*) | Poplar trees (*Populus deltoides x nigra*) |
| Bermuda grass (*Cynodon dactylon*) | Prairie buffalograss (*Buchloe dactyloides*) |
| Big bluestem (*Andropogon gerardi*) | Perennial ryegrass (*Lolium perenne*) |
| Bird's-foot trefoil (*Lotus corniculatus*) | Side oats grama (*Bouteloua curtipendula*) |
| Blue grama (*Bouteloua gracilis*) | Sorghum (*Sorghum bicolor*) |
| Bush bean (*Phaseolus vulgaris*) | Soybean (*Glycine max*) |
| Canada wild-rye (*Elymus canadensis*) | Sudangrass (*Sorghum vulgare*) |
| Carrot (*Daucus carota*) | Switchgrass (*Panicum virgatum*) |
| Common buffalograss (*Buchloe dactyloides*) | Tall fescue (*Festuca arundinacea*) |
| Duckweed (*Lemna gibba*) | Verde kleingrass (*Panicum coloratum*) |
| Indiangrass (*Sorghastrum nutans*) | Weeping grass (*Microlaena stipoides*) |
| Italian ryegrass (*Lolium multiflorum*) | Western wheatgrass (*Agropyron smithii*) |
| Lemon-scented grass (*Cymbopogon ambiguus*) | Willow (*Salix Viminalis*) |
| Little bluestem (*Schizachyrium scoparius*) | Winter rye (*Secale cereale*) |
| Maize (*Zea mays*) | Wheat (*Triticum aestivum*) |

Banks et al. 2003; Chen and Banks 2004; Frick et al. 1999; Gaskin and Bentham 2010; Hultgren et al. 2009; Peng et al. 2009; Shahsavari et al. 2013c; Yousaf et al. 2010

important substance in the mineralisation of hydrocarbons in contaminated soils (Adam and Duncan 2002).

Screening plants for tolerance against petrogenic hydrocarbons in soils represents the first and basic prerequisite step in any rhizoremediation project (Gaskin et al. 2008). The strategies used for screening plants can be as follows:

One strategy could be screening new plants which have not previously been evaluated for their ability to grow in hydrocarbon-contaminated soils and subsequently using the resistant plants in bioremediation projects. The second strategy could be the use of plants which have previously been identified as suitable for bioremediation and have been recommended in the literature (Table 26.6). In considering the potential application of phytoremediation technology, conditions such as soil moisture, soil pH, oxygen availability, and temperature are required to be studied; these conditions can be unique to a specific site or area. Screening tests are therefore required to fully assess the potential for phytoremediation in the specific case. The third strategy is the use of a combination of new (unevaluated) plants together with previously identified plants in a preliminary screening project.

In addition to assessing the potential for phytoremediation using a range of different hydrocarbons, researchers have used both single (Chen and Banks 2004; Kim et al. 2006; Kirk et al. 2005; Peng et al. 2009) and mixed (Cheema et al. 2010; Gaskin and Bentham 2010; Phillips et al. 2006; Wei and Pan 2010) plant species.

The effectiveness of single vs. mixed species on the degradation of petrogenic hydrocarbons is still open to question. For example, Gaskin and Bentham (2010) found that mixed planting with two Australian native grasses not only had no additional effect on TPH reduction but also led to a reduction in the hydrocarbon degradation process when compared to the remediation of hydrocarbons using single species. In contrast, Cheema et al. (2010) observed that degradation rates of PAHs were higher when mixed plant species were used (98.3–99.2 % degradation for phenanthrene and 88.1–95.7 % for pyrene) relative to single plants (90–98 % degradation for phenanthrene and 79.8–86 % for pyrene). However, in order to preserve site biodiversity in the natural contaminated environments (e.g. mine sites), using mixed plant species might be desirable (Gaskin and Bentham 2010).

Banks et al. (2003) evaluated four genotypes of sorghum in crude oil-contaminated soil at three stages of plant growth including five leaf, flowering, and maturity. They showed that the degradation of TPH varied among different treatments and plant stages. Overall, the results of this experiment revealed that the levels of TPH reduced on average by 69 % in soils amended with sorghum species while the reduction was only 35 % in unplanted controls.

Kim et al. (2006) used tall fescue plants for the remediation of PAH-contaminated soil. The results showed that the presence of tall fescue enhanced the degradation rate of PAHs relative to control by 36 %. Also, the authors observed that the reduction rate of <4-ring PAHs, 4-ring PAHs, and >4-ring PAHs of plant treated soil was higher with an average of 78, 68, and 61 % at the end of the study compared with rates in the unplanted control (70, 54, and 49 %, respectively).

Peng et al. (2009) evaluated the effect of an ornamental plant (*Mirabilis jalapa*) on the degradation of weathered petrogenic hydrocarbons (up to 2 % hydrocarbons) in a 127-day greenhouse experiment. Their results showed that the TPH reduction rate in planted treatments (average of 41.61–63.20 %) was significantly higher than that found in the corresponding controls (19.75–37.92 %). The results also indicated that the maximum reduction rate was observed for the saturated hydrocarbon fraction compared with other components of petrogenic contaminants. Gaskin and Bentham (2010) conducted an experiment to evaluate the potential of Australian native grasses in the rhizodegradation of 1 % (w/w) aliphatic hydrocarbons (60:40 diesel/oil). They reported that TPH reduction in the presence of grasses varied between species; TPH levels in planted treatments were lower relative to the unplanted control treatment for all species after 100 days. Their findings demonstrated that lemon-scented grass (*Cymbopogon ambiguus*) not only had the greatest TPH reduction rate (88 %) but also exhibited the fastest TPH reduction rate among grasses (about 95 % after 2 weeks).

However, the phytoremediation of hydrocarbon-contaminated soils is not always successful. For example, Ferro et al. (1994) reported that the presence of wheatgrass did not enhance the mineralisation rate of $^{14}$C-phenanthrene relative to a control (unplanted) soil. Zhang et al. (2012) also tested a wetland plant (*Juncus subsecundus*) for its ability to phytoremediate a soil contaminated with cadmium and PAHs (phenanthrene and pyrene). They found that the dissipation of PAHs from soils was not significantly affected after 70 days of plant growth. Interestingly, the authors also reported that the reduction rate of pyrene was significantly reduced in the rhizosphere when compared to the unplanted control soil (43 % for planted soil and 63 % unplanted soil) while the reduction rate for phenanthrene was 97 % for both soils. An explanation for this phenomenon could be that hydrophobic compounds (e.g. pyrene) are firstly accumulated in the rhizosphere, before being dissipated with time by the rhizodegradation process (Liste and Alexander 2000; Zhang et al. 2012).

## 26.6 Necrophytoremediation Vs. Phytoremediation

The toxicity related to hydrocarbon by-products towards plants as well as low organic matter content, poor structure, nutrient deficiency, and water stress, conditions which tend to be associated with many contaminated soils, represent important limitations to the application of phytoremediation (Wenzel 2009). One technology which possibly overcomes these issues is necrophytoremediation. Necrophytoremediation is defined as the use of dead plant biomass (e.g. hay and straw) for the remediation of contaminated soils.

Necrophytoremediation may have a number of advantages over the application of phytoremediation (Table 26.7). For example, necrophytoremediation is toxic independent and can be applied to any level of contamination. In necrophytoremediation, there is no need to consider the length of the growing season, rainfall, and temperature patterns. In addition, hydrocarbon-contaminated soil is frequently co-contaminated with high concentrations of soluble salts and other metal toxicities which may limit the use of phytoremediation (Hutchinson et al. 2004); in contrast, using necrophytoremediation may help to not only degrade the hydrocarbon but also enhance the desalination of contaminated soils (Zhang et al. 2008). The authors used wheat straw in combination with *Enterobacter cloacae* and *Cunninghamella echinulata* (hydrocarbonoclastic microorganisms) to remediate a petroleum- and salt-contaminated soil.

Their results from a field study showed that the concentration of Na and Cl ions in remediated soil decreased from 1,597 and 1,520 to 543 and 421 mg L$^{-1}$, respectively, in the top 25 cm of top soil. In addition, the amended treatment led to a decrease in TPH from 6,320 to 2,260 mg L$^{-1}$ after 45 days. There are only a few reports of research comparing the efficacy, in terms of hydrocarbon degradation of phytoremediation and necrophytoremediation. Kabay (2010) showed that a layer of sorghum straw enhanced the degradation of a broad range of PAHs (e.g. 2–4 rings PAHs) while the presence of the sorghum plant itself did not enhance the degradation rate relative to the control. The authors reported that the addition of the straw led to an increase in the naphthalene dioxygenase-bacterial population which led to an increased biodegradation rate of PAHs. In contrast, Hultgren et al. (2009) showed that the presence of willow plants increased PAHs significantly while the addition of wheat straw did not affect the degradation of PAHs.

## 26.7 Effect of Necrophytoremediation on the Remediation of Petrogenic Hydrocarbons

A variety of plant residues such as hay, shaved wood, and straw (e.g. pea straw) have been used in a number of hydrocarbon bioremediation studies (Adetutu et al. 2012; Hultgren et al. 2009; Lors et al. 2012; Morgan et al. 1993; Phillips et al. 2006; Rhykerd et al. 1999; Shahsavari et al. 2013b); however, the outcomes yielded mixed results. In some instances, the presence of plant residues accelerated the bioremediation rate of hydrocarbons in contaminated soils;

**Table 26.7** A comparison between phytoremediation and necrophytoremediation technologies

| Condition | Phytoremediation | Necrophytoremediation |
|---|---|---|
| Toxicity | Dependent | Independent |
| Climate | Dependent | Less dependent |
| Soil conditions (e.g. pH, aeration, and structure) | Need to be considered | Do not require consideration |
| Soil salinity | Limits to use | Does not limit application |
| Hydrocarbons levels | Limited to hydrocarbon levels (high levels kill plants) | Not limited to hydrocarbon levels |
| Plant husbandry | Required | Not required |
| Screening stage | Required | Not required |
| Usage in biopile | Cannot be used | Can be used |
| Price | – | Cheaper than phytoremediation as plant residues are also waste |

however, the mechanisms which bring about this are not well understood. Dead plant biomass consists largely of lignin, cellulose, and hemicellulose (Trigo and Ball 1994) and during the decay process, a substantial amount of degradation products from these biopolymers are released into the soil as either short chain sugars or small aromatic compounds. These degradation intermediates can be further degraded and can be used by microflora (e.g. hydrocarbon-utilising microorganisms) as nutrients available for growth and activity. In addition, saprophytic fungi (such as white rot fungi) are commonly associated with decaying lignocellulosic material where they play a vital role in the degradation of lignin; this is mediated through enzymes such as lignin peroxidase, manganese peroxidase, and laccase (Dinis et al. 2009; Hatakka 1994). It is well known that these enzymes also degrade PAHs (Haritash and Kaushik 2009; Mougin 2002). It is important to note that ligninolytic enzymes from fungi may play an important role in the degradation of highly recalcitrant PAHs (with five or more aromatic rings); bacteria are often unable to degrade these PAHs due to low bioavailability (Baldrian 2008; Field et al. 1992). Therefore, the addition of plant residues into the soil may accelerate the degradation of highly recalcitrant PAHs.

Plant residues also have their own associated microflora and when added to a soil lead to an increase in both the population and the activity of the soil microflora. This results in enhancement of the potential of microbes to remediate petrogenic hydrocarbons.

Aerobic conditions are an important factor in terms of maximising the degradation of petrogenic hydrocarbons in contaminated soils (Rhykerd et al. 1999). The oxygen concentration in soil is affected by microbial activity, soil structure, water content, and depth. It is known that the bioremediation of hydrocarbons in soils is significantly reduced when the amount of oxygen in soils is low (Rhykerd et al. 1999). Plant residues have low density and as a consequence when mixed with soils result in a reduced soil bulk density. Therefore, increased porosity, oxygen diffusion may result, which together with the formation of more water stable aggregates may stimulate microbial activity and possibly enhance the degradation of hydrocarbons (Rhykerd et al. 1999).

There are a number of published examples of the application of necrophytoremediation. Morgan et al. (1993) reported that the amendment of contaminated soil with wheat straw, hay, wood chips, and pine bark together with the inoculation of white rot fungi enhanced the remediation of benzo(a)pyrene; wheat straw showed the greatest contribution to the mineralisation. They concluded that successful inoculation and biodegradation of xenobiotics needs supplementary carbon sources.

Rhykerd et al. (1999) evaluated tillage, aeration, and the addition of bulking agents, including chopped Bermuda grass hay, sawdust, and vermiculite on the bioremediation of hydrocarbon (10 % w/w) contaminated soil. The results showed that amended soils had a more rapid reduction in TPH compared to the unamended control. The findings also indicated that the degradation rate of TPH was highest in the tillage-hay and tillage-vermiculite treatments (90 % degradation) when compared with unamended static treatment (77 % degradation) after 30 weeks.

Wu et al. (2011) evaluated the bioaugmentation of petroleum-contaminated soil using *Enterobacter cloacae* alone or in the presence of wheat straw (5 % w/w). The authors reported that the addition of wheat straw to a bioaugmentation microcosm resulted in a 56 % reduction in TPH compared with a 25 and 44 % reduction in TPH in soils which were unamended or only bioaugmented, respectively, after 56 days of incubation. It was concluded that the addition of wheat straw increased the proliferation of *E. cloacae* and produced a significantly enriched community.

Shahsavari et al. (2013b) recently investigated the effect of four types of plant residues including alfalfa hay, pea straw, wheat straw, and a combination of plant residues (containing 20 % hay, 37.5 % pea straw, 37.5 % wheat straw, and 5 % gypsum) on the bioremediation of an aliphatic hydrocarbon-contaminated soil. They mixed or covered the soil with dead plant biomass. The results of this necrophytoremediation study showed that all treatments amended with plant residues enhanced the TPH degradation significantly relative to control soil; the soil mixed with pea straw exhibited the highest effect and led to reduction in TPH of 83 % relative to the control (53 % reduction). The result also showed that the presence of plant residues led to an increase of hydrocarbon-utilising microorganisms relative to the control; a 12-fold increase in the hydrocarbonoclastic microbial population was observed when pea straw was mixed with contaminated soil.

In another report by (Shahsavari et al. 2013a), the effect of necrophytoremediation using pea and wheat straws on the remediation of phenanthrene and pyrene alone or in combination was investigated. The results showed that the presence of straw accelerated PAH degradation relative to their corresponding control. For example, pyrene-contaminated soil mixed with pea straw led to a 70 % pyrene reduction while the reduction in corresponding control was only 15 %. Again, the authors reported that the number of hydrocarbon-utilising microorganisms in contaminated soil amended with straw was higher than corresponding control. For example, in pyrene-contaminated soil, the abundance of PAH-utilising microorganisms in the soil amended with pea straw was 13-fold higher than in the corresponding control soil.

Like other bioremediation methods, the application of necrophytoremediation has not always been successful (Adetutu et al. 2012; Callaham et al. 2002; Hultgren et al. 2009; Phillips et al. 2006). For example, in a report by Hultgren et al. (2009), the degradation of PAHs in an aged creosote-contaminated soil in the presence of willow plants, wheat straw, and Triton X-100 (surfactant) was investigated in a greenhouse experiment. The results from this study demonstrated that the addition of wheat straw showed no positive effect on the degradation of PAHs compared with the control. Callaham et al. (2002) also found that wheat straw did not affect the degradation of TPH relative to control in the bioremediation of hydrocarbon-contaminated soils; the level of TPH in control and soil amended with wheat straw was 32.7 and 32.3 g $kg^{-1}$ (dry soil), respectively. Phillips et al. (2006) applied straw as an amendment in the phytoremediation of flare pit soil (the authors did not mention the type of straw). They reported that the presence of amendments resulted in inhibition of hydrocarbon degradation in unplanted treatments. However, hydrocarbon degradation showed an increase when phytoremediation with different plants was applied.

## 26.8 Hydrocarbon-Utilising Microorganisms

Hydrocarbon-utilising microorganisms, the main agents of remediation of hydrocarbons during phytoremediation and necrophytoremediation, are defined as microbes (mostly bacteria and fungi) that are capable of using petrogenic hydrocarbons as a source of energy. Representatives of many microbial genera have been reported to contain hydrocarbonoclastic strains, many of which have been isolated from either rhizosphere or bulk soils (Table 26.8). Common genera include *Pseudomonas, Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Nocardia*, and *Mycobacterium* (Germida et al. 2002). Fungi such as *Aspergillus ochraceus*, *Cunninghamella elegans*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, and *Syncephalastrum racemosum* have also been reported to exhibit hydrocarbonoclastic activity (Germida et al. 2002).

There are a number of reports which showed that the 'rhizosphere effect' led to increased microbial population of these hydrocarbon degraders (Gaskin et al. 2008; Kim et al. 2006; Kirk et al. 2005). It has also been shown that the population of *Pseudomonas, Arthrobacter*, and *Achromobacter* bacteria was found to be higher in rhizosphere soil than bulk soil (as reviewed by Frick et al. 1999).

**Table 26.8** Bacterial and fungal genera isolated from bulk or rhizosphere soil that have been shown to be hydrocarbonoclastic (Germida et al. 2002)

| Bacterial name | Fungal name |
|---|---|
| *Achromobacter* | *Acremonium* |
| *Acinetobacter* | *Aspergillus* |
| *Alcaligenes* | *Aureobasidium* |
| *Arthrobacter* | *Beauveria* |
| *Bacillus* | *Botrytis* |
| *Brevibacterium* | *Candida* |
| *Chromobacterium* | *Chrysosporium* |
| *Corynebacterium* | *Cladosporium* |
| *Cytophaga* | *Cochliobolus* |
| *Erwinia* | *Cunninghamella* |
| *Flavobacterium* | *Cylindrocarpon* |
| *Micrococcus* | *Debaryomyces* |
| *Mycobacterium* | *Fusarium* |
| *Norcardia* | *Geotrichum* |
| *Proteus* | *Gliocladium* |
| *Pseudomonas* | *Graphium* |
| *Rhodococcus* | *Humicola* |
| *Sarcina* | *Monilia* |
| *Serratia* | *Mortierella* |
| *Spirillum* | *Paecilomyces* |
| *Streptomyces* | *Penicillium* |
| *Vibrio* | *Phoma* |
| *Xanthomonas* | *Phanerochaete* |
| | *Rhodotorula* |
| | *Saccharomyces* |
| | *Scolecobasidium* |
| | *Sporobolomyces* |
| | *Sporotrichum* |
| | *Spicaria* |
| | *Syncephalastrum* |
| | *Tolypocladium* |
| | *Torulopsis* |
| | *Trichoderma* |
| | *Verticillium* |

## 26.9 Mechanisms of Microbial Degradation of Hydrocarbons

The mechanisms of degradation of hydrocarbon by microorganisms are varied, but one critical division of pathways is based around the oxic versus anoxic nature of the degradation. Under oxic (aerobic) conditions, the first step is incorporation of oxygen into the hydrocarbons; this is mediated through a broad range of monooxygenases and dioxygenases (hydroxylase) enzymes. In the degradation of n-alkanes (aliphatic hydrocarbons), degradation first occurs by oxidation of a terminal methyl group, resulting in the formation of a primary alcohol which is further oxidised to the corresponding aldehyde and finally transformed into a fatty acid (Fig. 26.2). Fatty acids are conjugated to CoA and enter the β-oxidation pathway to produce acetyl-CoA (Rojo 2010). Subterminal oxidation has also been detected in the degradation of longer chained alkanes (Fig. 26.2). Here, the secondary alcohols are converted to the corresponding ketone which is oxidised by a Baeyer–Villiger monooxygenase to an ester. Afterwards, the ester is hydrolysed with an esterase to produce an alcohol and a fatty acid (Fig. 26.2) (Van Beilen et al. 2003).

A variety of aerobic bacteria such as *Pseudomonas* and *Rhodococcus* start to oxidise benzene ring PAHs with the contributions of dioxygenase enzymes to produce *cis*-dihydrodiols. These dihydrodiols are subjected to dehydrogenases producing dihydroxylated intermediates; these intermediate chemicals are further metabolised via catechols to carbon dioxide and water (Fig. 26.3) (Bamforth and Singleton 2005). In contrast, a few bacteria such as *Mycobacterium* can oxidise PAHs through the cytochrome P450 monooxygenase enzyme to form *trans*-dihydrodiols (Fig. 26.3) (Bamforth and Singleton 2005). Fungi deal with PAHs using two pathways; while non-ligninolytic fungi used the P450 monooxygenase pathway, white-rot fungi (a ligninolytic fungus) degrade PAHs using ligninolytic enzymes (Fig. 26.3).

These ligninolytic enzymes include lignin peroxidase, laccase, and manganese peroxidase; these enzymes are involved in the oxidation of lignin in wood and other organic compounds (Bamforth and Singleton 2005). Under anoxic (anaerobic) conditions anaerobic degradation of hydrocarbons can also occur. Sulphate-reducing, denitrifying, and methanogenic bacterial communities all contribute to anaerobic degradation (Haritash and Kaushik 2009; Wentzel et al. 2007). However, although n-alkanes and PAHs (only ≤3 rings, there is no evidence the anaerobic degradation of >3 ring PAHs) can be degraded anaerobically, the process is slow and our knowledge is limited (see Haritash and Kaushik 2009 and Wentzel et al. 2007 for more details).

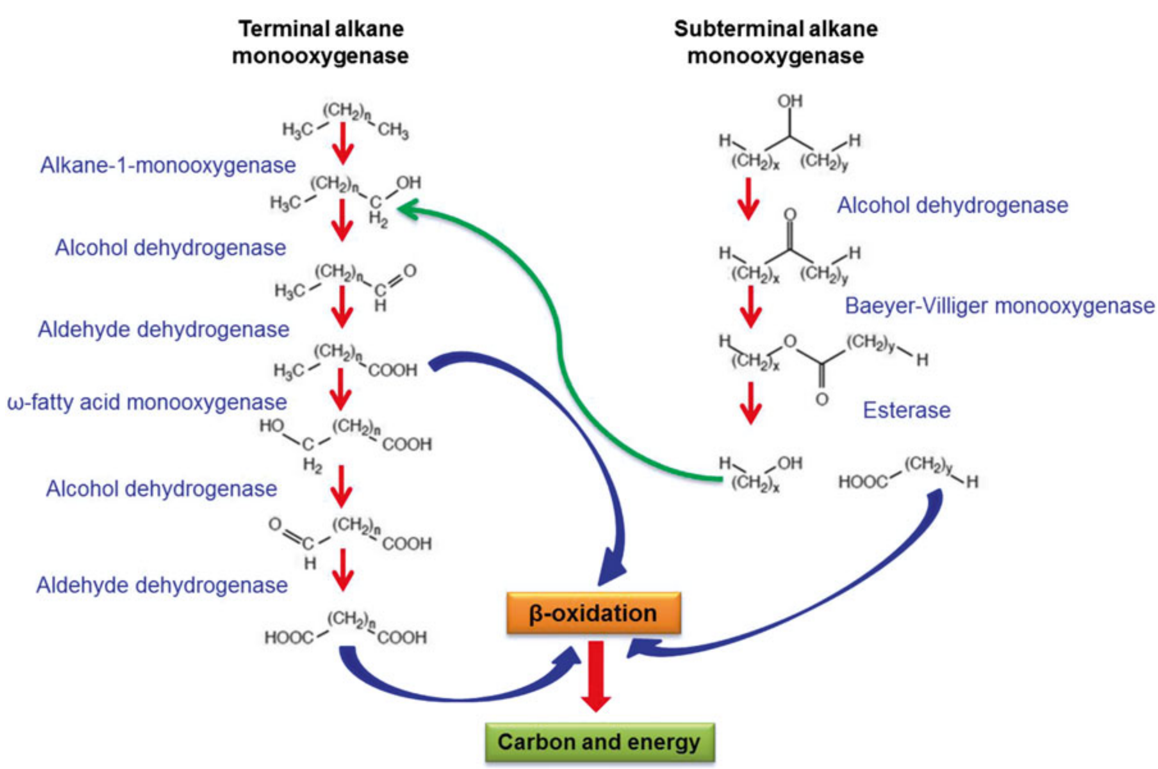



**Fig. 26.2** The main *n*-alkanes degradation pathways (adapted from Van Beilen et al. 2003)

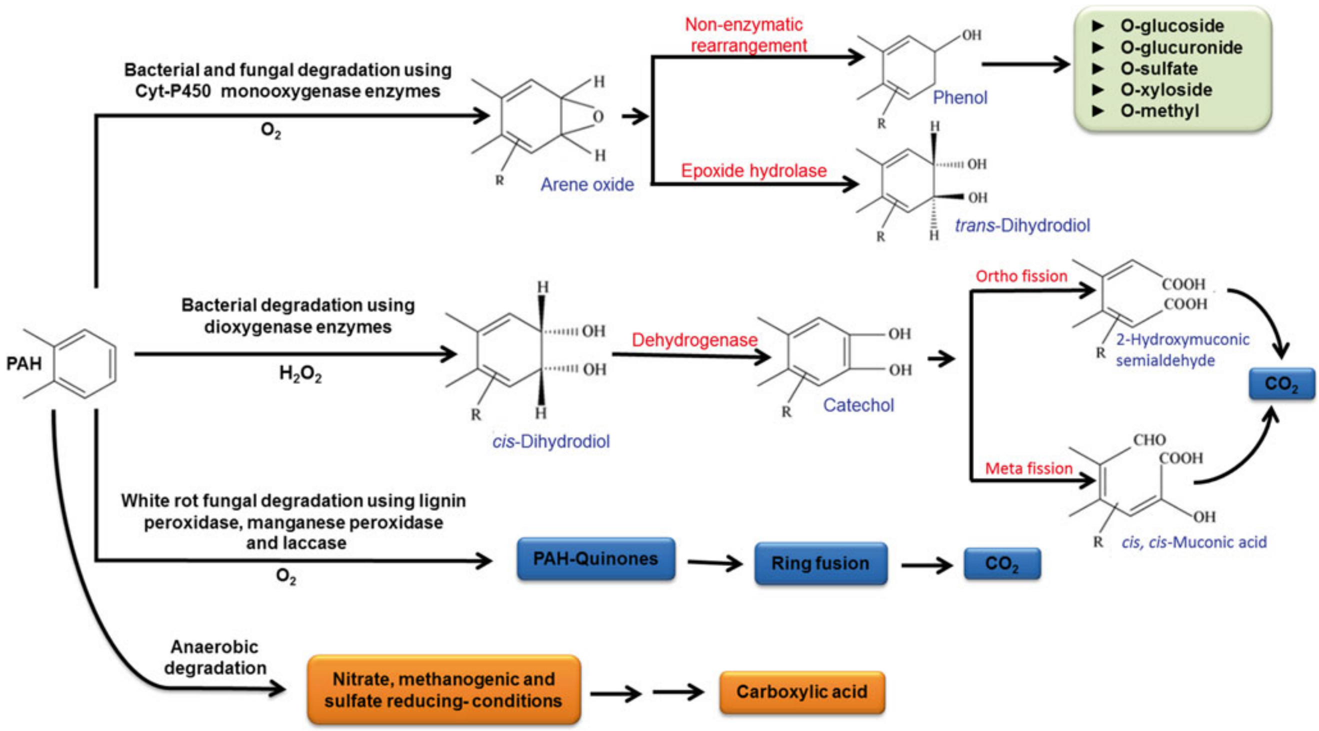



**Fig. 26.3** The main pathways of PAHs degradation in bacteria and fungi (adapted from Bamforth and Singleton 2005; Haritash and Kaushik 2009)

## 26.10 Conclusion

This review outlined the use of phytoremediation and necrophytoremediation to remediate the hydrocarbon-contaminated soils. In terms of hydrocarbon phytoremediation, plant roots (rhizosphere) mainly contribute to increased rate of degradation by increasing the microbial activities. However, the toxic nature of many hydrocarbon compounds (especially when the concentration of hydrocarbons in contaminated soil is too high) or unfavourable growth conditions (e.g. temperature, soil pH, and salinity) may lead to failure in the application of this technology. In this case, necrophytoremediation is an alternative method to remediate hydrocarbon-contaminated soils as it is a toxic-independent method and less affected by environmental factors.

## References

Adam G, Duncan H (2002) Influence of diesel fuel on seed germination. Environ Pollut 120:363–370

Adetutu EM, Ball AS, Weber J, Aleer S, Dandie CE, Juhasz AL (2012) Impact of bacterial and fungal processes on $^{14}$C-hexadecane mineralisation in weathered hydrocarbon contaminated soil. Sci Total Environ 414:585–591

Alcalde M, Ferrer M, Plou FJ, Ballesteros A (2006) Environmental biocatalysis: from remediation with enzymes to novel green processes. Trends Biotechnol 24:281–287

Aleer S, Adetutu EM, Makadia TH, Patil S, Ball AS (2011) Harnessing the hydrocarbon-degrading potential of contaminated soils for the bioremediation of waste engine oil. Water Air Soil Pollut 218:121–130

Baldrian P (2008) Wood-inhabiting ligninolytic basidiomycetes in soils: ecology and constraints for applicability in bioremediation. Fungal Ecol 1:4–12

Bamforth SM, Singleton I (2005) Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biotechnol 80:723–736

Banks M, Kulakow P, Schwab A, Chen Z, Rathbone K (2003) Degradation of crude oil in the rhizosphere of *Sorghum bicolor*. Int J Phytoremediation 5:225–234

Boyajian G, Carreira L (1997) Phytoremediation: a clean transition from laboratory to marketplace? Nat Biotechnol 15:127

Bozeman B (2011) The 2010 BP Gulf of Mexico oil spill: implications for theory of organizational disaster. Technol Soc 33:244–252

Callaham MA, Stewart AJ, Alarcón C, McMillen SJ (2002) Effects of earthworm (*Eisenia fetida*) and wheat (*Triticum aestivum*) straw additions on selected properties of petroleum-contaminated soils. Environ Toxicol Chem 21:1658–1663

Cheema SA, Imran Khan M, Shen C, Tang X, Farooq M, Chen L, Zhang C, Chen Y (2010) Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J Hazard Mater 177:384–389

Chen YC, Banks MK (2004) Bacterial community evaluation during establishment of tall fescue (*Festuca arundinacea*) in soil contaminated with pyrene. Int J Phytoremediation 6:227–238

Dinis MJ, Bezerra RMF, Nunes F, Dias AA, Guedes CV, Ferreira LMM, Cone JW, Marques GSM, Barros ARN, Rodrigues MAM (2009) Modification of wheat straw lignin by solid state fermentation with white-rot fungi. Bioresour Technol 100:4829–4835

Erkelens M, Adetutu EM, Taha M, Tudararo-Aherobo L, Antiabong J, Provatas A, Ball AS (2012) Sustainable remediation–the application of bioremediated soil for use in the degradation of TNT chips. J Environ Manage 110:69–76

Ferro A, Sims RC, Bugbee B (1994) Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. J Environ Qual 23

Field JA, De Jong E, Costa GF, De Bont J (1992) Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl Environ Microbiol 58:2219–2226

Frick CM, Farrel RE, Germida JJ (1999) Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Petroleum Technology Alliance of Canada (PTAC), Calgary

Gaskin S, Soole K, Bentham R (2008) Screening of Australian native grasses for rhizoremediation of aliphatic hydrocarbon-contaminated soil. Int J Phytoremediation 10:378–389

Gaskin SE, Bentham RH (2010) Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses. Sci Total Environ 408:3683–3688

Germida JJ, Frick CM, Farrell RE (2002) Phytoremediation of oil-contaminated soils. In: Violante A, Huang PM, Bollag JM, Gianfreda L (eds) Developments in soil science. Elsevier, Amsterdam, pp 169–186

Hall J, Soole K, Bentham R (2011) Hydrocarbon phytoremediation in the family Fabacea—a review. Int J Phytoremediation 13:317–332

Haritash A, Kaushik C (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol Rev 13:125–135

Hultgren J, Pizzul L, Castillo MP, Granhall U (2009) Degradation of PAH in a creosote-contaminated soil. A comparison between the effects of willows (*Salix viminalis*), wheat straw and a nonionic surfactant. Int J Phytoremediation 12:54–66

Hutchinson SL, Schwab AP, Banks MK (2004) Biodegradation of petroleum hydrocarbons in the rhizosphere. In: McCutcheon SC, Schnoor JL (eds) Phytoremediation: transformation and control of contaminants. Wiley, Hoboken, NJ, pp 355–386

Issoufi I, Rhykerd R, Smiciklas K (2006) Seedling growth of agronomic crops in crude oil contaminated soil. J Agronomy Crop Sci 192: 310–317

Iwamoto T, Nasu M (2001) Current bioremediation practice and perspective. J Biosci Bioeng 92:1–8

Kabay VG (2010) The fate of polycyclic aromatic hydrocarbons in plant-soil systems. PhD thesis. University of Melbourne, Australia

Kadali KK, Simons KL, Sheppard PJ, Ball AS (2012) Mineralisation of weathered crude oil by a hydrocarbonoclastic consortia in marine mesocosms. Water Air Soil Pollut 1–13

Kim J, Kang SH, Min KA, Cho KS, Lee IS (2006) Rhizosphere microbial activity during phytoremediation of diesel-contaminated soil. J Environ Sci Health A Tox Hazard Subst Environ Eng 41:2503–2516

Kirk JL, Klironomos JN, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ Pollut 133:455–465

Li X, Wu Y, Lin X, Zhang J, Zeng J (2012) Dissipation of polycyclic aromatic hydrocarbons (PAHs) in soil microcosms amended with mushroom cultivation substrate. Soil Biol Biochem 47:191–197

Liste H-H, Alexander M (2000) Accumulation of phenanthrene and pyrene in rhizosphere soil. Chemosphere 40:11–14

Liu W, Luo Y, Teng Y, Li Z, Ma LQ (2010) Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes. Environ Geochem Health 32:23–29

Lors C, Damidot D, Ponge JF, Périé F (2012) Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales. Environ Pollut 165:11–17

Lors C, Ryngaert A, Périé F, Diels L, Damidot D (2010) Evolution of bacterial community during bioremediation of PAHs in a coal tar contaminated soil. Chemosphere 81:1263–1271

Macek T, Mackova M, Káš J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18:23–34

Makadia TH, Adetutu EM, Simons KL, Jardine D, Sheppard PJ, Ball AS (2011) Re-use of remediated soils for the bioremediation of waste oil sludge. J Environ Manage 92:866–871

Militon C, Boucher D, Vachelard C, Perchet G, Barra V, Troquet J, Peyretaillade E, Peyret P (2010) Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil. FEMS Microbiol Ecol 74:669–681

Morgan P, Lee SA, Lewis ST, Sheppard AN, Watkinson RJ (1993) Growth and biodegradation by white-rot fungi inoculated into soil. Soil Biol Biochem 25:279–287

Mougin C (2002) Bioremediation and phytoremediation of industrial PAH-polluted soils. Polycycl Aromat Comp 22:1011–1043

Palmroth MRT, Pichtel J, Puhakka JA (2002) Phytoremediation of subarctic soil contaminated with diesel fuel. Bioresour Technol 84: 221–228

Peng S, Zhou Q, Cai Z, Zhang Z (2009) Phytoremediation of petroleum contaminated soils by *Mirabilis jalapa* L. in a greenhouse plot experiment. J Hazard Mater 168:1490–1496

Perelo LW (2010) Review: in situ and bioremediation of organic pollutants in aquatic sediments. J Hazard Mater 177:81–89

Phillips LA, Greer CW, Germida JJ (2006) Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biol Biochem 38:2823–2833

Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 56:15–39

Rhykerd R, Crews B, McInnes K, Weaver R (1999) Impact of bulking agents, forced aeration, and tillage on remediation of oil-contaminated soil. Bioresour Technol 67:279–285

Rojo F (2010) Enzymes for aerobic degradation of alkanes. In: Timmis K (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Heidelberg, pp 781–797

Ros M, Rodriguez I, Garcia C, Hernandez T (2010) Microbial communities involved in the bioremediation of an aged recalcitrant hydrocarbon polluted soil by using organic amendments. Bioresour Technol 101:6916–6923

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol 13:468–474

Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20: 243–248

Sarkar D, Ferguson M, Datta R, Birnbaum S (2005) Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. Environ Pollut 136:187–195

Schaefer M, Juliane F (2007) The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. Appl Soil Ecol 36:53–62

Shahsavari E, Adetutu EM, Anderson PA, Ball AS (2013a) Necrophytoremediation of phenanthrene and pyrene in contaminated soil. J Environ Manage 122:105–112

Shahsavari E, Adetutu EM, Anderson PA, Ball AS (2013b) Plant residues–a low cost, effective bioremediation treatment for petrogenic hydrocarbon-contaminated soil. Sci Total Environ 443:766–774

Shahsavari E, Adetutu EM, Anderson PA, Ball AS (2013c) Tolerance of selected plant species to petrogenic hydrocarbons and effect of plant rhizosphere on the microbial removal of hydrocarbons in contaminated soil. Water Air Soil Pollut 224:1–14

Sheppard PJ, Adetutu EM, Makadia TH, Ball AS (2011) Microbial community and ecotoxicity analysis of bioremediated, weathered hydrocarbon-contaminated soil. Soil Res 49:261–269

Simons KL, Ansar A, Kadali K, Bueti A, Adetutu EM, Ball AS (2012) Investigating the effectiveness of economically sustainable carrier material complexes for marine oil remediation. Bioresour Technol 126:202–207

Stroud J, Paton G, Semple KT (2007) Microbe-aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. J Appl Microbiol 102:1239–1253

Tang J, Wang R, Niu X, Zhou Q (2010) Enhancement of soil petroleum remediation by using a combination of ryegrass (*Lolium perenne*) and different microorganisms. Soil Tillage Res 110:87–93

Trigo C, Ball AS (1994) Is the solubilized product from the degradation of lignocellulose by actinomycetes a precursor of humic substances? Microbiology 140:3145–3152

Van Beilen JB, Li Z, Duetz WA, Smits THM, Witholt B (2003) Diversity of alkane hydroxylase systems in the environment. Oil Gas Sci Tech 58:427–440

Wasmund K, Burns KA, Kurtböke DI, Bourne DG (2009) Novel alkane hydroxylase gene *(alkB*) diversity in sediments associated with hydrocarbon seeps in the Timor Sea, Australia. Appl Environ Microbiol 75:7391–7398

Wei S, Pan S (2010) Phytoremediation for soils contaminated by phenanthrene and pyrene with multiple plant species. J Soils Sediments 10:886–894

Wellman DE, Ulery AL, Barcellona MP, Duerr-Auster S (2001) Animal waste-enhanced degradation of hydrocarbon-contaminated soil. Soil Sediment Contam 10:511–523

Wentzel A, Ellingsen T, Kotlar H-K, Zotchev S, Throne-Holst M (2007) Bacterial metabolism of long-chain n-alkanes. Appl Microbiol Biotechnol 76:1209–1221

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408

Wu Z, Dong H, Zou L, Lu D, Liu Z (2011) Enriched microbial community in bioaugmentation of petroleum-contaminated soil in the presence of wheat straw. Appl Biochem Biotechnol 164:1071–1082

Yousaf S, Andria V, Reichenauer TG, Smalla K, Sessitsch A (2010) Phylogenetic and functional diversity of alkane degrading bacteria associated with Italian ryegrass (*Lolium multiflorum*) and Birdsfoot trefoil (*Lotus corniculatus*) in a petroleum oil-contaminated environment. J Hazard Mater 184:523–532

Zhang K, Hua X-F, Han H-L, Wang J, Miao C-C, Xu Y-Y, Huang Z-D, Zhang H, Yang J-M, Jin W-B, Liu Y-M, Liu Z (2008) Enhanced bioaugmentation of petroleum- and salt-contaminated soil using wheat straw. Chemosphere 73:1387–1392

Zhang Z, Rengel Z, Chang H, Meney K, Pantelic L, Tomanovic R (2012) Phytoremediation potential of *Juncus subsecundus* in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs). Geoderma 175–176:1–8

Zhou W, Yang J, Lou L, Zhu L (2011) Solubilization properties of polycyclic aromatic hydrocarbons by saponin, a plant-derived biosurfactant. Environ Pollut 159:1198–1204

# An Integrated Electrochemical-Phytoremediation Process for the Treatment of Industrial Wastewater

27

Carlos Barrera Díaz, Gabriela Roa Morales, and Araceli Amaya Chávez

## 27.1 Physicochemical Characteristics of Industrial Wastewater

The aim of biological treatment is to oxidize the organic matter using microorganisms to reduce the biochemical oxygen demand (BOD). The reaction usually takes place in a reactor between microorganisms and wastewater with the input of dissolved oxygen in aqueous phase. The main products are carbon dioxide, water, and new cells (Duan and Gregory 2003; Puigdomenech 1997). The actual wastewater used in this study contains $SO_4^{-2}$, $Cl^-$, and $CO_3^{-2}$ with a concentration of about 0.01 M of each species and the raw pH is around 8 to 9. In the electrocoagulation treatment the colloidal matter is removed by the floc formation associated with the aqueous solution reaction of the anodic dissolution of Al(III) with the cathodic production of $OH^-$ which produces $Al(OH)_3$, as observed in Fig. 27.3. However, when industrial wastewater contains biorefractory compounds, the removal efficiencies are quite low. Therefore, coupled methods involving activated sludge have been used for enhancing pollutant removal efficiency in industrial wastewater (Duan and Gregory 2003; Benitez et al. 2000).

Industrial effluents that contain dyes and produce colored wastewater are highly toxic even after conventional wastewater treatments. Wastewater with highly toxic compounds, especially organic biorefractory compounds, leads to the partial inhibition of biodegradation, as microorganisms are sensitive to these pollutants. As biological treatments are insufficient to remove the color and meet current regulations, the application of special treatments is required (Villegas et al. 1999). The application of physicochemical pretreatments to industrial wastewater improves the water quality while enhancing the biodegradability (Wang et al. 2002).

It has been observed that conventional treatment processes which operate with physicochemical (coagulation-flocculation) and biological (aerobic and anaerobic) systems are not entirely efficient in the removal of industrial contaminants containing refractory organic compounds, which hinder or inhibit such treatments. As a result, the physicochemical parameters for environmental discharges are not always met (Barrera et al. 2005).

## 27.2 Electrocoagulation Treatment

Electrochemical methods have been used as coagulation processes to remove color and cloudiness from turbid industrial wastewater. In this application, the electrochemical process generated numerous flocculates, achieving high efficiency in clearing the wastewater (Ribeiro et al. 2000; Can et al. 2003). Electrochemical treatment techniques have attracted a great deal of attention because of their versatility and environmental compatibility, which makes the treatments of liquids, gases, and solids possible. In fact, the main reagent is the electron, which is a “clean reagent” (Roa et al. 2007; Janssen and Koene 2002).

Electrochemical reactions take place at the anode and the cathode of an electrolytic cell when an external direct current voltage is applied. The term electrocoagulation involves the in situ generation of coagulants by electrolytic oxidation of an appropriate sacrificial anode (iron or aluminum). The main stages involved in the electrocoagulation process using aluminum anodes have been previously identified (Can et al.

C.B. Díaz, Ph.D. • G.R. Morales, Ph.D.
Departamento de Química Ambiental, Facultad de Química, Universidad Autónoma del Estado de México
Paseo Colón esq. Paseo Tollocan s/n Col. Residencial Colón, Toluca, Estado de México, México

Centro Conjunto de Investigación en Química Sustentable UAEM – UNAM, Carretera Toluca-Atlacomulco, km 14.5, Unidad El Rosedal, Toluca, Estado de México C.P. 50200, México

A.A. Chávez, Ph.D. (✉)
Departamento de Farmacia de la Facultad de Química, UAEMex, Paseo Tollocan esq. Paseo Colón, Toluca, Estado de México C.P. 50100, Mexico
e-mail: aamayac@uaemex.mx

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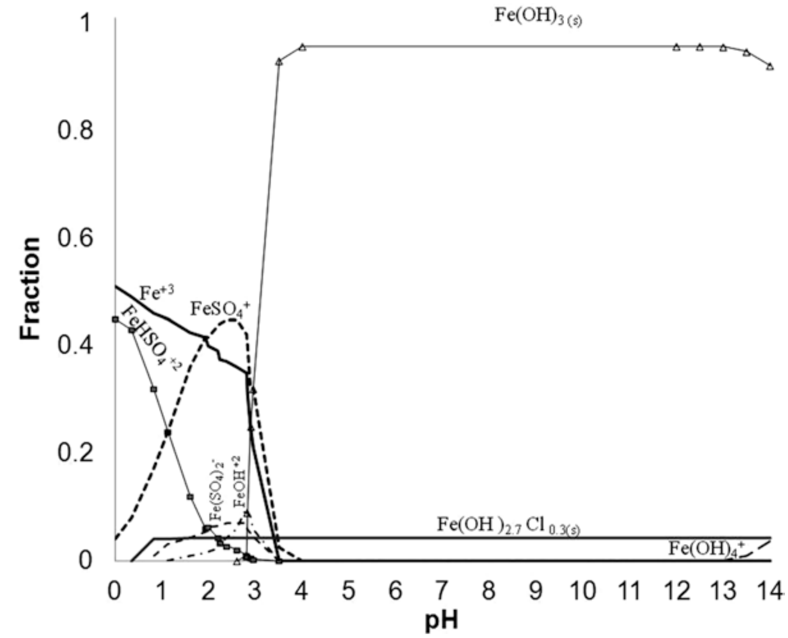


**Fig. 27.1** Species distribution diagram of Al 0.05 M in an aqueous medium with $SO_4^{-2}$, $Cl^{-}$, and $CO_3^{-2}$ 0.01 M each species (Puigdomenech 1997)

2006; Holt et al. 2002). The anodic process involves the oxidative dissolution of aluminum into aqueous solution as reaction (27.1) indicates and the reductive dissociation of water as reaction (27.2) shows

$$Al \rightarrow Al^{3+} + 3e^{-} \quad (27.1)$$

$$2H_2O \rightarrow O_{2(g)} + 4H^{+} + 4e^{-} \quad (27.2)$$

It has been reported that the species present during the electrocoagulation depend on the source of wastewater, as in the case of industrial wastewater with a high content of electrolytes such as sulfates, chlorides, and carbonates.

Residual water used in this study contains $SO_4^{-2}$, $Cl^{-}$, and $CO_3^{-2}$ with a concentration of about 0.01 M of each of these species. In the electrocoagulation treatment the colloids are eliminated by floc formation associated with $Al(OH)_3$ since the regular wastewater pH is between 8 and 9; the most probable chemical species formation in the aqueous system is shown in Fig. 27.1.

In the case of iron or steel anodes, two mechanisms for the production of the metal hydroxide have been proposed (Rajeshwar and Ibañes 1997). In Mechanism 1, common in high-pH media where oxygen can be involved for further $Fe^{2+}$ oxidation in $Fe^{3+}$,

$$4Fe_{(s)} \rightarrow 4Fe^{2+}_{(aq)} + 8e^{-} \quad (27.3)$$

In Mechanism 2, in lower-pH media, there is no further oxidation:

$$Fe_{(s)} \rightarrow Fe^{2+}_{(aq)} + 2e^{-}. \quad (27.4)$$

It is interesting that in electrocoagulation papers little attention has been paid on anodic reactions. Regardless of whether iron or aluminum is used, the main reaction that is reported is

$$2H_2O + 2e^{-} \rightarrow H_{2(g)} + 2OH^{-}_{(aq)} \quad (27.5)$$

However, this reaction has three important implications on the electrocoagulation technology: (a) provides hydroxyl ions which then react in bulk solution with iron or aluminum cations to form insoluble species; (b) hydrogen gas is produced which contributes in the destabilization of colloidal particles leading to flocculation; and (c) contribution to electroflotation which is a simple process that floats pollutants (or other substances) by their adhesion onto tiny bubbles formed by the hydrogen evolution (Casqueira et al. 2006).

Figure 27.2 shows a diagram of species distribution for Fe(II) indicating that at pH of 8, there is the presence of $Fe(OH)_2$ at 90 % and $FeCO_3$ with 10 %.

If there is sufficient water in oxygen, then the Fe(II) is oxidized to Fe(III) and the flocs thus formed during electrocoagulation can be associated with $Fe(OH)_3$ to 95 % and $Fe(OH)_{2.7}\,Cl_{0.3}$ according to species distribution diagram of Fe(III) in Fig. 27.3.

## 27.3 Plant Used in the Phytoremediation Experiments

The selection of the plant was based on the local availability; the relative abundance of this plant in the region makes it easy to get them. The choice was also made by the pollutant remotion capacity and your tolerance to exposure at contaminants.

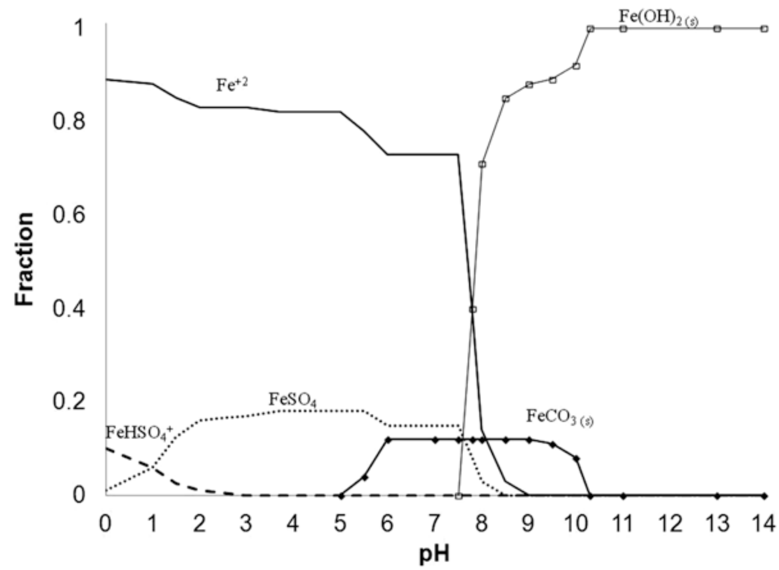


**Fig. 27.2** Species distribution diagram of Fe(II) 0.074 M in an aqueous medium with $SO_4^{-2}$, $Cl^-$, and $CO_3^{-2}$ 0.01 M each species (Puigdomenech 1997)

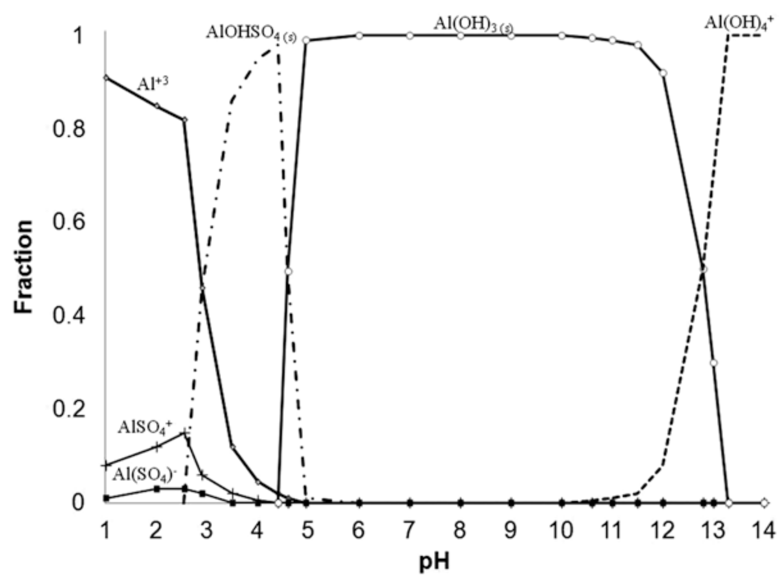


**Fig. 27.3** Species distribution diagram of Fe(III) 0.074 M in an aqueous medium with $SO_4^{-2}$, $Cl^-$, and $CO_3^{-2}$ 0.01 M each species (Puigdomenech 1997)

*Myriophyllum aquaticum* Vell. (Verdc) is a cosmopolite plant; it is a submersed macrophyte with emergent leaves, of easy propagation, and relatively fast growing. The species parrot feather has few roots, and it does not need to be rooted in a substrate (see Fig. 27.4). It is a native species of South America and its habitat is in lakes, ponds, streams, and canals (Susarla et al. 1999; Wersal 2010).

In previous studies, *Myriophyllum aquaticum* Vell. (Verdc) has shown a great potential to remove contaminants such as 2,4,6-trinitrotoluene (TNT), dichlorodiphenyltrichloroethane (DDT), perchlorate, pesticides, and antibiotics from water solutions and industrial wastewater (Susarla et al. 1999; Bhadra et al. 1999; Gao et al. 2000a, b; Turgut and Fomin 2002; Gujarathi et al. 2005; Turgut 2005).

## 27.4 Uptake Mechanism of Metal and Pollutant

Phytoremediation is used for the removal of various contaminants such as metals, pesticides, explosives, and hydrocarbons from soil and water; also they decrease the mobilization of the pollutants through the wind and water (EPA 2001). The main processes that are generated for bioremediation in

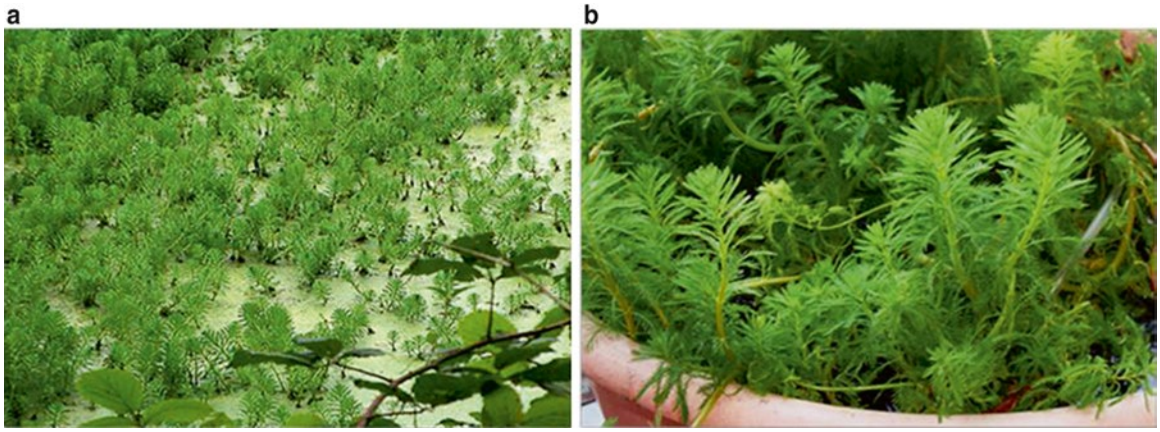


**Fig. 27.4** *Myriophyllum aquaticum* Vell. (Verdc) (**a**) natural habitat, (**b**) laboratory culture

plants are phytostabilization, rhizofiltration, phytoextraction, rhizodegradation, phytotransformation, and phytovolatilization (EPA 1999; Schnoor 1997).

Hall (2002) has described that the metal detoxification mechanism follows four major steps:

(a) Metal binding to the cell wall
(b) Reduced uptake or efflux pumping of metals at the plasma membrane
(c) Chelation of the metal in the cytosol by various ligands, such as phytochelatins, metallothioneins, and metal-binding proteins
(d) The compartmentation of metals in the vacuole by tonoplast-located transporters

Plants have evolved mechanisms to resist and survive to exposure to high concentrations of potentially toxic elements, as a result of an evolutionary process (Hall 2002). Some mechanisms that regulate the tolerance of the exposure to certain compounds have not been completely understood; however, the most studied are related to a) the metal bioaccumulation (via chelating or sequestration) in which the ligands metallothioneins and phytochelatins play an important role, b) antioxidant enzymatic using enzymes like superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, c) nonenzymatic antioxidant using glutathione, carotenoids, ascorbic acid systems, and d) homeostasis in the diffusion of cations, and the bacteria in the rhizosphere (Li et al. 2007; Márquez-García et al. 2012). Between the mechanisms for the detoxification of organic compounds, we can quote enzymatic oxidation, conjugation, reduction, or hydrolysis of organic pollutants. The biotransformation products are stored into vacuoles; they are distributed within the plant or are retained in insoluble cellular structures such as lignin (Gao et al. 2000b; Garcinuño et al. 2006).

Ederli et al. (2004) mentioned that the roots of *Phragmites australis* species were subjected to various concentrations of cadmium to prove the removal efficiency of the artificial aquatic system test, obtaining that the roots did not have structural changes at a concentration of 100 mM in 21 days. Amaya et al. (2006) reported that *Typha latifolia* has a high removal efficiency of methyl parathion in water and sediment samples, being tolerant to concentrations up to 200 mg $L^{-1}$ of the pesticide without significant changes in chlorophyll with $198.1 \pm 1.79$ g of biomass.

Torres et al. (2007) did a preliminary study using *Pistia stratiotes* for removal of divalent copper, which showed that in an aqueous solution having pH of 5 at a concentration of 1 mg $L^{-1}$, the plant survives for 2 days and is capable to uptake 70 % of dissolved copper.

Olguín et al. (2007) compared the $NH_4^+$ removal (initial concentration of 35 mg $L^{-1}$ ) using *Salvinia minima* and *Spirodela polyrrhiza*, they found that *Spirodela polyrrhiza* was more efficient. Romero (2007) conducted a study to remove chemical oxygen demand (COD) from municipal wastewater using two species, *Phragmites australis* and *Typha domingensis*, in a wetland system; it found that the COD removal efficiency was about 70 % in systems with the two species in a period of 5 days.

Carvalho et al. (2008) and Dordio et al. (2011) used *Typha* spp. for ibuprofen removal from wastewater. Dordion et al. (2011) observed a 60 % removal at 24 h and 99 % after 21 days of the plant treatment.

## 27.5 Electrocoagulation-Phytoremediation Coupled System

The electrocoagulation system used for the treatment of industrial water had good efficiency, but if the wastewater has a high concentration of refractory compounds, it is very difficult to remove totally the contaminants; therefore, it is necessary to use polishing treatments like phytoremediation, hence the relevance of hybrid system development to increase the efficiency remotion.

We worked with a hybrid system of electrocoagulation with iron and aluminum electrodes and phytoremediation using *Myriophyllum aquaticum* Vell. (Verdc) for the treatment of the wastewater, which comes from 150 industrial discharges of different types. Before the phytoremediation treatment, we diluted the electrocoagulated wastewater because the plants were tolerant at these concentrations of residual contaminants.

The wastewater physicochemical characteristics that were evaluated for the remotion are shown in Table 27.1 and Fig. 27.5, in which it is observed that the industrial wastewater contains a high amount of COD, coloration, and turbidity.

Table 27.2 shows the pollutant removal efficiency for the coupled techniques, electrocoagulation using aluminum electrodes and phytoremediation, for reducing the concentration of COD, color, and turbidity of industrial wastewater.

We observed that this process was more efficient than the coupled techniques, electrocoagulation using iron electrodes; it was obtained by a remotion of COD, color, and turbidity of 92.3, 94.9, and 94.7 %, respectively.

## 27.6 Tolerance of the Plants to the Electrocoagulated Wastewater

The evaluation of the tolerance of plants to exposure to residual contaminants in the electrocoagulated wastewater with iron electrodes at different concentrations was made by the determination, before and after the exposition time, the weight and longitude of the plants, chlorophyll content, and oxidative stress biomarkers (SOD, CAT activity, and lipid peroxidation). The results indicate that the toxicity of electrocoagulated wastewater to *M. aquaticum* was low when the plant was exposed to the dilution of 25–50 % during 15 days. For the electrocoagulation with iron electrode treatment, no significant differences were observed in weight and longitude. The basal average of total chlorophyll was $40.4 \pm 3.89$ mg mL$^{-1}$ and chlorophyll *a*/*b* ratio was $2.84 \pm 0.24$.

After 15 days of contact with the wastewater, there were no significant differences in the total chlorophyll content or the chlorophyll *a*/*b* ratio between the different concentrations. Research indicates that the chlorophyll end point, weight, and longitude are an important indication on the health status of the plants; the levels observed in this research

**Table 27.1** Removal efficiency of COD, color, and turbidity for coupled techniques, electrocoagulation with iron electrodes and phytoremediation from industrial wastewater

| Technique | COD (mg L$^{-1}$) | Color/PtCo (U) | Turbidity (NFU) |
|---|---|---|---|
| Raw wastewater | 1,680 | 2,489 | 168 |
| Electrocoagulation | 974 | 273 | 9 |
| Phytoremediation | 730 | 204 | 5 |

**Table 27.2** Removal efficiency of COD, color, and turbidity for coupled techniques, electrocoagulation with aluminum electrodes and phytoremediation from industrial wastewater

| Technique/value | COD (mg L$^{-1}$) | Color/PtCo (U) | Turbidity (NFU) |
|---|---|---|---|
| Raw wastewater | 1,921.04 | 2,036 | 79.67 |
| Electrocoagulation | 1,007.33 | 501 | 16.33 |
| Phytoremediation | 147 | 103 | 4.2 |


**Fig. 27.5** Image of industrial (**a**) raw wastewater, (**b**) after treatment

correspond to a good health level; changes in total chlorophyll or chlorophyll *a*/*b* ratio may directly affect $CO_2$ uptake during photosynthesis (Beatriz et al. 2008; Delgado 1993).

SOD, as an antioxidant enzyme, acts as the first line of defense against ROS damage, two superoxide dismutase radicals to $H_2O_2$ and $O_2$, allowing that levels of superoxide radicals to remain low in the cell, combating oxidative stress in plants (Wang et al. 2008; Dordio et al. 2009; Mishra et al. 2006).

Exposure of plants treated with concentrations of electrocoagulated water of 13 and 22 % did not cause significant alterations of the activity of SOD; however, the concentration of 16 and 19 % caused a significant increase; this observation may be due to the novel synthesis of enzymatic proteins by induction that suffers from being in contact with pollutants in the wastewater (Allen et al. 1997). The reduction of activity appeared higher (25 %) and is probably due to increased levels of $H_2O_2$ and its derivative ROS, causing damage to the enzyme system (Gallego et al. 1996). It is also considered that because of higher concentrations or a long-term exposure to any pollutant, SOD activity may be suppressed; further, efficiency of SOD is relatively higher for short periods of stress (Sánchez et al. 2004). The results obtained in this study are similar to those reported by Wang et al. (2008), who observed an increase in SOD activity in the macrophyte *Vallisneria natans*, when exposed to concentrations of 0.4 and 1.2 mM $NH_4Cl$, but the activity is reduced at higher concentrations (2, 2.8, 2 mM $NH_4Cl$).

Xu et al. (2010) found that SOD activity in *Phragmites australis* increases when exposed to water with a chemical oxygen demand (COD) of 200 mg $L^{-1}$ and decreases at 400 and 800 mg $L^{-1}$. In another study with *Typha angustifolia*, they observed the same behavior, SOD activity increased at concentrations of 600 mg $L^{-1}$ of COD, but decreases to 800 mg $L^{-1}$.

CAT is another important antioxidative enzyme, present in the peroxisomes and mitochondria of the cells, which degrades $H_2O_2$ to water and molecular oxygen that catalyzed reaction by SOD. CAT activity increase could be explained by a plant adaptive mechanism to maintain the $H_2O_2$ level in a steady state within the cells (Wang et al. 2008; Dordio et al. 2009; Mishra et al. 2006).

In this research, the enzymatic activity increased significantly to a concentration of 25 % of electrocoagulated water (Fig. 27.5). As noted, this residual concentration of contaminants was sufficient to produce oxidative stress in plant tissues, so that with the increase of enzyme activity, the plant tries to maintain its homeostasis.

The increase in catalase activity has been observed in several studies of aquatic plants exposed to various contaminants. In *Myriophyllum mattogrossense* there is an increased CAT activity when exposed to concentrations of 20 and 30 mg $L^{-1}$ for ammonia (N), since this compound triggers the production of peroxide and therefore oxidative stress (Nimptsch and Pflugmacher 2007). In *Vallisneria natans* the CAT activity increased at concentrations of 1.2, 2, and 2.8 mM $NH_4Cl$ (Wang et al. 2008). *Typha* spp. presented an increase of CAT when exposed to concentrations of 1 and 2 mg $L^{-1}$ clofibric acid (Dordio et al. 2009)as well as when exposed to water contaminated with ibuprofen at concentrations of 0.5, 1, and 2 mg $L^{-1}$ (Dordio et al. 2011).

Active oxygen radicals may induce chain-like peroxidation of unsaturated fatty acids in the membranes leading to formation of lipid peroxidation products like malondialdehyde (MDA). The effect of the water electrocoagulated toxicity on lipid peroxidation of *M. aquaticum* was determined by TBARS (thiobarbituric acid reactive substances). TBARS formation in plant exposed to environmental stress conditions is an indicator of free radical formation in the tissues and may be used as an index of lipid peroxidation (Singh et al. 2006).

The results obtained in this study showed that there is no significant difference when compared to control in lipid peroxidation levels in water electrocoagulated to concentrations until 19 %. This indicates a possible tolerance of *M. aquaticum* to exposure of residual pollutants in water pretreated with electrocoagulation.

## 27.7 Conclusions

The system of electrocoagulation with aluminum electrodes and phytoremediation used for the treatment of industrial wastewater had good remotion efficiency (COD 92.3 %, color 94.9 %, and turbidity 94.7 %). The plants were tolerant only at dilution of 19 % (185.06 COD) of pollutant residuals. The coupled techniques, electrocoagulation with iron electrodes and phytoremediation, had a lower efficiency of remotion COD (56.5 %), color (91.8 %), and turbidity (97 %) from industrial wastewater and were more toxic for the plants. This work finds that it is necessary to use polishing treatments like phytoremediation, hence the relevance of hybrid system development to increase the efficiency remotion.

## References

Allen RD, Webb RP, Schake SA (1997) Use of transgenic plants to study antioxidant defenses. Free Radic Biol Med 23:473–479

Amaya CA, Martínez TL, López LE, Galar MM (2006) Methyl parathion toxicity to and removal efficiency by Typha latifolia in water and artificial sediments. Chemosphere 63:1124–1129

Barrera DC, Roa MG, Ávila CL, Pavón ST, Bilyeu B (2005) Electrochemical treatment applied to food-processing industrial wastewater. Ind Eng Chem Res 45:34–38

Beatriz P, Andrea DS, Rosa R, Marisol C (2008) Biomarcadores de contaminación por cadmio en las plantas. Interciencia 33(2):112

Benitez FJ, Beltran HJ, Real FJ, Acero JL (2000) Wine vinasses treatments by ozone and an activated sludge system in continuous reactors. Bioprocess Eng 23:149–154

Bhadra R, Spanggord RJ, Wayment DG, Hughes JB, Shanks JV (1999) Characterization of oxidation products of TNT metabolism in aquatic phytoremediation systems of Myriophyllum aquaticum. Environ Sci Technol 33:3354–3361

Can OT, Bayramoglu M, Kobya M (2003) Decolorization of reactive dye solutions by electrocoagulation using aluminum electrodes. Ind Eng Chem Res 42:3391–3396

Can OT, Kobya M, Demirbas E, Bayramoglu M (2006) Treatment of the textile wastewater by combined electrocoagulation. Chemosphere 62:181–187

Carvalho A, Costa C, Dordio A, Ferro R, Pinto AP, Teixeira D (2008) Removal of ibuprofen from water by Typha spp. 35th International symposium on bioremediation Proceedings, Gdańsk, Poland

Casqueira RG, Torem ML, Kohler HM (2006) The removal of zinc from liquid streams by electroflotation. Miner Eng 19:1388–1392

Delgado B (1993) *Chlorophyll photosynthesis*, breathing and content and its relation with the yield of 16 genotypes of wheat (Triticum aestivum L.) worked in high temperature. Dissertation, School of Post-graduate, Institute of Education and Investigation in Agricultural Sciences

Dordio AV, Duarte C, Barreiros M, Carvalho AJP, Pinto AP, da Costa CT (2009) Toxicity and removal efficiency of pharmaceutical metabolite clofibric acid by Typha spp—potential use for phytoremediation? Bioresour Technol 100:1156–1161

Dordio A, Ferro R, Teixeira D, Palace AJ, Pinto AP, Dias CMB (2011) Study on the use of Typha spp for the phytotreatment of water contaminated with ibuprofen. Int J Environ Anal Chem 91:654–667

Duan J, Gregory J (2003) Coagulation by hydrolysing metal salts. Adv Colloid Interface Sci 100–102:475–502

Ederli L, Reale L, Ferranti F, Pasqualini S (2004) Responses induced by high concentration of cadmium in Phragmites australis roots. Physiol Plant 121:66–74

EPA (1999) Phytoremediation of organics action team. http://www.rtdf.org/public/phyto/phyto.pdf. Accessed 29 Oct 2011

EPA (2001) A citizen's guide to phytoremediation. http://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=10002SP5.PDF. Accessed 29 Oct 2011

Gallego SM, Benavídes MP, Tomaro ML (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Sci 121:151–159

Gao J, Garrison AW, Hoehamer C, Mazur CS, Wolfe NL (2000a) Uptake and phytotransformation of organophosphorus pesticides by axenically cultivated aquatic plants. J Agric Food Chem 48:6114–6120

Gao J, Garrison AW, Hoehamer C, Mazur CS, Wolfe NL (2000b) Uptake and phytotransformation of o,p′-DDT and p,p′-DDT by axenically cultivated aquatic plants. J Agric Food Chem 48:6121–6127

Garcinuño RM, Fernández HP, Cámara C (2006) Removal of carbaryl, linuron, and permethrin by Lupinus angustifolius under hydroponic conditions. J Agric Food Chem 54:5034–5039

Gujarathi NP, Haney BJ, Linden JC (2005) Phytoremediation potential of Myriophyllum aquaticum and Pistia stratiotes to modify antibiotic growth promoters, tetracycline, and oxytetracycline, in aqueous wastewater systems. Int J Phytoremediation 7:99–112

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Holt PK, Barton GW, Wark M, Mitchell CA (2002) A quantitative comparison between chemical dosing and electrocoagulation. Colloid Surf A 211:233–248

Janssen LJJ, Koene L (2002) The role of electrochemistry and electrochemical technology in environmental protection. Chem Eng J 85:137–146

Li WC, Ye ZH, Wong MH (2007) Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant, Sedum alfredii. J Exp Bot 58:4173–4182

Márquez-García B, Horemans N, Torronteras R, Córdoba F (2012) Glutathione depletion in healthy cadmium-exposed Erica andevalensis. Environ Exp Bot 75:159–166

Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK (2006) Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65:1027–1039

Nimptsch J, Pflugmacher S (2007) Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte Myriophyllum mattogrossense. Chemosphere 66:708–714

Olguín E, Sánchez GG, Pérez PT (2007) Assessment of the phytoremediation potential of *Salvinia minima* baker compared to Spirodela polyrrhiza in high-strength organic wastewater. Water Air Soil Pollut 181:135–147

Puigdomenech I (1997) Hydrochemical equilibrium constants database (MEDUSA)

Rajeshwar KA, Ibañes J (1997) Environmental electrochemistry; fundamentals and applications in pollution abatement. Academic, New York

Ribeiro AB, Mateus EP, Ottosen LM, Bech NG (2000) Electrodialytic removal of Cu, Cr, and As from chromated copper arsenate-treated timber waste. Environ Sci Technol 34:784–788

Roa MG, Campos ME, Aguilera CJ, Bilyeu B, Barrera DC (2007) Aluminum electrocoagulation with peroxide applied to wastewater from pasta and cookie processing. Sep Purif Technol 54:124–129

Romero AM (2007) Remoción de DQO de agua residual proveniente de un tratamiento primario por Phragmites australis (Cav.) Trin. Ex Steudel y Typha domingensis (Pers) Steudel en un humedal. Dissertation, Universidad Autónoma del Estado de México

Sánchez RMA, Santiago OE, Vargas LA, Mendoza NVM (2004) Propuesta de un constructo para evaluar integralmente el estrés oxidativo. Bioquímica 29:81–90

Schnoor J (1997) Phytoremediation: ground-water remediation technologies analysis center series. Technology Evaluation Report TE-98-01

Singh S, Eapen S, D'Souza SF (2006) Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, Bacopa monnieri L. Chemosphere 62:233–246

Susarla S, Bacchus ST, Wolfe NL, McCutcheon SC (1999) Phytotransformation of perchlorate and identification of metabolic products in Myriophyllum aquaticum. Int J Phytoremediation 1:97–107

Torres G, Navarro AE, Languasco J, Campos K, Cuizano NA (2007) Estudio preliminar de la fitoremediación de cobre divalente mediante *Pistia stratioides* (lechuga de agua). Rev Lat Rec Nat 3:13–20

Turgut C (2005) Uptake and modeling of pesticides by roots and shoots of parrotfeather (Myriophyllum aquaticum) (5 pp). Environ Sci Pollut R 12:342–346

Turgut C, Fomin A (2002) Sensitivity of the rooted macrophyte Myriophyllum aquaticum (Vell) Verdcourt to seventeen pesticides determined on the basis of EC50. Bull Environ Contam Toxicol 69:601–608

Villegas NA, González MCR, López ER, Aguilar RD, Marçal WS (1999) Evaluation of Daphnia magna as an indicator of toxicity and treatment efficacy of textile wastewaters. Environ Int 25:619–624

Wang C, Yediler A, Lienert D, Wang Z, Kettrup A (2002) Toxicity evaluation of reactive dyestuffs, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria Vibrio fischeri. Chemosphere 46:339–344

Wang C, Zhang SH, Wang PF, Hou J, Li W, Zhang WJ (2008) Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte Vallisneria natans (Lour) Hara. Aquat Toxicol 87:88–98

Wersal RM (2010) The conceptual ecology and management of parrotfeather [Myriophyllum aquaticum (Vell.) Verdc.]. Dissertation, Mississippi State University

Xu J, Zhang J, Xie H, Li C, Bao N, Zhang C, Shi Q (2010) Physiological responses of Phragmites australis to wastewater with different chemical oxygen demands. Ecol Eng 36:1341–1347

# 28 Metal Accumulation Capability of Weeds and Their Utilization in Phytoremediation Technology

Anand Mohan, Madhuri Girdhar, Hasibur Rehman, Anil Kumar, Shalini Saggu, and Abid Ali Ansari

## 28.1 Introduction

Phytoremediation is a cluster of technologies where plant-based accumulation and stabilization activities are utilized for remediation of environmental pollution and restoration of contaminated soil (Cunningham et al. 1997; Flathman and Lanza 1998). Decontamination of metal polluted soil through metal hyperaccumulating plants has gained increased attention these days due to their capability for accumulating heavy metals and having application in decontamination of metal polluted soil. Phytoremediation acts as an integrated multidisciplinary approach through which cleanup of contaminated soils can be achieved via combination of disciplines of plant physiology, soil microbiology, and soil chemistry (Cunningham and Ow 1996). The primary objective of phytoremediation becomes reduction and removal of toxic metals from the soil and restoration of soil to its natural state (Reeves and Baker 2000). The current works provide an informational insight into these technologies where eco-friendly techniques are implied to phytoremediation process, and the hyperaccumulating capability of various weeds (*Amaranthus* species, *Cannabis sativa*, *Solanum nigrum*, and *Rorippa globosa*) has been compared having enough capability for heavy metal accumulation. Weeds are suitable for this purpose because of their inherent resistant capability and their unsuitability for fodder purpose. Metals are required in different metabolic processes in all organisms with varied level of functions; however, since certain metals are toxic, plants have evolved systems for regulation, uptake, and distribution of metals. Uptake of metals occurs primarily through root system of plants; hence this becomes the primary site for regulation of accumulation. In extreme conditions, where metal concentration becomes very high in soil; leaves, roots and shoots display a unilateral effort by accumulating metal more than required by their physiology. Different studies have demonstrated that metal concentration in leaves is >0.1 mg/g dry weight for cadmium metal and >1 mg/g dry weight for lead, copper and nickel (Reeves and Baker 2000). Chemical and microbial entities of contaminated and distressed soil by heavy metals can be cleaned up and maintained by these accumulatory activities of such plants (Cunningham and Ow 1996). Tissues of higher plants accumulate a very high concentration of metals without showing toxicity (Klassen et al. 2000; Bennett et al. 2003).

Phytoremediation comprises various mechanisms: Phytoextraction, Phytovolatization, Rhizofiltration, and Phytodegradation (Fig. 28.1). Phytoextraction is a mechanism in which plant roots absorb contaminated groundwater and then transport it from roots to various parts of the plant (Salt et al. 1998). The cost involved in the phytoextraction as compared with the conventional soil remediation technique is tenfold less per hectare. It means phytoextraction is a cost-effective technique (Salt et al. 1995). The development of phytoextraction technique comes from the discovery of a variety of wild weeds, often endemic to naturally mineralized soils that concentrate high amount of essential and nonessential heavy metals. *Rorippa globosa* shows Cd-hyperaccumulation up to certain extent as shown in the work of Sun et al. (2007).

Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb and Zn (Ensley 2000). Rhizofiltration can be used for metals (Pb, Cd, Cu, Ni and Cr) which are retained only within the roots. It is a phytoremediative technique designed for the removal of metals in aquatic environments. Hydroponic technique is being used in which the plants first grow in nutrient medium and then they are transferred to the metal polluted sites, where the plants

A. Mohan, M.Sc., Ph.D. (✉) • M. Girdhar, M.Tech.
Department of Biotechnology, Lovely Professional University, Chehru, Phagwara, Punjab, India
e-mail: anandmohan77@gmail.com

H. Rehman, Ph.D. • S. Saggu, Ph.D. • A.A. Ansari, Ph.D.
Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia

A. Kumar
National Institute of Immunology, New Delhi, India

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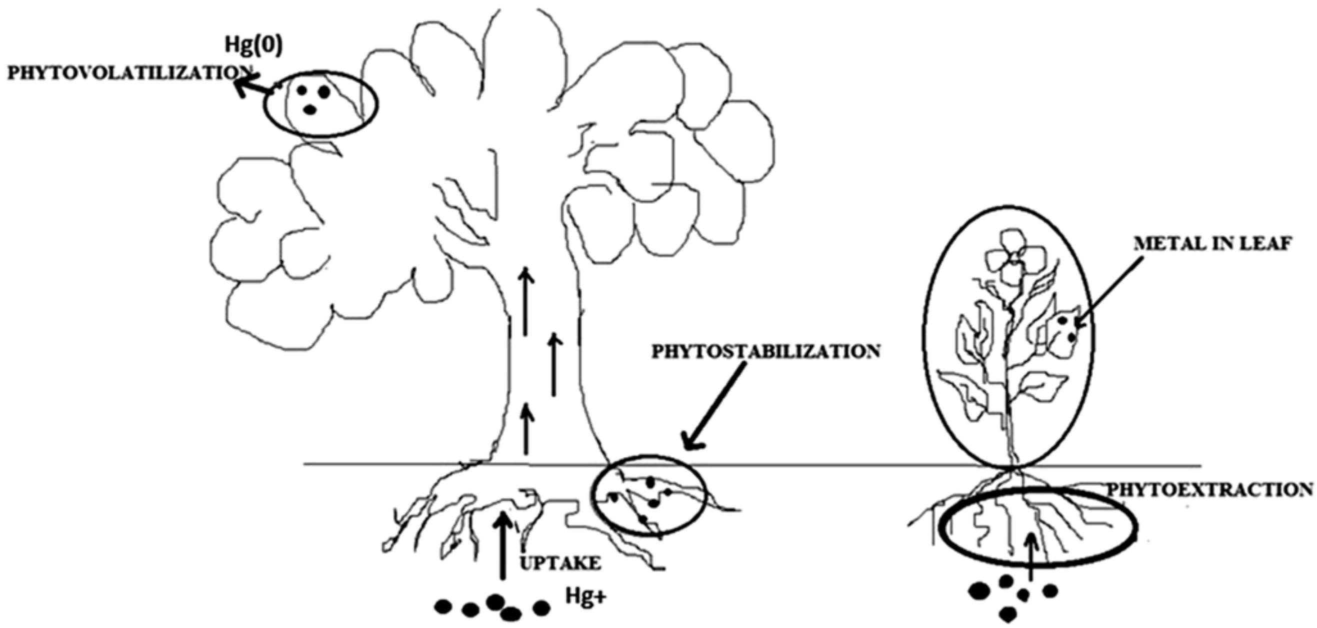


**Fig. 28.1** Phytoremediation processes and types, adapted from Singh et al. (2003) and Suresh and Ravishankar (2004)

**Table 28.1** Natural plant metal-hyperaccumulator species and their bioaccumulation potential

| Metals | Plant species | Amount [g/kg (d.m)] | Reference |
|---|---|---|---|
| Cd | *Rorippa globosa* | >1 | Sun et al. (2007) |
| Se | *Brassica juncea* | 2.0 | Orser et al. (1999) |
| Cr | *Salsola kali* | 2.9 | Gardea-Torresday et al. (2005) |
| Pb | *Thlaspi rotundifolium* | 0.13–8.2 | Reeves and Brooks (1983) |
| Cd | *Thlaspi caerulescens* | 10.0 | Lombi et al. (2001) |
| Ni | *Alyssum bertolonii* | >10.0 | Morrison et al. (1980) |
| Co | *Haumaniastrum robertii* | 10.2 | Brooks (1977) |
| Cu | *Ipomea* | 12.3 | Baker and Walker (1990) |
| Mn | *Phytolacca acinosa* | 19.3 | Xue et al. (2004) |
| As | *Pteris vittata* | 22.6 | Ma et al. (2001) |
| Zn | *Thlaspi caerulescens* | 30.0 | Baker and Walker (1990) |
| Zn | *Chenopodium album* L. | 33.5 | Malik et al. (2010) |
| Pb | *Amaranthus viridis* L. | >43.0 | Malik et al. (2010) |
| Cu | *Parthenium hysterophorus* L. | 59.3 | Malik et al. (2010) |

accumulate and concentrate the metals in their various body parts especially roots (Flathman and Lanza 1998; Salt et al. 1995; Dushenkov et al. 1995; Zhu et al. 1999) (Table 28.1).

Phytovolatization is a technique in which metals from the soil are taken up by the plant roots, and by the process of transpiration they are released in the environment. This process works only when the metals are volatile in nature (Hg and Se) (USPA 2000). Apart from metals, this has been established in case of organic contaminants also, as in the example of Poplar tree (*Liriodendron*) which is a phytovolatizer and volatilizes up to 90 % of the trichloroethane absorbed from contaminated soil (McGrath and Zhao 2003).

Phytodegradation which is also known as phytotransformation, is a process in which the breakdown of contaminants occurs by plants through metabolic processes within the plant, or in the close surrounding of the plant through plant root symbiotic associations (McGrath and Zhao 2003). A metabolic process known as *ex planta* occurs in which organic compounds are hydrolyzed into smaller units that can be absorbed by the plants. Some contaminants can be absorbed by the plant and are then broken down by plant enzymes. For the growth of the plant, these smaller pollutant molecules can be used as secondary metabolites (Prasad 1995).

## 28.2 Heavy Metal Uptake Mechanism by Plants

Hyperaccumulatory action of *Cannabis sativa* on cadmium contaminated soil has been shown in certain works. Some projects by U. S. Department of Energy have explored bioavailability of cadmium in the soil and they have suggested that it depends on soil pH, redox potential and rhizosphere chemistry. These factors determine the concentration of soluble Cd within the rhizosphere of the plant and the amount of Cd available for potential uptake by the plant. Soluble Cd could enter roots either by movement in the cell wall free space (apoplastic pathway) or by transport across the plasma membrane (PM) of root cells and movement through the cytoplasm (symplastic pathway). The large membrane potential which usually exists across the PM provides a driving force for the inward movement of Cd into cells. There are various types of channels that exist within the PM which allow the Cd transport into the different parts of the plant. Secondary level of accumulation has been observed in stems and leaves with accumulated amount of Cd found to a lesser extent (Linger et al. 2005). The metals are absorbed from the soil into the roots and shoots by the process of translocation (phytoextraction). After uptake by roots, translocation into shoots is desirable because the harvest of root biomass is generally not feasible (U. S. Department of Energy 1994). Plant uptake-translocation mechanisms are likely to be closely regulated. Plants generally do not accumulate elements beyond near-term metabolic needs, which are small ranging from 10 to 15 ppm of most trace elements, sufficient for most of the requirements (U. S. Department of Energy 1994). Hyperaccumulators are exceptions and can accumulate toxic metals much beyond these limits up to the levels of thousands of ppm. With this capability of hyperaccumulation, these plants could successfully be used for phytoremediation purposes. During the process; contamination is translocated from roots to shoots, which are harvested, causing contamination to be removed while leaving the original soil undisturbed (U. S. Department of Energy 1994).

Several physiological and biochemical rationales determine hyperaccumulatory behavior and activity. Poly chelatin formation is one of the important basic crucial factors for the hyperaccumulatory behavior. Polychelatins are the best characterized heavy metal chelator in plants, especially in the context of Cd tolerance (Cobbett 2000). Hemp (*Cannabis sativa*) roots demonstrated a strong resistance to heavy metals and have already shown hyperaccumulator-like potential (more than 100 mg/kg Cd in dry tissue), which more likely seems to depend on the plant development stage. High values of Cd accumulation achieved cannot be explained exclusively by passive ion uptake. Immobilization, by binding to the cell walls, is thought to play a minor role (Sanita di Toppi and Gabbrielli 1999). Polychelatins are known to be synthesized from glutathione (GSH) and its derivatives by enzyme phytochelatine synthase in the presence of heavy metal ions (Cobbett 2000; Rea et al. 2004). The synthesis of PC occurs in cytosol. With exposure of metal to the root of the plant, PCs coordinate to form ligand complexes with these metals, which are further sequestered into the vacuole. GSH also has a role in defense against heavy metals.

The other class of significantly notable chelating compound is metallothioneins. They are known to have a significant role in the detoxification of metals, and the induction of their synthesis in the plants occurs through exposure of root cells to heavy metals (Rauser 1999; Cobbett 2000; Clemens 2001; Hall 2002; Cobbett and Goldsbrough 2002; Rea et al. 2004). Metallothioneins (MTs) are sulfur-rich proteins of 60–80 amino acids, are known to contain 9–16 cysteine residues and are found in plants, animals and some prokaryotes (Rauser 1999; Cobbett 2000; Cobbett and Goldsbrough 2002). These cysteine-rich polypeptides exploit the property of heavy metals to bind to the thiol groups of proteins and detoxify them. Other well-known property of MTs is to participate in Cu homeostasis (Cobbett and Goldsbrough 2002).

Universal small polycations involved in numerous processes of plant growth and development are called as polyamines; they have anti-senescence and anti-stress effects. These distressing effects are owed due to their acid neutralizing and antioxidant properties, along with their membrane and cell wall stabilizing abilities (Zhao and Yang 2008). Technically polyamines strengthen the defense response of plants and modulate their activity against diverse environmental stresses including metal toxicity (Groppa et al. 2003), oxidative stress (Rider et al. 2007), drought (Yamaguchi et al. 2007), salinity (Duan et al. 2008) and chilling stress (Cuevas et al. 2008; Groppa and Benavides 2008). The accurate role of polyamines found in plants under metal stress has not been deduced yet. The most positive assumption regarding the functionality of polyamines is the protection of membrane systems and their stabilization from the toxic effects of metal ions particularly the redox active metals. Spermine, spermidine, putrescine and cadaverine are some of the important polyamines which have demonstrated the ability to scavenge free radicals in vitro (Drolet et al. 1986). Polyamines are also known to block the major vacuolar channels, the fast vacuolar cation channel. The accumulation of these vacuolar channels results in decreased ion conductance at the vacuolar membrane which facilitates metal ion compartmentation (Brüggemann et al. 1998).

### 28.2.1 General Properties of *Cannabis sativa*

*Cannabis sativa* is dioecious flowering herb. *Cannabis* is rich in Cannabinoids which are psychoactive and physiologically active chemical compound produced by the dioecious flowers of the herb. *Cannabis sativa* provides antispasmodic and muscle relaxant stimulating appetite (Zajicek et al. 2003). *Cannabis sativa* exhibits a great diversity with its prominence in both wild and cultivated areas, and hence can be utilized for the phytoremediation purpose (Mura et al. 2004). Native to central and southern Asia, *Cannabis* prefers a warm and humid climate, but are very resilient and can live in many habitats, so long as the soil pH is between 5 and 7.

### 28.2.2 *Cannabis sativa* Hyperaccumulative Action

Varied studies carried out on *Cannabis sativa* provide the leads that it can be used as a hyperaccumulator for different toxic trace metals like Lead, Cadmium, Magnesium, Copper, Chromium and Cobalt which pose a great risk to the ecological system. It has been established that the most of the sources of polluting metals are various anthropogenic activities such as smelting, sewage sludge distribution and automobile emissions (Foy et al. 1978; Chronopoulos et al. 1997; Prasad and Hagemeyer 1999; Dahmani-Muller et al. 2000). Hyperaccumulator plants can be used to remediate the metal contaminated soil from these anthropogenic activities. This technology implies on the aboveground harvestable plant tissues that have the phytoaccumulation capacity in the roots of plants to absorb, translocate and concentrate heavy metals from contaminated soil. The wild species which are endemic to metalliferous soil accumulate a very high concentration of metal from the soil (Baker and Brooks 1989). *Cannabis sativa* is also known as industrial hemp because it has the capability of hyperaccumulation of industrial waste. The potential of hemp crops is known to convert the waste land into cultivated land, especially the area contaminated with heavy metal pollution (lead, copper, zinc and cadmium) (Angelova et al. 2004). The hemp is well suited for phytoremediation and the fiber quality has not been negatively affected by uptake of metal.

Cd is known to be one of the most phytotoxic heavy metal (Salt et al. 1995; Prasad 1995). For the soil phytoremediation, a good alternative is provided by Hemp plant (*Cannabis sativa* L.). Except for roots, the highest concentrations of metal are found in leaves, whereas the lowest are typically observed in seeds (Ivanova et al. 2003). The photosynthesis pathway is influenced in two ways by the cadmium metal: (1) Cadmium metal disturbs indirectly water and ion uptake by the plant which has a negative effect on the plant water status (Seregin and Ivanov 2001). (2) It directly affects the chloroplast apparatus after entering the leaf cells. Cd concentrations of up to 72 mg/kg (soil) had no negative effect on germination of *Cannabis sativa*. It has been estimated from the post-conduction experiments that up to 100 ppm there is no effect of cadmium metal on the morphological growth of *Cannabis sativa*. The highest concentration of cadmium tolerance shown by the *Cannabis sativa* in roots is maximum 830 mg/kg and it does not affect the growth of the plant (Linger et al. 2005).

Plant viability and vitality is affected by cadmium metal in the leaves and stem of *Cannabis sativa* and was up to 50–100 mg Cd/kg [D.M] (dry mass). Control plants and plants growing on soil with 17 mg Cd/kg of soil show seasonal changes in photosynthetic performance. Under moderate cadmium concentrations, i.e., 17 mg Cd/kg of soil, hemp could preserve growth as well as the photosynthesis apparatus and long-term acclimatization at chronicle levels to Cd stress occurs. Growth on high Cd concentrations, i.e., above 800 mg/kg leads to a significant loss of vitality and biomass production. Shi et al. (2012) worked on 18 Hemp accessions in order to screen the accessions that can be cultivated in cadmium (Cd)-contaminated soils for biodiesel production. Pot experiments were carried out to evaluate the ability of Hemp for Cd tolerance and bioaccumulation when subjected to 25 mg Cd/kg (dry weight, DW) soil condition, in terms of plant growth, pigment contents, chlorophyll fluorescence and Cd accumulation at 45 days after seedling emergence. Pot experiment analysis was carried out and it was observed that most of the Hemp except USO-31, Shenyang and Shengmu, could grow quite well under 25 mg Cd/kg (DW) soil condition. A biomass of >0.5 g per plant, high tolerance factor (68.6–92.3 %), a little reduction in pigment content and chlorophyll fluorescence under 25 mg Cd/kg (DW) soil stress were observed in Yunma 1, Yunma 2, Yunma 3, Yunma 4, Qujing, Longxi, Lu'an, Xingtai, and Shuyang. The scientist concluded that these cultivars could be cultivated in Cd contaminated soils and had a strong tolerance to Cd stress (Shi et al. 2012). Hemp has been found to be highly cadmium-tolerant and very useful in bioaccumulation of cadmium with its superior ability to accumulate cadmium in shoots. Hemp does have a high capacity for phytostabilization. Hemp is tolerant to contaminants, has the ability to accumulate metals along with stabilization of contaminated areas and unlike most plants used in bioremediation, it offers additional end uses. The extraction capability for heavy metals from the soil makes the Hemp (*Cannabis sativa*) an excellent soil phytoremediation agent. Worldwide, Hemp can provide both an economic and sustainable solution to the contamination of soils. The utilization of various supplements of Hemp as a derived food has brought attention to the invisible negative effects that could be caused due to potential metal accumulation on the health of people of Romania reported in the recent years (Bona et al. 2007; Linger et al. 2002; Mihoc et al. 2012). The work done on the translocation rate of certain species showed that these species have high translocation rate as compared to other. The work concluded by Malik et al. (2010) shows that *Cannabis sativa* has high translocation rate as compared to other species for the metal Zn and

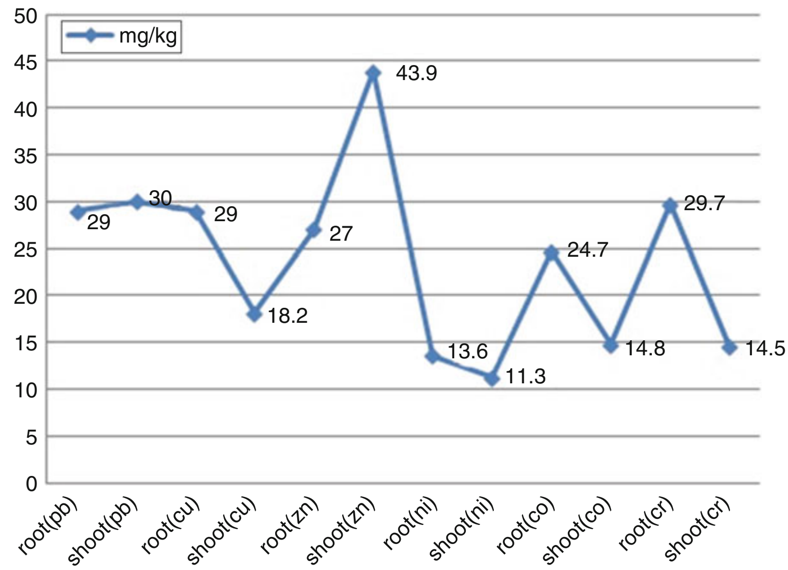


**Fig. 28.2** Representation of accumulation of toxic heavy metals by *Cannabis sativa* (Malik et al. 2010)

**Table 28.2** Hyperaccumulatory nature of *Cannabis sativa* depicted by accumulation of various metals (mg/kg) (d.m) in industrial areas (Malik et al. 2010)

| Concentration of metal (mg/kg) | Root | Shoot |
|---|---|---|
| Lead | 29 mg/kg | 30 mg/kg |
| Copper | 29 mg/kg | 18.2 mg/kg |
| Zinc | 27 mg/kg | 43.9 mg/kg |
| Nickel | 13.6 kg/mg | 11.3 mg/kg |
| Cobalt | 24.7 mg/kg | 14.8 mg/kg |
| Chromium | 29.7 mg/kg | 14.5 mg/kg |

could be used as a potential hyperaccumulator. It is estimated that the Translocation Factor value of *Cannabis sativa* for Zn is >1. Due to this the accumulation of metal Zn in shoots is high as compared to other heavy metals. The graph in Fig. 28.2 shows the hyperaccumulation by *Cannabis sativa* in its various tissues from contaminated soil having different heavy metals in elevated state. Investigation of metalliferous tissue of *Cannabis sativa* leads that accumulation of Zinc metal occurs maximum in shoots and it shows hyperaccumulating property by storing metal in their shoot. The least metal accumulated by *Cannabis sativa* was Ni in their shoot (Table 28.2). In another work it was reported that the geochemical characterization of soil and the nature of crops are the factors on which the accumulation of heavy metals depends; some of them have a high potential to accumulate higher concentrations of heavy metals (Linger et al. 2002). The researchers investigated and concluded that one of the Hemp varieties Zenit shows high bioaccumulation rate for iron, i.e., 1,859 mg/kg as compared to the other Hemp varieties, i.e., Diana, Denise, Armanca, Silvana (Mihoc et al. 2012) and this could possibly affect the health of people. This work brings into light the capability of specific *Cannabis* varieties for their extraction capability.

### 28.2.3 General Properties of *Solanum nigrum*

*Solanum nigrum* is commonly called as black nightshade. It belongs to the *Solanaceae* family. *S. nigrum* is widely used plant in ornamental medicine. *Solanum nigrum* L. is an annual herb 0.3–1.0 m in height (Wei et al. 2005). It is antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic and antipyretic herb. Compounds present in the *S. nigrum* are responsible for diverse activities. Ailments such as pain, inflammation and fever are treated with *S. nigrum* as traditionally acceptable method (Zakaria et al. 2006; Lee and Lim 2003; Raju et al. 2003). The flowering season of *Solanum nigrum* is from July to September and the seeds ripen from August to October. Herbal medication studies have proved that growth of cervical carcinoma in mice can be inhibited by this weed (Jian et al. 2008). Black nightshade is a fairly common herb found in many wooded areas. *Solanum nigrum* grows in damp shady spots (contaminated ground) and in waste lands (Wei et al. 2005). It also grows in cultivated lands. It is a native to Europe and Asia and further has been introduced in America, Australia and Africa through anthropogenic sources.

### 28.2.4 *Solanum nigrum* Hyperaccumulative Action

*Solanum nigrum* is a hyperaccumulator and has shown the effect of cadmium toxicity on the nitrogen metabolism in their leaves (Wang et al. 2007). Cadmium is very hazardous to human health adversely affecting kidney and lungs. The activity of nitrate reductase in plants is inhibited due to the effect of cadmium on the uptake and transportation of nitrates by affecting the nitrate assimilation (Hernandez et al. 1997).

*S. nigrum* could tolerate ≤12 mg/kg cadmium present in the soil and maintain N metabolism normal in the plant. However N metabolism is severely inhibited at levels of 48 mg Cd/kg. The nitrate reductase activity reduces significantly at 24 mg/kg but the activities of glutamine synthetase remain normal. *S. nigrum* appears to be an adequate species for phytoremediation of heavy metal contamination, especially Cd contamination and shows the hyperaccumulating properties. The effect of addition of different fertilizers on the phytoremediation capability of *S. nigrum* was investigated in a study conducted. Under pot-culture system, these experiments were carried out by fertilizer addition which increased the phytoextraction efficiencies of *S. nigrum* to Cd by increasing its shoot biomass. It was found that addition of chicken manure decreased Cd concentrations of *S. nigrum* but urea amendment did not affect organic Cd concentration. Considering the effect of decrease of available Cd in soil occurring by chicken manure, urea might be a better fertilizer for strengthening phytoextraction rate of *S. nigrum* to Cd, and chicken manure could be a better fertilizer for phytostabilization.

Zn is a micronutrient for plants and at higher concentrations it may become toxic (Broker et al. 1998; Ebbs and Cochin 1997). The Zn accumulation by *Solanum nigrum* decreases when manure or compost is added up to 40 % but there will be increase in the total biomass yield (Marques et al. 2007a).

Arbuscular mycorrhizal (AM) fungi support *Solanum nigrum* in its efforts to remediate the metal-contaminated soil. Arbuscular mycorrhizal (AM) fungi occur in the soil of most ecosystems, including polluted soils. The unique structures such as vesicles and arbuscules are formed by the fungi belonging to the phylum *Glomeromycota* (Burdett 2002). The wild weeds tolerance to biotic and abiotic stresses is enhanced by the presence of Arbuscular mycorrhizal fungi in the roots of the weeds present in contaminated area, as they provide a direct link between soil and roots (Joner and Leyval 1997). The availability of AMF in the roots increases the hyperaccumulation of metals by the plant. Arbuscular mycorrhizal fungi (AMF) are important root symbionts and partners that help in the removal of contaminants from the soil (Merharg and Cairney 2000). The fungal hyphae can extend into the soil and uptake large amounts of nutrients, including metals, to the host root. Plants provide important compounds for AMF survival; these fungi expand the contact surface between plants and soil, contributing to an enhanced plant uptake of macronutrients (Li et al. 1991) such as Zn (Burkert and Robson 1994). AMF helps plants adapt to metal-contaminated soils by either excluding the metals or enhancing their uptake by the plant. Arbuscular mycorrhizal fungi (AMF) are found everywhere in most terrestrial ecosystem, forming close or symbiotic associations with the roots of majority of plant (Smith and Read 1997). With the help of extended properties of metal absorption from soil through AMF, *Solanum nigrum* becomes highly capacitive for overall extraction of metal from contaminated soil. Apart from high absorption capacities, it also has sufficient accumulation capability and high translocation property which make *S. nigrum* an ideal tool for phytoremediation approach of metal-contaminated sites. Significantly higher level of zinc metal has been observed by workers (Marques et al. 2007b) in the *S. nigrum* supporting its hyperaccumulatory behavior. A good candidate for phytoremediation strategy would be a species that has good translocation of the metallic contaminant from the root to the stems and leaves, which means a higher translocation factor. High translocation factors (TF <1) obtained indicates that *S. nigrum* might be a good Zn phytoextractor, as the main metal accumulation occurs in the aboveground part of the plant.

Shenyang Zhangshi Irrigation area (SZIA) was polluted with high amount of cadmium and several set of studies have been carried out in this area. *Solanum nigrum* was used in situ as a phytoremediator in Cd polluted soil (Ji et al. 2011). Assessment of the performance of the plant over the whole growth stage was carried out. It was analyzed through experiments that aboveground biomass of single *Solanum nigrum* L. grew by a factor of 190, from $1.6 \pm 0.4$ g to $300.3 \pm 30.2$ g along with 141.2 times extractable Cd increase from $0.025 \pm 0.001$ to $3.53 \pm 0.16$ mg. The data analysis also showed that the percentage of biomass and extracted Cd in the stem increases from 20 to 80 % and from 11 to 69 %, respectively. After pot experimentations, analysis was carried out for highest Cd concentration in each part of *Solanum nigrum* plant and observed that at seedling stage the aboveground biomass was $16.1 \pm 1.1$ mg/kg, in stem it was observed as $12.4 \pm 1.1$ mg/kg and in leaf the values were $24.8 \pm 2.4$ mg/kg. The authors suggest that the results of their work provide reference values for the future research on the application of *Solanum nigrum* L. in phytoremediation or on agricultural strategies for phytoextraction efficiency enhancement. The pot experimentation establishes *Solanum nigrum* as a Cd hyperaccumulator with a maximum concentration of 125 mg/kg (Wei et al. 2005).

Graph describes the hyperaccumulating nature of *Solanum nigrum* in metal-contaminated soil (Fig. 28.3). Significant Zn metal accumulation occurs in the root tissue of *S. nigrum* and minimum accumulation is reported in their shoot tissue (Table 28.3).

### 28.2.5 General Properties of *Rorippa globosa*

*Rorippa globosa* an annual/perennial herb belonging to Brassicaceae family (*Mustard Family*) and the genus *Rorippa* (yellowcress), which grows to a height of 0.7 m (2 ft 4 in.). It is a flowering plant, usually with cross shape, yellow flowers,

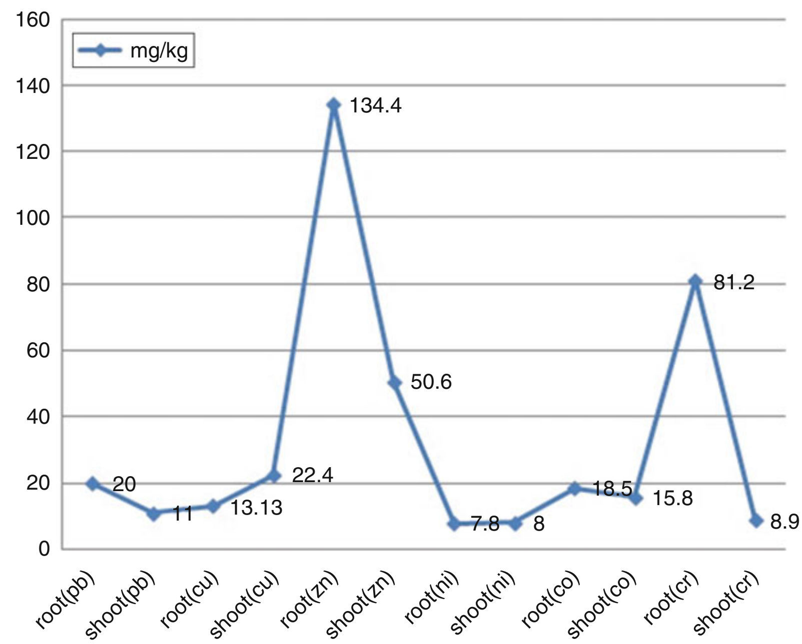


**Fig. 28.3** Representation of accumulation of toxic heavy metals by *Solanum nigrum* (Malik et al. 2010)

**Table 28.3** Hyperaccumulatory nature of *S. nigrum* depicted by accumulation of metals (mg/kg) (d.m) in industrial areas (Malik et al. 2010)

| Concentration of metal (mg/kg) | Root | Shoot |
|---|---|---|
| Lead | 20 mg/kg | 11 mg/kg |
| Copper | 13.13 mg/kg | 22.2 mg/kg |
| Zinc | 134.4 mg/kg | 50.6 kg/kg |
| Nickel | 7.8 kg/mg | 8 mg/kg |
| Cobalt | 18.5 mg/kg | 15.8 mg/kg |
| Chromium | 81.2 mg/kg | 8.9 mg/kg |

and peppery flavor. The flowering season of *Rorippa globosa* is from April to November. The *Rorippa globosa* is widely distributed in Europe through central Asia, Africa and North America. The habitat of this species is near river banks, moist areas, grasslands, and railroad embankments from near sea level to 2,500 m.

### 28.2.6 *Rorippa globosa* Hyperaccumulative Action

Phytoremediation-based studies work on the motive for screening out and breeding hyperaccumulative plants or hyperaccumulators that have an innate capacity to absorb and accumulate metals at higher levels (Baker and Brooks 1989). The leaves of *Rorippa globosa* show no phytotoxicity or biomass reduction when exposed to 25 µg Cd/g and the concentration of cadmium accumulated in leaves was up to 218.9 µg Cd/g dry weight (DW). Analysis of this study points towards strong self-protection ability of *Rorippa globosa* towards cadmium metal by adapting oxidative stress caused by the cadmium exposure (Sun et al. 2010). An attractive feature of *R. globosa* provides its plantation capability twice in a year in metal-contaminated soils. *R. globosa* can be harvested at its flowering phase based on the site climatic conditions and growth characteristics of the hyperaccumulator. This could result with an increase in the extraction efficiency of Cd in shoots of *R. globosa* by 42.8 % as compared to its single maturity state when the plant was transplanted into contaminated soils (Wei and Zhou 2006).

A comparative assessment of hyperaccumulative capability of two different species of *Rorippa* was carried out by Wei and Twardowska (2013). Six Cd treatments were experimentally designed and treatment was given to two species *Rorippa globosa* and *Rorippa palustri*. Different concentrations of Cd were used, i.e., 2.5, 5, 10, 20, and 40 mg/kg along with a control without Cd addition. The Cd hyperaccumulating properties of *R. globosa* showed that the cadmium in the aboveground organs was >100 mg/kg, with enrichment factor EF >1, translocation factor TF >1 with no significant biomass reduction at Cd doses >10 mg/kg, and lack of such properties in *R. palustri*, which made these species suitable for comparative studies (Wei and Twardowska 2013). The total root lengths were decreased by 39, 41.8, and 46.3 % expressing its tolerance limitation, when Cd concentrations were 10, 20, and 40 mg/kg. In *R. palustri* the total root lengths when compared with the control decreased by 55.3, 64.1, and 64.4 %, indicating its weak tolerance (Li et al. 2011). In comparative research analysis done by hydroponic experiments concluded that *R. globosa* showed high tolerance capability and acted as a good Cd hyperaccumulator as compared to the *R. palustri*.

Certain current studies show that the growth of *R. globosa* is skewed by the antagonist effect caused by the Cd and As metal exposure. It was observed that when the concentration of Cd in the soil was 10 mg/kg and the concentration of As was 50 mg/kg, the plant grows up to a height of 35.9 cm and the dry weight (d.w) of the shoots reaches up to 2.2 g per pot (Wei et al. 2005). At the same time the accumulation of cadmium in the leaves under the combined stress of Cd and As becomes higher as compared to the same level of stress caused by the cadmium itself. At low concentration of Cd and As in the soil, the height and the shoot biomass of *Rorippa globosa* increased but the high concentrations of As and Cd reduce the Cd accumulation in the shoot of the plant, which reflects a synergic effect on plant growth. Certain plants have the unique ability to transport, uptake and exclude various essential or nonessential metals through their roots (Fayiga et al. 2004). Exclusion and accumulation are the two main strategies that are essential to build a relationship between roots and metals. From the above study, another attractive feature of *Rorippa globosa* comes into the picture; it has the ability to accumulate Cd metal into their various body parts like stem, root, and leaves in the presence of arsenic metal but at the same time it has the ability to exclude arsenic metal (Yang et al. 2004).

Cd hyperaccumulation capacities in *Rorippa globosa* were investigated by Wei and Zhou (2006) in pot experiments and they concluded that in order to enhance the metal removing efficiency in a year, the two phase planting method can be utilized which measures the phytoextraction capability of plant by harvesting the plant at its flowering period. The biomass was 107.0 and 150.1 mg/kg of the Cd accumulation in stems and leaves respectively, when soil Cd added was concentrated to 25.0 mg/kg. *R. globosa* when harvested at its flowering phase yielded total dry stem and leaf biomass up to 92.3 % of its full maturity and the total cadmium concentration was up to 73.8 % and 87.7 % of that at the mature phase, respectively. Climatic condition of the site and the trait of the plant growth-based factors enable *R. globosa*, so that it could be transplanted into the contaminated soils twice in 1 year, by harvesting the hyperaccumulator at its flowering phase. Following the two phase planting method, the extraction efficiency of the plant increased by 42.8 % as compared to its single maturity state. This method of two phase planting significantly helps in increasing the Cd hyperaccumulation in contaminated sites by using the technique of phytoremediation over the course of a year.

Conclusion could be drawn that Cd-hyperaccumulator has the basic characteristics of weed plants and the benign feature of crops. Because of the unique features like nutrition-competitive ability, fast growth, high efficiency of photosynthesis, a short lifecycle and anti-pests capability. *Rorippa globosa* has incomparable advantages compared with other hyperaccumulators and its utilization as a potential phytoremediator could have substantial advantages (Sun et al. 2007) (Table 28.4 and Fig. 28.4).

**Table 28.4** Representation of the antagonistic effect showed by the Cd and As on the bioaccumulation potential of Cd hyperaccumulator *R. globosa* (Sun et al. 2007)

| Conc. of Cd and As (mg/kg) | Accumulation of Cd (mg/kg) in root | Accumulation of Cd (mg/kg) in shoot |
|---|---|---|
| Ck (control) | 0 | 0 |
| Cd10+As50 | 0.002 | 0.09 |
| Cd10+As250 | 0 | 0.025 |
| Cd25+As50 | 0.015 | 0.2 |
| Cd25+As250 | 0.01 | 0.1 |
| Cd50+As50 | 0.022 | 0.25 |
| Cd50+As250 | 0 | 0.12 |

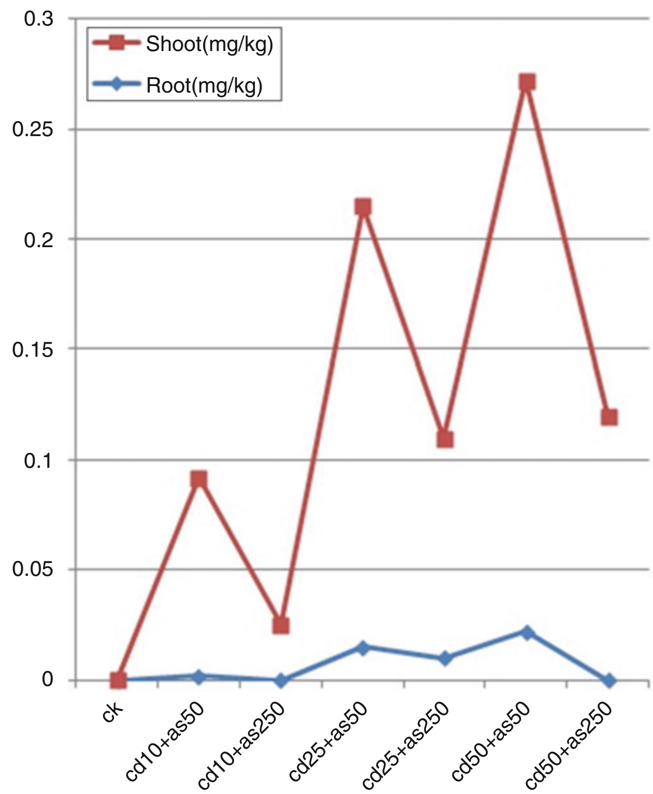


**Fig. 28.4** Representation of antagonistic effect by Cd and As on the bioaccumulation potential of Cd-hyperaccumulator *R. globosa* (Sun et al. 2007)

### 28.2.7 General Properties of *Amaranthus* sp.

Amaranthus belonging to the family Amaranthaceae comprises a series of wild, weedy, cultivated species and found worldwide in almost all agricultural environments. Amaranth is a very ancient crop. Its presence in Mexico dates from 4000 B.C. in Tehuacan, Puebla (Teutonico and Knorr 1985), and thus it is one of the oldest known plants. Amaranthus species have different centers of domestication and origin, being widely distributed in North America, Central America, and the South American Andes, India, and Nepal where the greatest genetic diversity is found (Sun et al. 1999; Xu and

Sun 2001; Zheleznov et al. 1997). It is estimated that there are 87 species of Amaranthus: 17 in Europe, 14 in Australia and 56 in America (Mujica and Jacobsen 2003). Three species of the genus *Amaranthus* produce edible seeds: *Amaranthus hypochondriacus*, grown in Mexico; *Amaranthus cruentus*, grown in Guatemala and Mexico; and *Amaranthus caudatus*, grown in Peru. This grain was an important nutrient for the Aztec, Maya and Inca. Due to its high-quality protein, especially its relatively high Lys content (Downton 1973) and the presence of sulfur amino acids (Segura-Nieto et al. 1992), this crop has received considerable attention as a supplement to cereals and legumes to prevent protein malnutrition and is known as pseudocereal (Barba de la Rosa et al. 1992; Zheleznov et al. 1997; Gorinstein et al. 2001).

### 28.2.8 *Amaranthus* sp. Hyperaccumulative Action

Hyperaccumulatory activity of various *Amaranthus* sp. has been investigated by various researchers. More researches have been carried out on the weed *Amaranthus* and it has been concluded that in a test of phytoextraction, the scientists found a crop (*Amaranthus hypochondriacus*) which not only grows rapidly but also accumulates high levels of Cd metal (Li et al. 2009). When intercropped with maize, *A. hypochondriacus* accumulated over 50 mg/kg Cd in shoots and over 90 mg/kg Cd in roots from soil containing 3 mg/kg Cd. *Amaranthus hypochondriacus* has been widely used as forage for cattle. It grows very fast with a very high biomass, having an average biomass yield of 10–15 t h/m$^2$ (DW). In another experiment of monoculture, *A. hypochondriacus* accumulated more than 100 mg/kg Cd from soil containing 5 mg/kg Cd. Since this plant has long been used as forage species, the cultivation systems of the crop are well established and highly mechanized. It has great potential to efficiently extract Cd from contaminated soils. *Amaranthus* has been cultivated for a long time on a large scale in China, producing a good quality of forage. They can grow in regions from the southernmost to the northernmost in China. The results suggested that amaranth has great potential in phytoremediation of farmlands in China, where Cd contamination largely ranged from 2 to 8 mg/kg. The tested varieties of Amaranth were introduced from America, and they can grow in many places of the world. In the pot experiment, Amaranth had a dry biomass of 42–54 g per plant in treatment of NPK fertilizer. In the farmland, tested varieties of Amaranth could achieve a fresh shoot biomass of 120,000–225,000 kg h/m$^2$ for K112, 150,000–300,000 kg h/m$^2$ for K472, and 120,000–195,000 kg h/m$^2$ for R104 (Kong and Yue 2003). Obviously, the biomass accumulation of the Amaranth was much larger than any other Cd hyperaccumulators like *Viola baoshanensis* of 6.5 t/ha (DW), *Sedum alfredii* of 5.5 t/ha (DW) and *Vertiveria zizanioides* of 30 t/ha (DW) (Zhuang et al. 2007). Easy cultivation of Amaranth in farmland, together with large biomass, is an important merit superior to many Cd hyperaccumulators in removing heavy metal of farmland. Workers conducted another experiment (Li et al. 2010) and showed that Amaranth could tolerate Cd contamination as high as 16 mg/kg. It has been further reported that amaranth favors high K (Li et al. 2006). Potassium might play a vital role in the large biomass achieved by Amaranth, a possibility that needs further rework to optimize biomass yields. Biomass of heavy metal accumulators is a key factor in phytoremediation, however, application rates of fertilizers is an another factor. High aboveground tissue concentrations of heavy metal and high biomass production are both critical for successful Phytoextraction.

Researchers worked on three amaranth cultivars and tested that they could accumulate high levels of Cd in their tissues, especially leaves, from rather low levels of Cd in soil (5 mg/kg). Leaf Cd generally exceeded 100 mg/kg, the criterion for a Cd hyperaccumulator. In phytoextraction, Amaranth cultivars might be superior to *Solanum nigrum* L., another reported Cd hyperaccumulator. This species could accumulate 103.8 mg/kg Cd in stems and 124.6 mg/kg Cd in leaves from soil containing 25 mg/kg Cd (Wei et al. 2005), a lower extraction efficiency than amaranth. In this study, the BCF (bio concentration factor) and TF (translocation factor) of the three amaranth cultivars ranged from 17.7 to 29.7 and 1.0 to 2.0, respectively. The BCFs of amaranth were higher than many other previously reported Cd accumulators, for instance, 2.4 for *Viola baoshanensis* (Liu et al. 2004), 3.5 for *Cardaminopsis halleri* (Kupper et al. 2000), 5.0 for *Solanum nigrum* L. (Wei et al. 2005) and 6.0 for *Rorippa globosa* (Wei and Zhou 2006). Application of fertilizers often increased Cd content in leaf and decreased Cd in root, resulting in a higher TF. However, fertilizers did not greatly impact BCF in most cases. Lower BCFs were observed in K112 (20.1) and R104 (20.9) with NPK treatment compared to the control. The BCF values are more important than shoot concentration, when one considers the potential of phytoextraction for a given species (Zhao et al. 2003). Large BCFs of amaranth for Cd, combined with TF >1, suggest the species has great potential for practical use in phytoremediation of Cd contaminated soil. In summary, amaranth cultivars (K112, R104, and K472) could grow in soil containing 5 mg/kg Cd without visible toxic symptom, accumulating Cd in their harvestable aboveground tissues ranging from 95.1 to 179 mg/kg, with BCFs of 17.7–29.7 and TF of 1.0–2.0. Increased Cd availability, as a result of lowered pH caused by fertilizers, ensured a good uptake of Cd when fertilized. Fertilizers containing only N or N and P combined did not markedly increase dry biomass of the three cultivars, leading to a limited increment of Cd accumulation. NPK fertilizer greatly increased dry biomass, by factors of 2.7–3.8, resulting in a large increment of Cd accumulation. The work has been carried out in greenhouse pot experiment, where phytoextraction potential for Cd for three amaranth cultivars (*Amaranthus hypochondriacus* L. Cvs. K112, R104 and K472) was conducted and the effect of application of N, NP and NPK fertilizer on Cd uptake of the three cultivars from soil contaminated with 5 mg/kg Cd was observed (Li et al. 2012). All three amaranth cultivars had high levels of Cd concentration in their tissues, which ranged from 95.1 to 179.1 mg/kg in leaves, 58.9 to 95.4 mg/kg in stems and 62.4 to 107.2 mg/kg in roots, resulting in average bioaccumulation factors ranging from 17.7 to 29.7. Application of N, NP or NPK fertilizers usually increased Cd content in leaves but decreased Cd content in stem and root. Fertilizers of N or NP combined did not substantially increase dry biomass of the three cultivars, leading to a limited increment of Cd accumulation. Amaranth cultivars (K112, R104, and K472) have great potential in phytoextraction of Cd contaminated soil. They have the merits of high Cd content in tissues, high biomass, easy cultivation, and little effect on Cd uptake by fertilization (Li et al. 2012).

Another experiment was carried out by the workers in order to prove the success of Amaranth weed as a good accumulator of heavy metals. The experiment conducted on the three amaranth hybrids (*Amaranthus paniculatus* f. *cruentus* (Vishnevyi dzhem), *A. paniculatus* (Bronzovyi vek) and *A. caudatus* f. *iridis* (Izumrud)) was grown in the climate controlled chamber on Jonson nutrient medium supplemented with 2 μM $Fe^{3+}$ EDTA. The red leaf hybrid Vishnevyi dzhem accumulated the greatest amount of nickel. Already at 150 μM $NiCl_2$ in medium, its content within the plants was about 2 mg/g dry wt, whereas in the presence of 250 μM $NiCl_2$, it was equal to 4 mg/g dry wt, which exceeds fourfold the admissible level of toxicity for grain crops (Liphadzi and Kirkham 2006). Such high content of Ni in the aboveground biomass in amaranth, the intensive crop produces about 100 t/ha of green mass, acts promising for its usage as phytoremediation of Ni contaminated territories. At the same time, in the range of relatively low $NiCl_2$ concentrations (50–100 μM) in medium, the amaranth hybrid Vishnevyi dzhem accumulated the lower biomass and manifested chlorosis of young leaves, a typical symptom of Fe deficit, which resulted from a disturbance of Fe influx to them. At present, low availability of Fe leading to the yield losses is characteristic of one third of agricultural areas with high content of calcium and alkaline pH (Briat et al. 2007). As evident from the research, plant growing in the presence of 100 μM $Fe^{3+}$-EDTA resulted in the marked increase in the biomass of upper leaves and roots at similar $NiCl_2$ concentrations as in the presence of 2 μM Fe. In this case, the content of iron in the upper leaves rose sharply at 50 μM $NiCl_2$ in medium, as compared with plants grown at 2 μM $Fe^{3+}$-EDTA, which suffered from Fe deficit to the greatest degree. At the high iron dose in medium, nickel accumulation in leaves was slightly reduced, although the total nickel efflux remained unchanged due to the increase in the aboveground biomass. All data obtained indicate that the intensity of nickel accumulation in plants and plant tolerance to it depends largely on the level of iron in the cells (Shevyakova et al. 2011b).

On the basis of data obtained, it seems possible that amaranth plant tolerance to relatively high nickel concentrations (150–200 μM) in medium accompanied by its enhanced accumulation in plants might be determined by PA (Polyamine) accumulation in leaves. It is not excluded that, under conditions of heavy metal accumulation in the cells, PA functions as antioxidants, protecting cells against metal-induced oxidative stress (Shevyakova et al. 2011a; Stetsenko et al. 2011). A capacity of exogenous PA to increase the phytoremediation potential of amaranth plants allows us to explain results. According to obtained data, the relatively low $NiCl_2$ concentration (50 μM) suppressed substantially shoot biomass accumulation. However, at 150–250 μM $NiCl_2$, the degree of suppression was reduced in spite of the presence of high nickel concentrations in medium. The results of experiments with PA treatments permit a suggestion that, in response to severe stress exerted by 150–250 μM $NiCl_2$, amaranth plants synthesized low-molecular organic compounds with chelating or protective action, such as for example, polyamines and this provides for active shoot biomass accumulation under conditions of severe stress. Thus, the experiments performed showed that when you study the phytoremediation potential of plants accumulating nickel in the aboveground organs, their capacity to maintain iron homeostasis and antioxidant system functioning should be taken into consideration. The accumulation of polyamines and other low-molecular compounds manifesting chelating or stress-protectory properties should be also analyzed (Shevyakova et al. 2011b).

Some other workers investigated metal accumulating capability of another species of *Amaranthus* viz., *Amaranthus paniculatus* L. These plants were grown for 1 week in Ni-spiked growth solutions at 0, 25, 50, 100, 150 M $NiCl_2$ in hydroponics under controlled climate conditions. Results showed a high tolerance to Ni in plants exposed to low Ni concentrations. Tolerance decreased as Ni concentration in the growth solutions enhanced. Ni concentrations in plant organs (root, stem and leaves) revealed a trend to increase in parallel with the enhancement of Ni content in the growth solution. The ability to accumulate Ni in plants was also evaluated by calculating the bioconcentration factor (BCF). An inverse relation between BCF and Ni concentrations in the growth solution was evidenced. Ni phytoremoval ability of *A. paniculatus* plants was particularly appreciable at 25 M $NiCl_2$, where more than 65 % of the initial Ni amount was taken up by plants in 1 week of treatment. The capability of plants to translocate Ni from roots to shoots (stem + leaves) was evaluated by the translocation factor (Tf). Results displayed a low Tf in plants exposed to low Ni concentration, suggesting a tolerance mechanism to protect physiological processes occurring in leaves. Overall, *A. paniculatus* plants showed a valuable capability to phytoremediate and decontaminate Ni-polluted waters, particularly at low Ni concentrations.

*A. paniculatus* plant showed a good tolerance to Ni, evaluated by the Tolerance index (Ti). Ti was higher than 0.8 at 25 M $NiCl_2$, decreasing at 50 and 100 M $NiCl_2$. A marked reduction of Ti was observed in plants exposed at 150 M Ni-spiked growth solution, with a value lower than 0.4. In particular, the highest Ni concentrations were detected in the roots of plants exposed to 100 and 150 M $NiCl_2$, but a remarkable Ni concentration was also measured at 50 M $NiCl_2$. In stems, Ni concentration was particularly high in plants treated with 150 M $NiCl_2$. Lower Ni concentrations were found in 50 and 100 M Ni-treated plants (Brooks et al. 1977). In leaves, a higher Ni concentration was found in plants exposed to 150 M $NiCl_2$ in comparison with plants treated with the other Ni concentrations. To evaluate the ability of plants to concentrate Ni from the external solutions in their tissues, the bioconcentration factor (BCF) was calculated. An ability to concentrate Ni more than 30-fold from the nutrient solution was observed in *A. paniculatus* plants exposed to 25 and 50 M $NiCl_2$. However, this value is on average near tenfold lower than that reported in Ni-hyperaccumulating plants (Galardi et al. 2007). The capability of *A. paniculatus* plants to remove Ni from the Ni-spiked growth solution was followed along the experimental time interval. A different Ni phytoremoval trend was exhibited by *A. paniculatus* plants depending on the Ni concentration in the growth solution. In accordance with the tolerance responses, plants exposed to 25 M $NiCl_2$ exhibited a higher and more constant Ni removal ability along time compared to plants exposed to 50, 100 and 150 M $NiCl_2$, succeeding in removing more than 65 % of the initial Ni content of the growth solution. Lower removal abilities were found in plants treated with 50 and 100 M $NiCl_2$. Plants exposed to 150 M $NiCl_2$ showed the lowest Ni removal ability, being the Ni content at the end of the treatment period near 70 % of the initial one. Moreover, these plants exhibited a strong reduction of Ni removal ability just after 2 days from the start of the Ni treatments, evidencing metabolic disturbances exerted by Ni to root uptake processes. The absorbed metal can be translocated from roots to the aerial plant organs through the xylem fluid, commonly bound to metal chelating compounds. To measure the capability of plants to transfer the absorbed metals to the shoots, the translocation factor (Tf) was applied. Data showed a remarkable higher Tf in control plants, where Ni concentration in roots depended only on the seed supply compared to that of plants treated with different Ni concentrations. This result was consistent with Galardi et al. (2007), even if in that work the ratio between Tf of control plants and that of Ni-treated plants was far lower than that found in these experiment. Among Ni-treated plants, a higher Tf was calculated in plants exposed to 150 M $NiCl_2$. The Tf values observed for *A. paniculatus* in this experiment are notably lower than those calculated from data reported by Galardi et al. (2007) in different ecotypes of *Alyssum bertolonii*, a Ni hyperaccumulator plant, exposed to Ni in similar experimental conditions. In control plants, the highest Tf can be ascribed to the physiological Ni demand of shoots to sustain leaf metabolic functions, such as enzyme activities and photosynthetic reactions. On the contrary, in 150 M Ni-treated plants, a higher Tf can be associated with an impairment of the metabolic processes regulating metal transport, due to the extremely high Ni concentration in the roots. The lower Tf observed in plants exposed to 25, 50 and 100 M $NiCl_2$, unrelated to the metal concentration in the growth solution, can be attributed to a tolerance response aiming at reducing metal presence in leaf cells, preserving physiological functions (Seregin and Kozhevnikova 2006). Results evidenced that, up to a concentration of 50 M $NiCl_2$ in the growth solution (a Ni concentration near 150-fold higher than that allowed in waters by Italian law), plants can maintain adequate physiological functions, allowing to accumulate remarkable amounts of Ni in their tissues while tolerating them. Although the Ni bioconcentration potential expressed by this plant species was far lower than that reported for Ni-hyperaccumulation plants, the good ability to remove Ni from contaminated solutions, especially at low Ni concentrations, represents a valuable characteristic to exploit for the utilization of this plant species for the decontamination of Ni-polluted waters (Pietrini et al. 2013).

The results obtained from plant analysis showed that concentration of cadmium in shoots of *Amaranthus* in all treatments was very high. It is concluded that *Amaranthus* had suitable ability for phytoremediation by Phytoextraction method, transmitting more cadmium from root to shoot. In *Amaranthus*, the highest iron was observed in the strains, i.e., B2Cd50, B2Cd200, and B1Cd200 treatments respectively. The highest concentration of zinc in *Amaranthus* was observed in the strains, i.e., B2Cd200, B2Cd100, and B1Cd0 respectively. Zinc concentration in different treatments of *Amaranthus* had significant difference in terms of concentration of cadmium and consumption of inoculant. In a calcareous soil of Karaj region (Fine Loamy, Mixed Super Active Thermic Xeric Haplocambids) and in green house conditions, effect of culture of the plant, i.e., *Amaranthus* and three levels of control inoculants (BO), *Bacillus mycoides M1* (B1), *Micrococcus roseus M2* (B2), and four levels of control cadmium concentration (0, 50, 100, and 200 mg/kg) was studied in a factorial experimental design with random blocks basic design with three replications. Concentration of cadmium, iron and zinc was measured in shoot and root as a function of dry material and photosynthetic chemical efficiency. The analysis of variance analysis showed that application of inoculant significantly ($P<0.01$) increased phytoremediation efficiency and effect of *Amaranthus* in cadmium phytoextraction was high as studied in the research. Treatments of cadmium increased concentration of this element in plant and decreased photosynthetic quenching (Fv/Fm) (Zadeh et al. 2008).

## 28.3 Conclusion

Cleanup of heavy metal-contaminated soils can be achieved through the Phytoremediation-based technologies which are emerging and promising bio-based technique with low-cost inputs as compared to chemical-based technologies. Advancement is going on in various aspects of phytoremediation and for the absorption of heavy metals, where basic process understanding responsible for the remediation processes is being addressed. Weeds can act at potential level in metal-contaminated soil accumulating metals through their hyperaccumulation characteristics and hence proving their phytoremediation capability. In case of *Solanum nigrum*, arbuscular mycorrhizal fungi play an important role in the accumulation of Zinc metal. Addition of fertilizers including chicken manure plays an important role in stabilization and extraction protocols. *Cannabis sativa* shows its hyperaccumulation nature by accumulating cadmium metal. *Rorippa globosa* also shows Cd-hyperaccumulation characteristic by showing a unique property of antagonistic effect on the growth and biomass concentration in the presence of arsenic metal. Two phase planting is proposed in case of *Rorippa globosa* for effective accumulation and extraction-based phytoremediation protocols for cadmium metal. The hyperaccumulating capabilities of *Amaranthus species* are quite noticeable for Ni and Cd metals. The role of NPK fertilizer addition has also been positive on the removal capabilities. The hyperaccumulating nature of plants also depends on type of species, soil quality, and its inherent control. Weeds undertaken in the current study are capable at sufficient level for bioaccumulation and still they are capable of maintaining their growth rates and reproduction levels as compared with controls in studies undertaken. Pilot-scale studies will bring out the true application of these weeds; hence they should be carried out by researchers for recording more analysis, so they can be part of systemic metallic component removal treatment plants in industrial and municipal level wastewaters.

**Conflict of Interest** No conflict of interest reported in the current work.

## References

Angelova V, Ivanova R, Delibaltova V, Ivanov K (2004) Bioaccumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp). Ind Crops Prod 19:197–205

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC, Boca Raton, FL, pp 155–177

Barba de la Rosa AP, Paredes-Lopez O, Gueguen J (1992) Globulins fraction from amaranth: studies on subunit composition by ultracentrifugation and chromatography. J Agric Food Chem 40: 937–940

Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M, Pilon-Smits EA (2003) Analysis of transgenic Indian Mustard plants for phytoremediation of metals-contaminated mine tailings. J Environ Qual 32:432–440

Bona E, Marsano F, Maria Cavaletto D, Berta G (2007) Proteomic characterization of copper stress response in Cannabis sativa L. roots. Proteomics 7(7):1121–1130

Briat JF, Curie C, Gaymard F (2007) Iron utilization and metabolism in plants. Curr Opin Plant Biol 10:276–282

Broker CM, Cox FR, Tucker MR (1998) Zinc and copper toxicity in peanut, soybean, rice and corn in soil mixtures. Commun Soil Sci Plant Anal 29:2991–3005

Brooks RR (1977) Copper and cobalt uptake in *Haumaniastrum* sp. Plant Soil 48:541–544

Brooks RR, Lee J, Reeves RD, Jaffrè T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57

Brüggemann LI, Pottosin II, Schönknecht G (1998) Cytoplasmic polyamines block the fast-activating vacuolar cation channel. Plant J 16:101–105

Burdett MC (2002) Convolution of roots and mycorrhizas of land plants. New Phytol 154(2):275–304

Burkert B, Robson A (1994) Zn uptake in subterranean clover (*Trifolium subterraneum L.*) by three vesicular arbuscular mycorrhizal fungi in a root free sandy soil. Soil Biol Biochem 26:1117–1124

Chronopoulos J, Haidouti C, Chronopoulou-Sereli A, Massas I (1997) Variations in plant and soil lead and cadmium content in urban parks in Athens, Greece. Sci Total Environ 196:91–98

Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212(4):475–486

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cuevas JC, Lopez-Cobollo R, Alcazar R, Zarza X, Koncz C, Altabella T et al (2008) Putrescine is involved in *Arabidopsis* freezing tolerance and cold acclimation by regulating ABA levels in response to low temperature. Plant Physiol 148:1094–1105

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719

Cunningham SD, Shann JR, Crowley D, Anderson TA (1997) Phytoremediation of contaminated water and soil. In: Kruger EL, Anderson TA, Coats JR (eds) Phytoremediation of soil and water contaminants, vol 664, American Chemical Society symposium series. American Chemical Society, Washington, DC, pp 2–19

Dahmani-Muller H, Van Oort F, Balabane M (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Pollut 109:231–238

Downton WJS (1973) Amaranthus edulis: a high lysine grain amaranth. World Crops 25:20

Drolet G, Dumbroff EB, Legge RL, Thompson JE (1986) Radical scavenging properties of polyamines. Phytochemistry 25:367–371

Duan JJ, Li J, Guo SR, Kang YY (2008) Exogenous spermidine affects polyamine metabolism in salinity-stressed Cucumis sativus roots and enhances short-term salinity tolerance. J Plant Physiol 165:1620–1635

Dushenkov V, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239–1245

Ebbs SD, Cochin LV (1997) Toxicity of zinc and copper to Brassica species: implications for phytoremediation. J Environ Qual 26:776–781

Ensley BD (2000) Rational for use of phytoremediation. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York, pp 3–12

Fayiga AO, Ma LQ, Cao XD et al (2004) Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator *Pteris vittata L.* Environ Pollut 132(2):289–296

Flathman PE, Lanza GR (1998) Phytoremediation: current views on an emerging green technology. J Soil Contam 7:415–432

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29:511–566

Galardi F, Corrales I, Mengoni A, Pucci S, Barletti L, Barzanti R, Arnetoli M, Gabbrielli R, Gonnelli C (2007) Intra-specific differences in nickel tolerance and accumulation in the Ni-hyperaccumulator Alyssum bertolonii. Environ Exp Bot 60:377–384

Gardea-Torresday JL, De la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jiminez G, Cano-Aguilera I (2005) Differential uptake and transport of trivalent and hexavalent chromium by tumble weed (Salsola kali). Arch Environ Contam Toxicol 48:225–232

Gorinstein S, Delgado-Licon E, Pawelzik E, Heriyati Permandy H, Weisz M, Trakhtenberg S (2001) Characterization of soluble amaranth and soybean protein based on fluorescence, hydrophobicity, electrophoresis, amino acid analysis, circular dichroism, and differential scanning calorimetry measurements. J Agric Food Chem 49:5595–5601

Groppa MD, Benavides MP (2008) Polyamines and abiotic stress: recent advances. Amino Acids 34:35–45

Groppa MD, Benavides MP, Tomaro ML (2003) Polyamine metabolism in sunflower and wheat leaf discs under cadmium or copper stress. Plant Sci 161:481–488

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Hernandez LE, Grate A, Carlene-Ruiz R (1997) Effects of cadmium on the uptake, distribution and assimilation of nitrate in *Possum sativa*. Plant Soil 189:97–106

Ivanova R, Angelova V, Delibaltova V, Ivanov K, Shamov D (2003) Accumulation of heavy metals in fibre crops flax, cotton and hemp. J Environ Prot Ecol 4:31–38

Ji P, Song Y, Sun T, Liu Y, Cao X, Xu D, Yang X, McRae T (2011) In-situ cadmium phytoremediation using Solanum nigrum l.: the bio-accumulation characteristics trail. Int J Phytoremediation 13:1014–1023

Jian L, Qingwang L, Tao F, Kun L (2008) Aqueous extract of *Solanum nigrum* inhibit growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia 79(7–8):548–556

Joner EJ, Leyval C (1997) Uptake of Cd by roots and hyphae of *Glomus mosseae/Trifolium subterraneum mycorrhiza* from soil amended with high and low concentration of cadmium. New Phytol 135:353–360

Klassen SP, McLean JE, Grossel PR, Sims RC (2000) Fate and behavior of lead in soils planted with metal-resistant species (River birch and smallwing sedge). J Environ Qual 29:1826–1834

Kong FL, Yue SX (2003) Characteristics of different varieties of amaranth and feeding effect for cattle. Shan Dong Forage 10:27–28

Kupper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator Arabidopsis halleri. Planta 212:75–84

Lee SJ, Lim KT (2003) Antioxidative effects of glycoprotein isolated from *Solanum nigrum Linne* on oxygen radicals and its cytotoxic effects on the MCF-7 cell. J Food Sci 68:466–470

Li XL, Marschner H, George E (1991) Acquisition of phosphorus and copper by VA mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil 136:49–57

Li TX, Ma GR, Zhang XZ (2006) Root exudates of potassium-enrichment genotype grain amaranth and their activation on soil mineral potassium. Chin J Appl Ecol 17:368–372

Li NY, Li ZA, Zhuang P, Zou B, McBride M (2009) Cadmium uptake from soil by maize with intercrops. Water Air Soil Pollut 199:45–56

Li NY, Lu HP, Li ZA, Zhuang P, Qiu J (2010) Tolerance and accumulation of cadmium in soil by *Amaranthus hypochondriacus* L. Chin J Appl Environ Biol 16(1):028–032

Li T, Di Z, Islam E, Jiang H, Yang X (2011) Rhizosphere characteristics of zinc hyperaccumulator *Sedum alfredii* involved in zinc accumulation. J Hazard Mater 185:818–823

Li NY, Fu QL, Zhuang P, Guo B, Zou B, Li ZA (2012) Effect of fertilizers on Cd uptake of Amaranthus hypochondriacus, a high biomass, fast growing and easily cultivated potential Cd hyperaccumulator. Int J Phytoremediation 21:23–36

Linger P, Mussing J, Fisher H, Kobert J (2002) Industrial hemp (*Cannabis sativa L.*) growing on heavy metal contaminated soil fibre quality and phytoremediation potential. Ind Crop Prod 16(1):33–42

Linger P, Ostwald A, Haensler J (2005) *Cannabis sativa L.* growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. Biol Plant 49(4):567–576

Liphadzi MS, Kirkham MB (2006) Physiological effects of heavy metals on plant growth and function. In: Huang B (ed) Plant–environment interactions. CRC, Boca Raton, FL, pp 243–270

Liu W, Shu WS, Lan CY (2004) Viola baoshanensis, a plant that hyperaccumulates cadmium. Chin Sci Bull 49:29–32

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30:1919–1926

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kenelly ED (2001) A fern that hyperaccumulates arsenic. Nature 409:579–582

Malik RN, Husain SZ, Nazir I (2010) Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad. Pak J Bot 42(1):291–301

Marques APGC, Oliveira RS, Rangel AOSS, Castro PML (2007a) Application of manure and domestic sludge compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated with different arbuscular mycorrhizal fungi. Environ Pollut 151:608–620

Marques APGC, Oliveira RS, Rangel AOSS, Castro PML (2007b) EDDS and EDTA-enhanced zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi grown in contaminated soil. Chemosphere. doi:10.1016/j.chemosphere.2007.08.045

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282

Meharg AA, Cairney JWG (2000) Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv Ecol Res 30:69–112

Mihoc M, Pop G, Alexa E, Radulov I (2012) Nutritive quality of Romanian hemp varieties (Cannabis sativa L.) with special focus on oil and metal contents of seeds. Chem Cent J 6:122

Morrison RS, Brooks RR, Reeves RD (1980) Nickel uptake by *Alyssum* sp. Plant Sci Lett 17:453–457

Mujica A, Jacobsen SE (2003) The genetic resources of Andean grain amaranths (*Amaranthus caudatus* L., *A. cruentus* L. and *A. hypochondriacus* L.) in America. Plant Genet Resour Newsl 133:41–44

Mura P et al (2004) *Cannabis sativa* var. indica: une plante complexe aux effets pervers. Ann Toxicol Anal 16:7–17

Orser CS, Salt DE, Pickering II, Epstein A, Ensley BD (1999) Brassica plants to provide enhanced mineral nutrition: selenium phytoenrichment and metabolic transformation. J Med Food 1:253–261

Pietrini F, Iori V, Cheremisina A, Shevyakova NI, Radyukina N, Kuznetsov VLV, Zacchini M (2013) Metal tolerance and phytoremoval ability in *Amaranthus paniculatus* L. grown in nickel-spiked nutrient solution. doi:10.1051/e3sconf 05/201301130

Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. Environ Exp Bot 35:525–545

Prasad MNV, Hagemeyer J (eds) (1999) Heavy metal stress in plants—from molecules to ecosystems. Springer, New York, p 401

Raju K, Anbuganapathi G, Gokulakrishnan V et al (2003) Effect of dried fruits of *Solanum nigrum Linn* against CCl4-induced hepatic damage in rats. Biol Pharm Bull 26:1618–1619

Rauser WE (1999) Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin, and metallothioneins. Cell Biochem Biophys 31:19–48

Rea PA, Vatamaniuk OK, Rigden DJ (2004) Weeds, worms, and more. Papain's long-lost cousin, phytochelatin synthase. Plant Physiol 136:2463–2474

Reeves RD, Baker M (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, London, pp 193–230

Reeves RD, Brooks RR (1983) Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. Environ Pollut A 31:277–285

Rider JE, Hacker A, Mackintosh CA, Pegg AE, Woster PM, Casero RA (2007) Spermine and spermidine mediate protection against oxidative damage caused by hydrogen peroxide. Amino Acids 33:231–240

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley D, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Mol Biol 49:643–668

Sanita di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Segura-Nieto M, Vazquez-Sanchez N, Rubio-Velazquez H, Olguin-Martinez LE, Rodriguez-Nester CE, Herrera-Estrella L (1992) Characterization of amaranth (*Amaranthus hypochondriacus* L.) seed proteins. J Agric Food Chem 40:1553–1558

Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 4:523–544

Seregin IV, Kozhevnikova AD (2006) Physiological role of nickel and its toxic effects on higher plants. Russ J Plant Physiol 53:257–277

Shevyakova NI, Cheremisina AI, Kuznetsov Vl V (2011a) Phytoremediation potential of Amaranthus hybrids: antagonism between nickel and iron and chelating role of polyamines. Russ J Plant Physiol 58(4):547–557

Shevyakova NI, Il'ina EN, Stetsenko LA, Kuznetsov VV (2011b) Nickel accumulation in rape shoots (*Brassica napus* L.) increased by putrescine. Int J Phytoremediation 13:345–356

Shi G, Liu C, Cui M, Ma Y, Cai Q (2012) Cadmium tolerance and bioaccumulation of 18 hemp accessions. Appl Biochem Biotechnol 168(1):163–173

Singh OV, Labana S, Pandey G, Budhiraja R, Jain RK (2003) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61:405–412

Smith SE, Read DJ (1997) Micorrhizal symbiosis, 2nd edn. Academic, London

Stetsenko LA, Shevyakova NI, Rakitin VY, Kuznetsov VV (2011) Proline protects Atropa belladonna plants against nickel salt toxicity. Russ J Plant Physiol 58:337–343

Sun M, Chen H, Leung FC (1999) Low-Cot DNA sequences for fingerprinting analysis of germplasm diversity and relationships in Amaranthus. Theor Appl Genet 99:464–472

Sun Y, Zhou Q, Wei S, Ren L (2007) Growth responses of the newly-discovered Cd-hyperaccumulator *Rorippa globosa* and its accumulation characteristics of Cd and As under joint stress of Cd and As. Front Environ Sci Eng 1:107–113

Sun R, Jin C, Zhou Q (2010) Characteristics of cadmium accumulation and tolerance in *Rorippa globosa* (Turcz.) Thell., a species with some characteristics of cadmium hyperaccumulation. Plant Growth Regul 61:67–74

Suresh B, Ravishankar GA (2004) Phytoremediation—a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24:97–124

Teutonico RA, Knorr D (1985) Amaranth: composition, properties, and applications of a rediscovered food crop. Food Technol 39:49–61

U. S. Department of Energy (1994) Plume Focus Area, December. Mechanisms of plant uptake, translocation, and storage of toxic elements. Summary Report of a workshop on phytoremediation research needs. www.osti.gov/scitech/servlets/purl/10109412

United States Protection Agency (USPA) (2000) Introduction to phytoremediation. EPA 600/R-99/107. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH. nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30003T7G.TXT

Wang L, Zhou Q, Ding L, Suna Y (2007) Effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum L.* as a newly found cadmium hyperaccumulator. J Hazard Mater 154:818–825

Wei S, Twardowska I (2013) Main rhizosphere characteristics of the Cd hyperaccumulator *Rorippa globosa* (Turcz.) Thell. Plant Soil 372(1–2):669–681

Wei S, Zhou QX (2006) Phytoremediation of cadmium-contaminated soils by Rorippa globosa using two-phase planting (5 pp). Environ Sci Pollut Res 13(3):151–155

Wei SH, Zhou QX, Wang X et al (2005) A newly-discovered Cd hyperaccumulator *Solanum nigrum L.* China. Chin Sci Bull 50:33–38

Xu F, Sun M (2001) Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (Amaranthus; Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. Mol Phylogenet Evol 21:372–387

Xue SG, Chen YX, Reeves RD, Lin Q, Fernando DR (2004) Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa Roxb*. (Phytolaccaeae). Environ Pollut 131:393–399

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi T, Michael AJ, Kusano T (2007) A protective role for the polyamine spermine against drought stress in *Arabidopsis*. Biochem Biophys Res Commun 352:486–490

Yang XE, Long XX, Ye HB et al (2004) Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii Hance*). Plant Soil 259:181–189

Zadeh BM, Savaghebi-Firozabadi GR, Alikhani HA, Hosseini HM (2008) Effect of sunflower and Amaranthus culture and application of inoculants on phytoremediation of the soils contaminated with cadmium. Am Eurasian J Agric Environ Sci 4(1):93–103

Zajicek J, Fox P, Sanders H et al (2003) Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo controlled trial. Lancet 362:1517–1526

Zakaria ZA, Gopalan HK, Zainal H et al (2006) Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* chloroform extract in animal models. Yakugaku Zasshi 26:1171–1178

Zhao H, Yang H (2008) Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in Malus hupehensi Rehd. Sci Hort 116(4):442–447

Zhao FJ, Lombi E, McGrath SP (2003) Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249:37–43

Zheleznov AV, Solonenko LP, Zheleznova NB (1997) Seed proteins of the wild and the cultivated Amaranthus species. Euphytica 97:177–182

Zhu YL, Bayed AM, Quean JH, De Souza M, Terry N (1999) Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J Environ Qual 28:339–344

Zhuang P, Yang QW, Wang HB, Shu WS (2007) Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Pollut 184:235–242

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