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

# Phytoremediation

## Management of Environmental Contaminants, Volume 6

Springer

# Phytoremediation

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

# Phytoremediation

## Management of Environmental Contaminants, Volume 6

Springer

*Editors*
Abid A. Ansari
Faculty of Science, Department of Biology
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

ISBN 978-3-319-99650-9 ISBN 978-3-319-99651-6 (eBook)
https://doi.org/10.1007/978-3-319-99651-6

Library of Congress Control Number: 2014952730

© Springer Nature Switzerland AG 2018
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Preface

> Ecological restoration to predisturbance condition, or an approximation thereof, is possible.
>
> John Cairns, Jr.

Volume VI of the series *Phytoremediation: Management of Environmental Contaminants* provides a global selection of research results from 15 countries on several continents. Laboratory and field studies including case histories of applications to contaminated sites describe an array of basic phytoremediation approaches, mechanisms, and potential applications to clean up and monitor aquatic and terrestrial ecosystems. Chapters include the landscape redesign of abandoned gas stations using practical phytotechnologies, the interaction of plants and microbes to remove different contaminants, new information on soil sorption-release mechanisms as they affect phytoremediation efficiency, and plant surveys of metal/metalloid uptake and tolerance at contaminated sites including landfills and agricultural areas. The use of constructed wetlands to treat water contaminated with fuel and oil hydrocarbons and the removal of metals from industrial and municipal wastewater are covered in several chapters. One chapter describes the PGPR activity associated with the use of wastewater contaminated with metals to irrigate crops. Another chapter details the potential of using lichens in the detection and treatment of cancers associated with toxic metal contaminants. Two chapters report the use of trees and other woody species to clean up contaminated sites, while seven chapters describe the study of both engineered nanoparticles and basic nano-phytoremediation approaches to treat environmental contamination including metals, pharmaceutical residuals, and pesticides.

The development and use of phytotechnologies continues to move forward at a steady pace. More ecologists, engineers, and government officials now recognize the potential of phytoremediation to provide a green, cost-effective, and viable application to address some of the world's many environmental challenges. The editors of *Phytoremediation: Management of Environmental Contaminants* Volumes I–VI have provided important studies of the basic approaches of phytoremediation in a diverse global context. The development and acceptance of genetic editing such as CRISPR and other dynamic new approaches to modify plant/microbe biochemistry and growth bode well for the future of phytoremediation and other phytotechnologies. It is our hope as editors that much of the basic information provided by this series of books can serve as the foundation for the development of new applications that feature the integration of modern research discoveries into new methods to remediate contaminated ecosystems.

| | |
|---|---|
| Tabuk, Kingdom of Saudi Arabia | Abid A. Ansari |
| Rohtak, Haryana, India | Sarvajeet Singh Gill |
| Rohtak, Haryana, India | Ritu Gill |
| Syracuse, NY, USA | Guy R. Lanza |
| Syracuse, NY, USA | Lee Newman |

# Contents

Part I Phytoremediation Applications: An Update

Part II Phytoremediation Applications for Contaminated Soils

Part III Phytoremediation Applications for Contaminated Waters

**Part IV Phytoremediation and Microbial Applications**

**Part V Phytoremediation Applications of Organic Contaminants**

**Part VI Specialized Plant Species for Phytoremediation**

**Part VII Nano-phytoremediation Applications**

# Contributors

**Neama Abdalla** Genetic Engineering Division, Plant Biotechnology Department, National Research Center, Giza, Egypt

**Maha I. Alkhalf** Applied Biochemistry Department, Science Faculty, University of Jeddah, Jeddah, Jeddah, Saudi Arabia

Biochemistry Department, Science Faculty, King Abdulaziz University, Jeddah, Saudi Arabia

**Tarek Alshaal** Faculty of Agriculture, Soil and Water Department, Kafrelsheikh University, Kafr El-Sheikh, Egypt

Agricultural Botanic, Plant Physiology and Biotechnology Department, University of Debrecen, Debrecen, Hungary

**Rita Aromolo** Centro di ricerca Agricoltura e Ambiente, Rome, Italy

**Saule Atabayeva** Al-Farabi Kazakh National University, Research Institute of Ecology Problems, Almaty, Kazakhstan

**Asghari Bano** Department of Biosciences, University of Wah, Wah Cantt, Pakistan

**M. Barbafieri** Institute of Ecosystem Study, CNR, Pisa, Italy

**Gabriel Basílico** Laboratorio de Bioindicadores y Remediación, Universidad de Flores, Ciudad Autónoma de Buenos Aires, Argentina

**Madhulika Bhati** National Institute of Science, Technology and Development Studies [CSIR-NISTADS], New Delhi, India

**Sylwia Budzyńska** Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland

**Demet Cansaran-Duman** System Biotechnology Advance Research Unit, Biotechnology Institute, Ankara University, Ankara, Turkey

**Guido Capotorti** Syndial Servizi Ambientali, Rome, Italy

**Laura de Cabo** Museo Argentino de Ciencias Naturales – Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina

**A. de Varennes** Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

**Elif Değerli** System Biotechnology Advance Research Unit, Biotechnology Institute, Ankara University, Ankara, Turkey

**C. M. B. Dias** Chemistry Department, University of Évora, CLAV, Évora, Portugal

HERCULES Laboratory, University of Évora, CLAV, Évora, Portugal

**Nevien Elhawat** Agricultural Botanic, Plant Physiology and Biotechnology Department, University of Debrecen, Debrecen, Hungary

Faculty of Home Economic, Department of Biological and Environmental Sciences, Al-Azhar University, Cairo, Egypt

**Eman El-Nahrawy** Agricultural Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt

**Sahar El-Nahrawy** Agricultural Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt

**Hassan El-Ramady** Faculty of Agriculture, Soil and Water Department, Kafrelsheikh University, Kafr El-Sheikh, Egypt

**Tamer Elsakhawy** Agricultural Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt

**Ana Faggi** Laboratorio de Bioindicadores y Remediación, Universidad de Flores, Ciudad Autónoma de Buenos Aires, Argentina

Museo Argentino de Ciencias Naturales – Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina

**Muhammad Faisal** Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan

**Miklós Fári** Agricultural Botanic, Plant Physiology and Biotechnology Department, University of Debrecen, Debrecen, Hungary

**Monika Gąsecka** Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland

**Azza Ghazi** Agricultural Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt

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

**M. Grifoni** Institute of Ecosystem Study, CNR, Pisa, Italy

**Neha Gupta** Institute of Environment and Development Studies, Bundelkhand University, Jhansi, Uttar Pradesh, India

**P. S. Harikumar** Centre for Water Resources Development and Management, Kozhikode, India

**Matthew Hisle** Department of Landscape Architecture & Regional Planning, University of Massachusetts, Amherst, MA, USA

**Valentina Iori** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

**K. Jesitha** SreeSankara College, Ernakulam, India

**Janina Kaniuczak** Department of Soil Science, Environmental Chemistry and Hydrology, University of Rzeszów, Rzeszów, Poland

**Ravi Katiyar** Center for Environmental Science and Climate Resilient Agriculture, Indian Agriculture Research Institute, New Delhi, India

**Fares K. Khalifa** Biochemistry Department, Science Faculty, Alsolimania, King Abdulaziz University, Jeddah, Saudi Arabia

Biochemistry and Nutrition Department, Womens College, Ain Shams University, Cairo, Egypt

**Hera Naheed Khan** Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan

**Naeem Khan** Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan

**Katarína Kráľová** Faculty of Natural Sciences, Institute of Chemistry, Comenius University in Bratislava, Bratislava, Slovak Republic

**Amit Kumar** Center for Environmental Science and Climate Resilient Agriculture, Indian Agriculture Research Institute, New Delhi, India

**Vinit Kumar** Institute of Environment and Development Studies, Bundelkhand University, Jhansi, Indian Agriculture Research Institute, Uttar Pradesh, India

**M. E. Lopes** Chemistry Department, University of Évora, CLAV, Évora, Portugal

HERCULES Laboratory, University of Évora, CLAV, Évora, Portugal

**Xingmao Ma** Zachry Department of Civil Engineering, Texas A&M University, College Station, TX, USA

**Zuzanna Magdziak** Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland

**Elena Masarovičová** Faculty of Natural Sciences, Department of Soil Science, Comenius University in Bratislava, Bratislava, Slovak Republic

**Angelo Massacci** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

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

**Alfreda Ogochukwu Nwadinigwe** Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria

**Alaa El-Dein Omara** Agricultural Microbiology Department, Soil, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt

**Laura Passatore** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

**F. Pedron** Institute of Ecosystem Study, CNR, Pisa, Italy

**G. Petruzzelli** Institute of Ecosystem Study, CNR, Pisa, Italy

**Fabrizio Pietrini** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

**Lucia Pietrosanti** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

**A. P. Pinto** Chemistry Department, University of Évora, CLAV, Évora, Portugal

Institute of Mediterranean Agricultural and Environmental Sciences (ICAAM), University of Évora, Évora, Portugal

**Radhika Rai** National Institute of Science, Technology and Development Studies [CSIR-NISTADS], New Delhi, India

**I. Rosellini** Institute of Ecosystem Study, CNR, Pisa, Italy

**Paweł Rutkowski** Department of Forest Sites and Ecology, Poznań University of Life Sciences, Poznań, Poland

**Ayesha Siddiqa** Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan

**Jitendra Kumar Singh** School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, India

**Frank Sleegers** Department of Landscape Architecture & Regional Planning, University of Massachusetts, Amherst, MA, USA

**Akansha Srivastav** Center for Environmental Science and Climate Resilient Agriculture, Indian Agriculture Research Institute, New Delhi, India

**Alexandros I. Stefanakis** Bauer Resources GmbH, Schrobenhausen, Germany

Bauer Nimr LLC, Muscat, Sultanate of Oman

Department of Engineering, German University of Technology in Oman (GUtech), Halban, Sultanate of Oman

**Małgorzata Szostek** Department of Soil Science, Environmental Chemistry and Hydrology, University of Rzeszów, Rzeszów, Poland

**Vildan Torun** System Biotechnology Advance Research Unit, Biotechnology Institute, Ankara University, Ankara, Turkey

**Emmanuel Chibuzor Ugwu** Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria

**Xiaoxuan Wang** Zachry Department of Civil Engineering, Texas A&M University, College Station, TX, USA

**Krishna Kumar Yadav** Institute of Environment and Development Studies, Bundelkhand University, Jhansi, Uttar Pradesh, India

**Sunita Yadav** Center for Environmental Science and Climate Resilient Agriculture, Indian Agriculture Research Institute, New Delhi, India

**Massimo Zacchini** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy

**Maria Clara Zuin** Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Legnaro, Agripolis, Legnaro (PD), Italy

# Part I
# Phytoremediation Applications: An Update

# Chapter 1
# Redesigning Abandoned Gas Stations Through Phytotechnologies

**Frank Sleegers and Matthew Hisle**

## 1.1 Scope and Introduction

Brownfields are defined as real property, the expansion, redevelopment, or reuse of which may be complicated by the presence or potential presence of a hazardous substance, pollutant, or contaminant [1]. One commonly occurring brownfield site with a history of perpetuating contaminated land is the gas station. Gas stations proliferated throughout the United States in the twentieth century as major oil companies overbuilt their chains attempting to succeed in the battle for territorial gain. This competition created an overt presence in the American landscape, and in recent decades the abandoned gas station has become just as significant a symbol in our culture as they have brought a certain dereliction to almost every American neighborhood [2]. Phytotechnologies have the potential to fulfill the growing need for an innovative, sustainable, low-cost method to address the contamination issues prevalent in soils and groundwater. Upon full remediation, a gas station can offer recreation as public green space or ecological functions that benefits society. “Phytotechnology is about using specifically selected plants, installation techniques, and creative design approaches to rethink the landscapes of the post-industrial age” ([3], p. xxv). This definition targets the discipline of landscape architecture as it includes natural systems, considers multiple scales between site and region, emphasizes prophylactic approaches, includes green infrastructure, and addresses the need to incorporate cultural values [4, 5]. Phytotechnology utilizes vegetation to remediate, contain, or prevent contaminants in soils, sediments, and groundwater and/or add nutrients, porosity, and organic matter.

The objective of this design research project is to showcase new design models and strategies for abandoned, existing, and planned gas stations through

---

F. Sleegers (✉) · M. Hisle
Department of Landscape Architecture & Regional Planning, University of Massachusetts, Amherst, MA, USA
e-mail: sleegers@larp.umass.edu

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_1

**Fig. 1.1** Aerial photograph of the larger project area. The former gas station is located close to wooded marshlands that connect to the Connecticut River in Hadley, MA (USA) [6]

phytotechnologies as a tool for aesthetic experience, ecological performance, and social resilience in the context of brownfield remediation and adaptive reuse. The project uses an abandoned gas station located on Massachusetts Route 9 outside of Amherst, MA, as an exemplary and typical study area (Fig. 1.1). The outcomes of this project were developed from a Master's Project by Matthew Hisle in the discipline of landscape architecture [7].

## 1.2 Method

This project applied a mixed method containing a review of relevant literature on urban brownfield remediation and their potential for providing ecosystem services and new green spaces, the problem of abandoned gas stations in northern America and their typical contaminants, and the study of phytotechnologies as an inclusive approach of applying phytoremediation. *Phyto*, a book by landscape architects Kennen and Kirkwood [4], described the subject with a more approachable set of planning, engineering, and design tools. They developed a toolset of 18 phytotypologies and recommended 9 of these typologies for the remediation of gas stations. This design research project studied all typologies in more detail and selected seven phytotypologies considering the specific conditions of the case study and explored them with other design strategies common to landscape architecture. Further methods that have been applied in the analysis are relevant to the profession of landscape architecture such as regional context including existing greenways and trails, watershed, land uses, traffic and walkability, and visual-spatial quality.

## 1.3 Background

### *1.3.1 Benefits of Reclaiming Urban Brownfields into Public Open Space*

There is considerable literature supporting the benefits of turning urban brownfields into public green space [8–14]. This research purports a variety of different reasons for these benefits ranging from visual preference [11], urban biodiversity [8], increasing property values [10], and positive community surveys [9]. There is unquestionable doubt that brownfields possess the intrinsic potential for becoming environmental or community green space that can clearly stimulate a city's built environment while at the same time remediate contaminants and transform socially and environmentally neglected urban areas [9]. With that said a significant minority, comprising 3–4%, of brownfield redevelopment projects are intended for these uses [9]. One of the major reasons for this is the enormous cost associated with many brownfield remediation projects. Revitalization powered by the promise of housing opportunities and economic gains through jobs, tax revenues, and increased revenues because of residential and commercial redevelopment often overshadows the invisible, qualitative, and long-term benefits associated with green space development [14]. To influence further prosperity in returning urban brownfields to green spaces, the difficulties associated with that transition must be addressed specifically cost and expectation.

### *1.3.2 Abandoned Gas Stations and Their Contaminants*

Abandoned gas stations are establishments that proliferated throughout the United States in the twentieth century as major oil companies overbuilt their chains attempting to succeed in the battle for territorial gain. This competition created an overt presence in the American landscape, and in recent decades the abandoned gas station has become just as significant a symbol in our culture as they have brought a certain dereliction to almost every American neighborhood [2]. The major problem at these locations is instances of fuel leaking underground storage tanks (LUSTs). From 1984 to 2011, the United States saw 500,000 instances of LUSTs. This has created a growing need for innovative, sustainable, low-cost methods to address the contamination issues prevalent throughout these sites' soils and groundwater [4].

Most of pollutants seen at typical gas stations are organic chemicals that are derived from petroleum sources and come in many forms. These chemicals enter the environment through fuel spills, leaking underground storage tanks, and botched fuel deliveries. The substances contain hundreds of hydrocarbon compounds that create unfavorable outcomes when they come in contact with people, animals, or the surrounding landscape. Typical for gas stations are petroleum hydrocarbon compounds that are considered lighter fractions, meaning they have characteristics that allow them to be more easily broken down. These are gasoline and gasoline additives like MTBE (methyl, tertiary butyl, ether) and BTEX (benzene, toluene, ethyl benzene, xylene), as well as diesel fuel. They often have chemical makeups with single molecule chains which are more water-soluble and therefore more easily degradable. Another group of contaminants typical for gas stations are chlorinated solvents. They occur in thinners and degreasers used during repairs and maintenance on cars. All lighter fractions of petroleum hydrocarbons and the group of chlorinated solvents can be targeted through phytotechnologies. In an ideal scenario, it is possible that organic pollutants are broken down to the point where they are degraded and eliminated as a harmful substance from the soil or groundwater.

Gas stations do incur inorganic pollution, especially if they were built prior to 1970. While this project does not deal directly with inorganic contamination, it is applying prophylactic measures. Inorganics are elements that cannot be broken down or degraded as they are at their most basic state. Methods for dealing with inorganic contaminants through the use of phytoremediation are much less available and less successful. One way to address the issue of inorganic pollution is through extraction and removal of plant material. Extraction allows for plants to uptake the inorganic chemicals and holds them until the plant can be harvested and transported elsewhere for disposal. The field application of phytoextraction is challenging as it depends on many factors like bioavailability of heavy metals, soil properties, speciation of the heavy metals, as well as the plant's ability to absorb and accumulate metals in its aboveground parts [15, 16]. Phytostabilization, though, has been proven to be a successfully applied technique within the field of phytoremediation ([17], pp. 142–145). It prevents inorganics from moving into vital water sources or areas by holding these pollutants in place with the assistance of vegetation.

### *1.3.3 Phytotypologies as Phytotechnology Planting Types*

Phytotypologies or phytotechnology planting types are a way to organize approaches to remediation in a spatial way to meet design goals while considering the functional requirements. Kennen and Kirkwood [4] created a toolbox of 18 phytotypologies. Each one serves a specific role in the landscape depending on (a) the primary mechanisms such as phytodegradation and rhizodegradation, phytovolatilization, phytometabolism, phytoextraction, phytohydraulics, and phytostabilization; (b) the location of the contaminant such as soil, groundwater, wastewater, and air; and (c) the contaminant that is being addressed. Different phytotypologies can be combined with each other if the situation needs it, or they can be integrated with non-remediation planting methods to integrate aesthetical and ecoservice functions that are relevant to landscape architecture. In this way a site with a complex pattern of contamination can be treated with a variety of methods to better incite remediation while responding to other, site-relevant issues.

### *1.3.4 Application of Phytotypologies for Gas Stations and Auto-Repair Shops*

Kennen and Kirkwood have described 16 land use categories typical for contamination and crafted scenarios of possible combinations of phytotypologies for their application. Gas stations are one of these categories ([4], pp. 266–267). They suggest nine phytotypologies to remediate gas stations: planted stabilization mat, phytoirrigation, green and blue roof, interception hedgerow, degradation bosque, degradation hedge/living fence, degradation cover, airflow buffer, and stormwater filter (Table 1.1). All plants should be petroleum-tolerant species (Table 1.2).

#### 1.3.4.1 Planted Stabilization Mat

A thickly *planted stabilization mat* holds pollutants on-site and prevents migration to minimize human and environmental contact. Metal excluder plant species prevent mobility of pollutants into aboveground plant tissues and therefore minimize wind and soil erosion. The plants have to tolerate the specific level of contamination.

#### 1.3.4.2 Phytoirrigation

*Phytoirrigation* retrieves polluted groundwater from subsurface contamination plumes and pumps it to the surface to be reused for irrigation. This typology should be administered through a drip irrigation system to prevent the release of contaminants above the surface where they can be hazardous to site visitors or wildlife. After the contaminants are pumped to the surface and released through the drip system, they can come in contact with the plantings' root zones [4]. The selected plants must be quick-growing and petroleum-tolerant species with a high evapotranspiration rate to absorb and process water quickly and efficiently. This is applicable to up to 30 ft deep-rooting phreatophytes.

**Table 1.1** Description and overview of 18 phytotypologies, their mechanism, target contaminants and media, general criteria for plant selection and function

| **Phytotypology** [a] | **Phytotechnology** | **Contaminant targeted** [b] | **Contaminant subject** | **Plants suitable** | **Function and description** |
|---|---|---|---|---|---|
| 1.Planted stabilization mat | Rhizofiltration<br>Phytostabilization | Metals[c]<br>POP's<br>Salts in soils | Soil<br>0-18 in. below surface | Metal excluders<br>Contaminant-tolerant plants. | Holds contaminants on site. |
| 2.Evapotranspiration cover | Phytostabilization<br>Phytohydraulics | All types of contaminants | Stormwater | Plants with deep tap roots and high evapotranspiration rates – phreatophytes. | Minimizes water infiltration and prevents contaminant mobilization. |
| 3.Phytoirrigation | Phytodegradation<br>Rhizodegradation<br>Phytovolatilization | **Petroleum**<br>**Chlorinated Solvents**<br>Nitrogen<br>Selenium<br>Tritium | Wastewater or groundwater | Petroleum tolerant/ chlorinated solvent tolerant.<br>Plants grow fast with high evapotranspiration. | Irrigating plants with contaminated water. |
| 4.Green and blue roof | Phytohydraulics | All types of contaminants | Stormwater | Draught-tolerant species. | Minimize stormwater runoff. |
| 5.Groundwater migration tree stand | Phytohydraulics | **Petroleum**<br>**Chlorinated Solvents**<br>Nitrogen<br>Radionuclides<br>POP's<br>Metals | Groundwater<br>0-20 ft below surface | Trees with deep tap roots and high evapotranspiration rates – phreatophytes. | Trees pump and treat groundwater. |
| 6.Interception hedgerow | Phytodegradation<br>Rhizodegradation<br>Phytovolatilization<br>Phytohydraulics<br>Phytometabolism | **Petroleum**<br>**Chlorinated Solvents**<br>Pesticides<br>Nitrogen | Groundwater<br>0-20 ft below surface | Trees with deep tap roots and high evapotranspiration rates – phreatophytes. | Roots tap into groundwater and keep fractions of contaminants from migrating. |
| 7.Degradation bosque | Phytodegradation<br>Rhizodegradation<br>Phytovolatilization<br>Phytometabolism | **Petroleum**<br>**Chlorinated Solvents** Pesticides<br>Nitrogen | Soils<br>(0-10 ft) | Petroleum tolerant/chlorinated solvent tolerant.<br>Plants grow fast with high evaptranspiration. | Deep rooted trees and shrubs degrade contamination within the soil profile. |

| | | | | | |
|---|---|---|---|---|---|
| 8.Degradation hedge/living fence | Phytodegradation<br>Rhizodegradation<br>Phytovolatilization<br>Phytometabolism | **Petroleum**<br>**Chlorinated Solvents**<br>Pesticides<br>Nitrogen | Surface Soils (0-4 ft) | Petroleum tolerant/chlorinated solvent tolerant.<br>Plants grow fast with high evapotranspiration. | Shrub species degrade contamination. |
| 9.Degradation cover | Phytodegradation<br>Rhizodegradation<br>Phytovolatilization<br>Phytometabolism | **Petroleum**<br>Nitrogen | Soil (0-5 ft) | Deep rooted drought tolerant prairie grass species. Mixed species. | Deep-rooted herbaceous species remove contaminants. |
| 10.Extraction plots | Phytoextraction<br>Phytometabolism | Arsenic<br>Selenium<br>Nickel<br>Long-term:<br>Cadmium<br>Zinc | Soil (0-3 ft) | Limitations through climate, toxicity and bioavailability. | Hyperaccumulator plants or high-biomass crop species extract<br>inorganic pollutants or organic pollutants<br>Harvesting necessary. |
| 11.Multi-mechanism mat | Phytoextraction<br>Phytometabolism<br>Phytodegradation<br>Phytostabilization<br>Phytovolatilization | All types of contaminants | Soil<br>(0-5 ft) | Selection based on contaminants, overall soil conditions and overall targets for the area. | Combination of phytotypologies as mixed herbaceous planting using a broad range of phytotechnology mechanisms while providing other ecosystem services. |
| 12.Air flow buffer | Phytoaccumulation | Air pollution particulate matter | Air | Species with a high rate of accumulating particulate matter. | Interception of particulate matter from moving air through leaf surfaces of vegetation. |
| 13.Green wall | Rhizodegradation<br>Phytosequestration<br>Phytometabolism<br>Rhizofiltration | VOC's<br>Air pollution particulate matter | Air and/or water | Diversity of species to intercept particulate matter and cleanse it in the root system of the plant. | Installations of plants on vertical surfaces with or without soils. |
| 14.Multi-mechanism buffer | Phytostabilization<br>Phytohydraulics<br>Rhizodegradation<br>Phytodegradation<br>Phytovolatilization<br>Phytometabolism | All types of contaminants | Soil and air | Selection based on contaminants, overall soil conditions and overall targets for the area. | Combination of phytotypologies as mixed planting using all phytotechnology mechanisms while providing other ecosystem services. |

**Table 1.1** (continued)

| | | | | | |
|---|---|---|---|---|---|
| 15.Stormwater filter | Rhizofiltration | Nitrogen<br>**Petroleum**<br>**Chlorinated Solvents**<br>Pesticides<br>Metals<br>Phosphorus<br>POP's | Stormwater | Plants that thrive with substantial water and withstand periods of drought. Plants with deep tap roots and high evapotranspiration rates. | Plantings and soil remove and trap contaminants from stormwater.<br>Extraction possible through harvesting. |
| 16.Surface-flow constructed wetland | Rhizofiltration | Nitrogen<br>**Petroleum**<br>**Chlorinated Solvents**<br>Pesticides<br>Metals<br>Phosphorus<br>POP's | Stormwater<br>Wastewater<br>Groundwater | Plants suitable for constructed wetlands.<br>Selection based on contaminants and overall targets for the area. | Water is directed through a series of planted marshes and engineered soil media to remove contaminants. |
| 17.Subsurface gravel wetland | Rhizofiltration<br>Phytostabilization | Nitrogen<br>**Petroleum**<br>**Chlorinated Solvents**<br>Metals<br>Phosphorus<br>POP's | Stormwater<br>Wastewater<br>Groundwater | Plants suitable for constructed wetlands/ deeper rooting depth. | Contaminated water is treated by pumping the water slowly through subsurface gravel beds. Filtering through plant roots and soil media. |
| 18.Floating wetland | Rhizofiltration | Nitrogen<br>**Petroleum**<br>**Chlorinated Solvents**<br>Pesticides<br>Metals<br>Phosphorus<br>POP's | Existing surface water bodies | Selection based on contaminants and overall targets for the area. | Plantings installed on structures are floated on existing water bodies to filter contaminants out of the water |

Typologies that specifically target the most common contaminants at gas stations are highlighted in bold letters. Rows with pattern overlays (1, 3, 4, 6, 7, 8, 9, 12, 15) indicate Kennen and Kirkwood's suggested nine phytotypologies for the land use category of gas stations and auto-repair shops (2015, pp. 266–267). Rows in green indicate the six phytotypologies explored in this design research project (4, 7, 9, 12, 13, 15)

[a] Eighteen phytotechnology planting types after Kennen and Kirkwood [4], pp. 201–244

[b] (1) Petroleum hydrocarbons, pp. 65–94; (2) chlorinated solvents, pp. 94–103; (3) explosives, pp. 103–110; (4) pesticides, pp. 111–117; (5) persistent organic pollutants (POP's), pp. 118–123; (6) volatile organic compounds (VOC's), pp. 124–125; (7) nitrogen, pp. 126–131; 125–136; (8) phosphorus, pp. 131–135; (9) metals, pp. 136–179; salt, pp. 179–181; (10) radioactive isotopes, pp. 182–189; (11) air pollutants, pp. 189–198 [4]

[c] Contaminated sites should be screened for heavy metals that may still occur on gas stations. Specific remediation techniques may apply including conventional remediation

Sequence of order follows the referenced literature

**Table 1.2** List of petroleum-tolerant plant species recommended for remediation with focus on indigenous North American plants within Plant Hardiness Zone 6a [18]

| Plant species | |
|---|---|
| Herbaceous plants | *Andropogon gerardii*—Big bluestem<br>*Bouteloua curtipendula*—Side oat grass<br>*Bouteloua dactyloides*—Buffalo grass<br>*Bouteloua gracilis*—Signal grass<br>*Carex cephalophora*—Ovalhead sedge<br>*Carex stricta*—Sedge<br>*Elymus canadensis*—Canada wild rye<br>*Elymus hystrix*—Bottlebrush grass<br>*Festuca rubra*—Red fescue<br>*Geranium viscosissimum*—Sticky geranium<br>*Panicum virgatum*—Switchgrass<br>*Agropyron smithii*—Western wheatgrass<br>*Schizachyrium scoparium*—Little bluestem<br>*Scirpus atrovirens*—Green bulrush<br>*Solidago* ssp.—Goldenrod<br>*Sorghastrum nutans*—Indiangrass<br>*Triglochin striata*—Three-rib arrowgrass<br>*Trifolium* spp.—Clover<br>*Tripsacum dactyloides*—Eastern gamagrass<br>*Typha* spp.—Cattail |
| Trees and shrubs | *Betula nigra*—River birches<br>*Celtis occidentalis*—Hackberry<br>*Cercis canadensis*—Eastern redbud<br>*Fraxinus pennsylvanica*—Green ash<br>*Juniperus virginiana*—Eastern red cedar<br>*Morus rubra*—Red mulberry<br>*Gleditsia triacanthos*—Honey locust<br>*Pinus banksiana*—Jack pine<br>*Populus deltoides*—Eastern cottonwood<br>*Populus* spp.—Hybrid poplars<br>*Pinus virginiana*—Virginia pine<br>*Quercus macrocarpa*—Bur oak<br>*Robinia pseudoacacia*—Black locust<br>*Salix alaxensis*—Arrowhead<br>*Salix nigra*—Black willow<br>*Salix* spp.—Willows |

List selected from Kennen and Kirkwood ([4], pp. 74–85)

#### 1.3.4.3 Green and Blue Roof

*Green and blue roofs* evapotranspire water and minimize stormwater runoff and thus reduce the impact of surface pollutants. The *blue roof* provides short-term detention of rainfall and promotes evaporation without plants. This has the advantage of maximizing the evaporation rate in comparison to *green roofs*, while the latter has aesthetic and environmental benefits. Plants on *green roofs* are drought-resistant species and typically not selected for contaminant removal.

#### 1.3.4.4 Interception Hedgerow

Another method of targeting contaminated groundwater is the *interception hedgerow*. This treatment is suitable for situations with a limited amount of space and therefore very applicable to gas stations in denser urban conditions. A single row of trees is planted, usually around the perimeter of a site and downgradient of the source of the contamination to prevent contaminated groundwater leaching off-site. Phreatophytes with deep ending root systems are recommended. As these hedgerows are implemented on the perimeter of sites, they can serve as a buffer for contaminants as much as they can serve as a visual or aesthetic screen.

#### 1.3.4.5 Degradation Bosque

A *degradation bosque* is very similar to an *interception hedgerow*. The same plants that are applicable to the hedgerow are expanded into a grid of trees. The grid of trees maximizes the effectiveness by ensuring that every available portion of the land being planted is targeted. Under the surface, the root systems of these trees become interconnected and are essentially in contact with the complete volume of soil in the root zone. The *degradation bosque* provides constant layers of activity through multiple rows of trees, contrary to the *interception hedgerow* which has only one layer.

#### 1.3.4.6 Degradation Hedge: Living Fence

Similar to *interception hedgerows*, *degradation hedges* and *living fences* are valuable methods of targeting contaminants in areas where space is limited, specifically on the edges of sites and in areas where spatial definition is needed. These two methods vary in the type of species planted and what depth they target. Whereas *interception hedgerows* utilize trees with deep-rooted phreatophyte species, *degradation hedges* use shrub and grass species that are intended on targeting surface soils up to 4 ft deep (120 cm). These species are typically prairie grasses which have root systems that plunge deep below the surface and have high surface fibrous root systems. In nature these species exist in dry prairie conditions and develop their extensive root systems to find water. This provides the intrinsic ability to seek out contaminated water for mitigation.

#### 1.3.4.7 Degradation Cover

The *degradation cover* is similar to a *degradation hedge*. As with the similarities between *degradation bosques* and *interception hedgerows*, *degradation covers* are essentially a more expansive version of *degradation hedges*. This application uses thick, deep-rooted shrub and grass species to target soils from 0 to 5 ft deep

(0–150 cm). The predominant plants used for *degradation covers* are the same deep-rooted and drought-tolerant prairie grass species mentioned with the *degradation hedges*.

#### 1.3.4.8 Airflow Buffer

This typology uses vegetation to trap particulate matter in the air on leaf surfaces and keep pollutants on-site. The contaminants are not degraded and are washed off the plants. Therefore, it is recommended to pair this phytotypology with other systems such as *stormwater filters*. Multilayered plantings and plant species with big leaves seem to be more successful in accumulating particular matter than others.

#### 1.3.4.9 Stormwater Filter

*Stormwater filters* generally consist of plantings that tolerate an extreme amount of water as they are placed downgradient of impervious surfaces. There the contaminants are removed and immobilized by the plants and thus do not migrate off-site and pollute the groundwater. To maximize degradation, it is suggested to select a diverse plant palette that breaks down petroleum and produces high biomass. This will allow for a mixture of root types to target the contamination while having the capacity necessary for high rainfall events. Inorganics are also stabilized through *stormwater filters* and are prevented from leaching off-site. Plants will need to be harvested to remove the contaminants from the soil.

### *1.3.5 Plant Selection*

The plants listed below are petroleum-tolerant plant species recommended for remediation with focus on indigenous North American Plants within Plant Hardiness Zone 6a [18]. Further selection is necessary following site-specific criteria, maintenance, and aesthetic principles.

## 1.4 Application of Phytotypologies at a Former Gas Station in Hadley, MA

### *1.4.1 Site Description and Analysis*

The case study area is a former Getty gas station on Route 9 in Hadley, Western Massachusetts (USA). This gas station was selected because of the size and location. The small size of 12 acres (0.5 ha) is typical for smaller-scale gas station and exemplary for many abandoned gas stations due to the growing competition [2]. The exposed location at the entry of a town or city underpins the importance for cleaning up a derelict site and showcases new design models.

Hadley, MA, has an average low temperature of 20 °F (−6.7 °C) and an average high temperature of 78 °F (25.3 °C) [19]. The area falls within the Plant Hardiness Zone 6a (−20 to −15 °F; −28.9 to −26.1 °C) [18] and is approximately 600 ft (200 m) away from the Connecticut River as part of the Connecticut River watershed. It is located 60 ft (20 m) to an adjacent wooded marshland that connects to the River. The water table is 48–72 inches (120–180 cm) below ground. The prime soil in the area is fertile Hadley silt loam. On-site, the upper horizons are silty loams from 0 to 68 inches (0–173 cm) and loamy fine sands below with a high permeability. Land use activities that cause leaking of petroleum-related substances thus create a potential danger for the environment. Likely locations and quality of pollutants that exist in common gas stations were retrieved from the prevailing literature such as Kennen and Kirkwood (pp. cc [4]) because core samples of the soil itself could not be taken (Fig. 1.2). This figure shows the primary culprits of contamination found at a typical gas station with the leaking underground fuel tanks and the commonly forgotten surface spills.

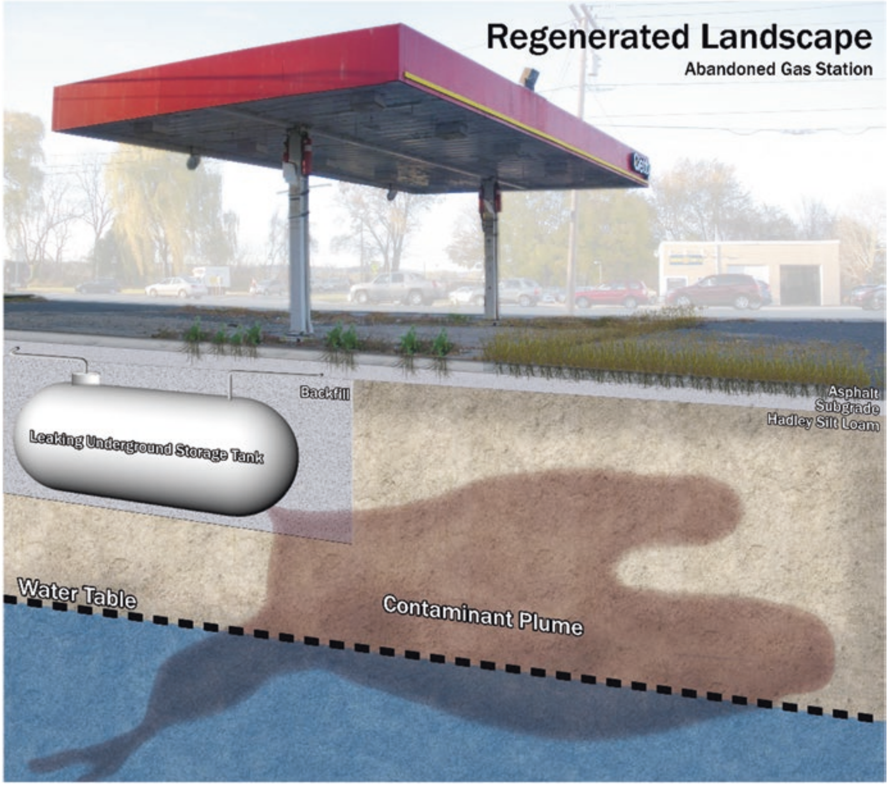


**Fig. 1.2** Existing contamination—illustration of contaminations found on-site [7]

### *1.4.2 Overall Design Description and Considerations*

The redesigned former gas station is a new public green space that invites visitors from the nearby recreational trail (Fig. 1.3). Safe road crossings on Route 9 make the place accessible. From there the visitors reach entry spaces that are secluded from the busy street and are invited to take a journey over a winding boardwalk to experience the diverse landscape elements that perform the cleansing of water and soils. The elevated boardwalk prevents physical contact with potentially harmful substances.

Many of the planting typologies that are applied in the design have tangible spatial qualities such as the *airflow buffer* and the *interception hedgerow* along the Road 9, the *degradation bosque* to the east, or the *green walls* that are attached to the roof structure of the former gas station. Other elements such as the *stormwater filter* create a network of vegetated swales that connects to the larger landscape and the adjacent wooded marshland.

The roof structure references the history of the site. One might think that erasing all traces of a bleak history might be a good thing for a site that is deemed inhospitable. This is understandable, but it would regrettably limit a connection with the landscape's story in its truest sense. The large overhead structure that serves as

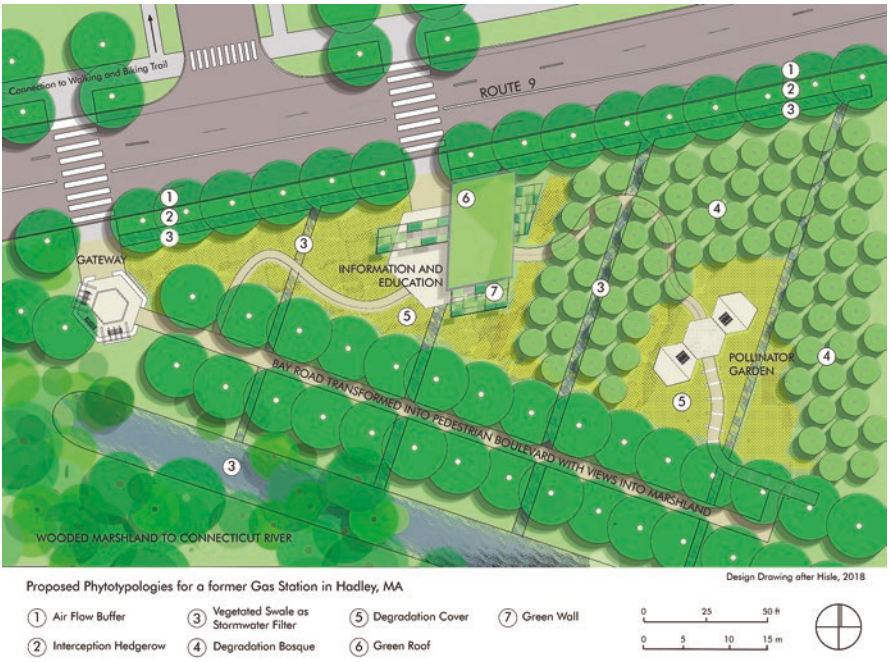


**Fig. 1.3** Design plan—proposed phytotypologies for a former gas station in Hadley, MA. Drawing after Hisle [7]

shelter during fuel pumping was therefore retained and transformed with a *green roof* and *green walls*. It encourages a dialogue between the user and the landscape's past and present. In this way one can begin to understand the place more thoroughly and begin to piece together clues as to why it might not look like a place they have been before and that perhaps the nuanced abnormalities of the site are indicative of the impacts beheld upon the site prior to introduction [5].

### 1.4.3 Selection and Application of Phytotypologies

Seven phytotypologies were selected for the project in Hadley, MA. These are *airflow buffer*, *interception hedgerow,* stormwater *filter*, *degradation bosque, degradation cover*, *green roof, and green wall.* The plan and section illustrate the spatial relationship between the applied typologies. The typologies are described in a sequential order from the edge of the road, across the former gas station to the marshland from north to south (Figs. 1.3, 1.4, and 1.5). While it is helpful to distinguish typologies, the qualities can overlap and hybridize. For example, the *airflow buffer* along a busy street's edge can contain functions of an *interception hedgerow*. Another example is the *degradation bosque* that evolves into an *interception hedgerow* with a single or double row of larger trees to provide more spatial definition.

*Airflow Buffer*—A row of big-leafed trees and a layer of understory grasses with shrubs of different heights accumulates traffic-related particular matter from Route 9. Possible trees are *Fraxinus pennsylvanica* (green ash) or *Quercus rubra* (red oak).

*Interception* Hedgerow—A screen of robust and petroleum tolerant shrubs such as *Salix alaxensis* (feltleaf willow) - prevent contaminated groundwater leaching offsite. The interception hedgerow also serves as an *airflow buffer*.

*Stormwater Filter*—A vegetated swale treats contaminants that are washed off the street and the plants from the *airflow buffer*. It provides a buffer between Route 9 and the abandoned gas stations to prevent any further contamination from accessing the site.

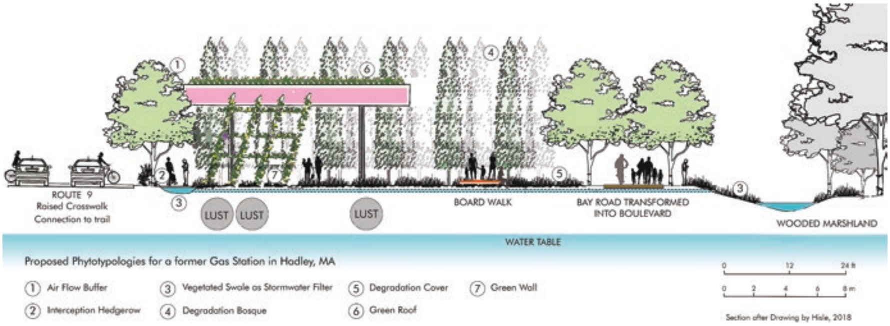


**Fig. 1.4** Design section—proposed phytotypologies for a former gas station in Hadley, MA. Drawing after Hisle [7]

**Fig. 1.5** Design perspective of former gas station in Hadley, MA, (USA) and selected phytotypologies [7]

A second *stormwater filter* is added in the transitional strip adjacent to the wooded marshland to the south to protect the surface water bodies that connect directly to the Connecticut River.

*Degradation Bosque*—A grid of large trees such as Hybrid Poplars *(Populus trichocarpa x deltoides)* treats a large portion of the property and is paired with publicly visible monitoring devices that display the progress of the reme-diation. At the edge of the property the design features a linear line or double line of trees along the boundaries. There the *Degradation Bosque* shares qualities of the *Degradation Hedge* and the *Air Flow Buffer*.

*Degradation Cover*—A mix of deep-rooting prairie grasses targets contamination up to 5 ft (150 cm) below the surface and allows visitors to grasp the spatial expansion of the site.

*Green Roof*—The existing roof of the gas station is transformed into a green roof that evapotranspires water and minimizes stormwater runoff. The impact of still existing surface pollutants is reduced, while the roof provides shelter for visitors and other functions for adaptive reuses such as educational purposes. Slow-growing and drought-tolerant species (*Saxifraga* spp., *Sedum* spp.) are applied to reduce maintenance. While it is not feasible at this specific site to see the roof from above, it is possible to install cameras that showcase this application for visitors.

*Green Wall*—This technology is not an application specific to gas stations. The *green wall* adds aesthetic quality and provides natural habitat for birds and insects while filtering particular matter. It can be recommended as an attachment to existing architectural elements that were not removed from a site or as new constructed freestanding elements. In our project the *green wall* reduces impact from Route 9 and could be attached to the still existing roof structure.

*Phytoirrigation*—This application retrieves polluted groundwater from subsurface contamination plumes. It can be recommended for sites or areas that are not accessible without supervision. The contact of individuals with contaminated irrigation water creates a potential hazard. A potential exploration of this technology on the site in Hadley, MA, could be facilitated through proper fencing.

## 1.5 Concluding Remarks

The project demonstrates how phytotypologies, as a series of selected phytotechnology planting types, can be used in combinations for varying spatial situations due to their ability to remediate the types of contaminants present at typical gas stations. They create modules that can be easily combined and expanded through exploration of legible design elements with spatial and aesthetical qualities. Upon full remediation, a gas station can offer recreation as public green space or ecological functions that benefits society. Contributions include scenic beauty, appeal, and improved access to trails, recreation space, and connection with nature which would boost pride and remove blight.

The project also showcases that interdisciplinary collaborations between soil scientist and designers have the potential to integrate the phytoremediation technology with aesthetic and cultural values to widespread the treatment of planned and abandoned gas stations and make to raise their acceptance. This discourse is needed to limit solutions that may work on a technological level while otherwise exclude cultural factors. Changing public perception is inevitably to provide a site that benefits the community. Cleanup goals seem to be achievable and could resolve in largely applied Best Management Practices (BMP), transferable to multiple sites and areas worldwide. Phytotechnology as a means for remediating small sites polluted with organic chemicals is a step in promoting this technology and proving its worth for other, larger, and more complicated brownfields.

## References

1. USEPA (2008) Design principles for stormwater management on compacted, contaminated soils in dense urban areas. Office of Solid Waste and Emergency Response, Washington, DC

2. Jakle JA, Sculle KA (1994) The gas station in America. Johns Hopkins University Press, Baltimore
3. Rock S (2015) Foreword. In: Kennen K, Kirkwood N (eds) Phyto: principles and resources for site remediation and landscape design. Routledge, New York, NY
4. Kennen K, Kirkwood N (2015) Phyto: principles and resources for site remediation and landscape design. Routledge, New York, NY
5. Sleegers F (2014) Phytoremediation: visions of growing frameworks for landscapes of urban revitalization. In: Ansari A et al (eds) Phytoremediation: management of environmental contaminants, Part I, Overview of phytoremediation application, vol 1. Springer International Publishing, Switzerland, pp 57–68
6. Google Maps (2018) Hadley. Available from https://www.google.com/maps/place/Hadley,+Massachusetts/@42.3388799,-72.6115035,253m/data=!3m1!1e3!4m5!3m4!1s0x89e6d16cec600e0f:0x73cce046957acb06!8m2!3d42.3417565!4d-72.5884222
7. Hisle M (2018) Using phytotechnology to redesign abandoned gas stations. Masters Project, University of Massachusetts Amherst
8. Bonthoux S, Brun M, Di Pietro F, Greulich S, Bouché-Pillon S (2014) How can wastelands promote biodiversity in cities? A review. Landsc Urban Plan 132:79–88
9. De Sousa CA (2006) Unearthing the benefits of brownfield to green space projects: an examination of project use and quality of life impacts. Local Environ 11(5):577–600
10. Kaufman D, Cloutier N (2006) The impact of small brownfields and greenspaces on residential property values. J Real Estate Financ Econ 33(1):19–30
11. Mathey J, Rößler S, Banse J, Lehman I, Bräuer I (2015) Brownfields as an element of green infrastructure for implementing ecosystem services into urban areas 2. J Urban Plan Dev 141(3):1–13
12. Harnik P, Donahue R (2011) Turning brownfields into parks. Planning 77(10):13–17
13. Kattwinkel M, Biedermann R, Kleyer M (2011) Temporary conservation for urban biodiversity. Biol Cons 144(9):2335–2343
14. Siikamäki J, Wernstedt K (2008) Turning brownfields into greenspaces: examining incentives and barriers to revitalization. J Health Polit Policy Law 33(3):559–593
15. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals – concepts and applications. Chemosphere 91:869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075
16. Bhargava A, Carmona FF, Bhargava M, Srisvastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manag 105:103–120
17. Pinto AP, de Varennes A, Fonseca R, Teixeira DM (2014) Phytoremediation of soils contaminated with heavy metals: techniques and strategies. In: Ansari A et al (eds) Phytoremediation: management of environmental contaminants, Part I, Overview of phytoremediation application, vol 1. Springer International Publishing, Switzerland, pp 133–155
18. USDA (2018) http://planthardiness.ars.usda.gov/PHZMWeb/#. Accessed 2 Apr 2018
19. USCLIMATEDATA (2018) https://www.usclimatedata.com/climate/hadley/massachusetts/united-states/usma0907/2018/1. Accessed 15 Apr 2018

# Chapter 2
# Microbial-Assisted Phytoremediation: A Convenient Use of Plant and Microbes to Clean Up Soils

**A. P. Pinto, A. de Varennes, C. M. B. Dias, and M. E. Lopes**

## 2.1 Introduction

Environmental pollution by metal(loid)s (e.g., heavy metals—HMs) is a severe problem worldwide, as soils and aquatic resources became increasingly contaminated, threatening land ecosystems, surface and groundwater, as well as food safety and human health [1]. The primary sources contributing to this extended pollution are anthropogenic inputs related to the burning of fossil fuels, mining and continued industrial activities, disposal of municipal solid wastes and wastewater discharges or use for irrigation, and excessive utilization of fertilizers and pesticides [1–9]. A consequence of these anthropogenic activities is an increase of contaminated areas, which should be remediated to prevent or mitigate transfer of contaminants into terrestrial, atmospheric, or aquatic environments. Point and diffuse contamination by organic and inorganic pollutants causes wide concerns, and intentional or accidental introduction of these substances in the environment may represent serious impacts on public health.

Soil contamination is an important issue across the European Union (EU). About 3.5 million sites in the EU were estimated to be potentially contaminated

---

A. P. Pinto (✉)
Chemistry Department, University of Évora, CLAV, Évora, Portugal

Institute of Mediterranean Agricultural and Environmental Sciences (ICAAM), University of Évora, Évora, Portugal
e-mail: app@uevora.pt

A. de Varennes
Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

C. M. B. Dias · M. E. Lopes
Chemistry Department, University of Évora, CLAV, Évora, Portugal

HERCULES Laboratory, University of Évora, CLAV, Évora, Portugal

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_2

with 0.5 million sites being highly contaminated and in need of remediation. About 400,000 polluted sites were already identified in Germany, England, Denmark, Spain, Italy, the Netherlands, and Finland. Sweden, France, Hungary, Slovakia, and Austria have at least 200,000 contaminated sites. Greece and Poland reported 10,000 contaminated land areas, while Ireland and Portugal reported fewer than 10,000 contaminated sites [10, 11]. Soils act as a final acceptor of toxic substances/trace elements (e.g., HMs; nonessential metal(loid)s), and these inorganic pollutants can limit development and growth of plants and pose a health hazard to humans and animals because some may be bioaccumulated and bio-magnified along the whole food chain [3, 12–15].

When the concentration of these pollutants is above a defined legal standard value, water, air, and soils are described as polluted, and environmental remediation becomes fundamental to decrease the potential risk of food chain contamination and other associated health risks [13, 16, 17]. Thus, it is necessary to use efficient soil cleanup techniques to restore heavy metal-polluted soils. Over the last decades, several physical, chemical, and biological approaches have been attempted to achieve this goal. Conventional treatments include excavation and transfer of soil to landfills, soil washing with water and solubilizing agents, building a physical cover, solidification through use of stabilizing agents, vitrification at high temperatures, electrochemical separation, etc. which may rapidly remediate soils, but irreversibly damage the ecological environment [18–21].

Between the different approaches for the reclamation of metal(loid)s contaminated soils, special attention is drawn to the technologies of phytoremediation (green and clean technologies) [10]. Phytoremediation is based on efficient, inexpensive, and eco-friendly rehabilitation strategies that use plants and associated soil microorganisms to absorb, accumulate, immobilize, or biodegrade organic and inorganic pollutants, present in different environmental matrices (air, soil, and water), through physical, chemical, and biological processes. Phytoremediation is proposed as a relatively recent technology with sustainable costs [13, 22, 23] that improves the native microflora and the physical, chemical, and biological properties, thus enhancing soil health and fertility [1, 20, 21, 23–27]. Moreover, phytoremediation appears to benefit plant growth and carbon sequestration, because harvested biomass can be used to produce renewable energies like biofuel production [13, 23, 28–30].

Phytoremediation is therefore a suitable option to clean metal(loid)s soil contamination. However, metal(loid)s are immutable, and therefore several of the low-cost phytoremediation options that are available for the remediation of organic contamination, such as phytodegradation and rhizodegradation, are not applicable to metal(loid)s-contaminated soils [31, 32]. Moreover, another phytoremediation technique, phytovolatilization, can only be used for some metals like mercury (Hg) and selenium (Se) which have volatile forms [24, 31]. Remediation options that remain are phytostabilization and phytoextraction [31, 32]. Based on economic implications, the aim of phytoremediation can be (1) plant-based extraction of metals with financial benefit (phytoextraction), (2) risk minimization (phytostabilization), and (3) sustainable soil management in which phytoremediation steadily increases soil fertility allowing growth of crops with added economic value [10, 32].

It can be an effective strategy for in situ or ex situ stabilization, removal, or biodegradation of a great range of pollutants in the different environmental compartments, including trace elements (HMs and nonessential metal(loid)s), radionuclides, excess nutrients, salts, and recalcitrant organic pollutants, like petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated solvents, and explosives (e.g., 2,4,6-trinitrotoluene) [1, 33]. Phytoremediation includes a range of plant-based remediation processes, and the most usual are presented in Table 2.1.

Phytoremediation reduces the risks of pollutants dispersion, and it is applicable for the decontamination of soils or wastewaters with mixed pollutants [13, 34]. Mechanisms and efficiency of phytoremediation depend on several factors such as the pollutant class, its bioavailability especially in soils, physical and chemical characteristics of the matrix (soil, water, and wastewaters), and plant species [13, 35].

The plants considered more efficient for phytoremediation are the metallophytes. These are able to survive and reproduce on metal-polluted soils [31, 36]. However, a great number of known metallophytes have small biomass and slow growth, characteristics that are not advantageous for phytoremediation technologies [31, 37]. Some metallophytes can be further classified as metal hyperaccumulators [31, 36]. Content of specific metal(loid)s in these plants exceeds levels that are usually required for normal growth and development. Hyperaccumulators belong to distantly related families, but share the ability to grow on metalliferous soils and accumulate metals in levels far in excess of those found in the majority of species, without suffering phytotoxic effects [1]. Three basic hallmarks distinguish hyperaccumulators from related non-hyperaccumulating plants: a strongly enhanced rate of metal(loid)s uptake, a faster root-to-shoot translocation, and a greater ability to detoxify and sequester metals in leaves [1].

These plants can be used in phytoextraction applications, but in most cases, hyperaccumulator plants are only able to accumulate one metal, while metal-polluted soils often contain a mixture of metals [31, 32, 36, 38]. This mixed pollution is not only challenging for phytoremediation with hyperaccumulator plants but also for metal-tolerant plants in general as plant metal tolerance mechanisms are

**Table 2.1** Overview of most employed phytoremediation techniques (Adapted from [1])

| Technique | Description |
|---|---|
| Phytostabilization | Immobilization of pollutants in the root zone while stabilizing the soil, thus reducing metal leaching and aerial dispersion of contaminated soil particles |
| Phytoextraction | Uptake of pollutants by plant roots and their translocation and subsequent accumulation in aboveground tissues |
| Rhizodegradation | Breakdown of organic pollutants through rhizospheric microbes |
| Phytodegradation | Plant metabolism transforms, breaks down, stabilizes, or volatilizes organic compounds from the soil and groundwater into harmless by-products |
| Phytovolatilization | Uptake of pollutants by plant roots and their transformation into volatile forms that transfer into the atmosphere |

usually metal-specific. Another group of metallophytes, the metal excluders, are also considered appropriate for phytoremediation. These plants accumulate metals from the soil into their roots, but restrict metal(loid)s transport inside the plant. Such plants cannot be used for phytoextraction, rather they can be effective in phytostabilization techniques [31, 39]. Other plants used for phytoextraction of metals are high biomass-producing non-hyperaccumulator plants [32]. In comparison with hyperaccumulators, these plants accumulate a lower concentration of metal(loid)s but produce higher aboveground biomass, removing similar quantities of metals as hyperaccumulators [24, 31]. In conclusion, there are a large number of different plant species and ecotypes referred in the literature with the potential for phytoremediation purposes (phytoextraction or phytostabilization).

The success of phytoremediation is strongly determined by the amount of plant biomass present and the concentration of metal(loid)s in plant tissues. Therefore, high uptake and an efficient root-to-shoot transport system combined with enhanced metal tolerance provide hyperaccumulators with a high potential detoxification potential. However, high levels of metal(loid)s are toxic to most plants and can impair cell metabolism, reduce plant growth, and restrict metal phytoextraction. Physiological mechanisms that may be affected include enzymatic activity, protein structure, water balance, respiration and ATP content, photosynthesis, plant division, and morphogenesis [1, 5]. Phytoremediation has some disadvantages: naturally occurring hyperaccumulators grow slowly, the remediated area is only that close to the root, harvestable aboveground biomass is low, and numerous species cannot be planted in places strongly polluted. So genetic engineering approaches to develop transgenic plants with more favorable characteristics such as high biomass production, more metal accumulation, tolerance against metal toxicity, and well adapted to a variety of climatic conditions might be more beneficial in this respect [40].

Further research is needed in the field of genetic engineering to improve the phytoremediation abilities of transgenic plants and to understand the mechanisms and effectiveness of phytoremediation techniques in order to make these technologies more effective, timesaving, and economically feasible [40].Accordingly, it is necessary to develop other phytoremediation strategies for metal(loid)s-contaminated soils. Strategies to improve phytoremediation efficiency are the use of soil amendments (to increase or decrease metal availability) and the use of the plants' associated microorganisms. The rhizospheric environment is an essential habitat for different microbes including protozoa, algae, fungi, and bacteria. Such microorganisms exhibit a diversity of associations with plants [41, 42]. Microbes have the ability to synthesize and sense signaling molecules that trigger microbial populations to form a biofilm around the root surface and induce a related response. A number of plant-associated microorganisms are favorable because they can enhance the bioavailability of nutrients and mitigate the negative effects of metal(loid)s on plants. Rhizospheric microorganisms possess potential to biodegrade organic pollutants, through rhizodegradation, biotransformation, and volatization [41].

Numerous studies have demonstrated the adverse effect of different metal(loid) s on the soil microbial diversity and their disastrous interaction with plants in polluted soils. Excessive concentrations of metal(loid)s in soils can affect the growth, morphology, and metabolism of microorganisms mainly through destruction of cellular membranes and organelles, enzyme denaturation, and functional or conformational disturbance [41, 43]. To optimize the results of the application of phytoremediation techniques, many researchers have analyzed the dynamics between plants, microorganisms, and metal(loid)s in rhizospheric environments [43–54]. Recently, inoculation of plants with selected and acclimatized microbes (bioaugmentation) has attained salience for phytoremediation of metal(loid)s polluted soils, and assisted phytoremediation techniques are starting to be used to decontaminate polluted soils on large scales [40, 48, 50, 52, 55].

The interface between microbes and plant roots in the rhizosphere is believed to vastly influence the growth and survival of plants. The biotechnological potential of microorganisms to resist and/or remove metals directly from polluted media and their beneficial effects on plant growth may lead to environmental-friendly and cost-effective strategies toward reclamation of polluted soils. Rhizosphere microbes can determine metal availability and change speciation of HMs by producing biosurfactants, organic ligands via microbial degradation of soil organic matter, and exudates (e.g., metabolites, microbial siderophores), which can modify HMs bioavailability and uptake by plants [25, 44, 56–58]. Thus, microbial activities strongly influence metal speciation and transport in the environment. Different organisms exhibit diverse responses to toxic ions, which confer upon them a certain range of metal tolerance.

In conclusion, microbe-assisted phytoremediation has emerged as a sustainable soil cleanup technology with reduced soil disturbance, low maintenance, and overall low costs [25]. Recent studies have demonstrated that microorganisms play an important role in phytoremediation technology. Some species of microorganisms, including biodegradative bacteria, plant growth-promoting bacteria (PGPB), and filamentous fungi, appear to be beneficial in phytoremediation by changing rhizospheric environment, increasing biomass production and bioavailability or stabilization of HMs, and reducing the respective toxicity [23, 59]. Therefore, they can be used in soil amelioration. Microorganisms can produce organic compounds that solubilize and/or stabilize HMs by changing pH and oxidation-reduction potential of their soil environment.

Some polysaccharides secreted by microorganisms can easily bind soil particles, thereby improving the formation of soil aggregates. For example, glomalin and other glycoproteins released by arbuscular mycorrhizal fungi (AMF) may improve soil structure by increasing particle aggregation and aggregating stabilization against wind and water erosion. In addition, some soil bacteria are able to biodegrade toxic organic compounds, including solvents, produced in mineral processing [23].

Microorganisms further play an important role in removing or detoxifying HMs during the phytoremediation process [23]. Although phytoremediation is a sustainable and inexpensive technology for the removal of pollutants from the environment by plants, it is nevertheless a slow process.

Thus, it is important to improve the efficiency and increase the level of the stabilization or removal of toxic metal(loid)s from soils by plants. For this reason, greater attention has been paid to the role that fungi play in plants grown on metal-contaminated soils that have poor nutrients, low water-holding capacity, and adverse physical conditions [60].

## 2.2 Phytoremediation of Metals Assisted by Fungi

The microbiota can contribute to plant growth, productivity, carbon sequestration, and phytoremediation. Fungi are generally more tolerant to metals than bacteria [44, 61]. Fungi can efficiently explore soil microsites that are not accessible for plant roots due to the small diameter of the mycelia and can compete with other microorganisms for water and metal uptake, protect the roots from direct interaction with the metals, and hinder metal transport through increased soil hydrophobicity [44, 58]. Fungi have been defined as eukaryotic, heterotrophic, and absorptive organisms, which typically develop a branched, tubular body called a mycelium and reproduce by means of sporulation. Furthermore, the ability of fungi to form extended mycelial networks makes them well suitable for bioremediation processes. The application of filamentous fungi can be a promising alternative or a valuable complement in situations of bacterial malfunction [46, 47, 62]. Indeed, they play an important role in organic and inorganic transformation, element cycling, rock and mineral transformations, bio-weathering, mycogenic mineral formation, fungal-clay interactions, and metal-fungal interactions. The extensive hyphal networks in the soil can also significantly contribute to stabilization of soil aggregates; they modify the chemical composition of root exudates and soil pH and control metal(loid)s bioavailability in the soil [60].

Many fungi, such as *Trichoderma*, *Aspergillus*, and the arbuscular mycorrhizal fungi (AM), have shown the potential to improve phytoremediation processes in metal-contaminated soils [45, 63], because they have high ability to immobilize toxic metals by either the formation of insoluble oxalate, biosorption, or chelation onto melanin-like polymers. In fact, vascular plants host a great variety of fungi. In addition to being susceptible to soilborne pathogens, plant roots are also colonized by nonpathogenic or mutualistic fungi, such as AM fungi, ectomycorrhizae (EM), and dark septate endophytes (DSE). The AM fungi comprise about 150 species of zygomycetous fungi, and EM fungi include about 6000 species that are primarily basidiomycetes along with a few ascomycetes and zygomycetes [44].

The AM fungi are associated with most of herbaceous plants and with various woody plant families, while the EM fungi are mainly associated with a limited number of woody plant families [44, 64]. The mycorrhizal fungi facilitate the absorption of nutrients from the soil and help their translocation to host plants, sequester potentially deleterious metal(loid)s, and can stimulate soil microbial activity contributing to the overall biodegradation of soil pollutants [3, 40, 44, 65, 66]. Some DSE have been found as fungal symbionts in members of the *Cruciferae* species, although these are known as non-mycorrhizal plants [44]. The DSE are broadly classified as conidial and sterile septate fungal endophytes, which form melanized structures, such as inter- and intracellular hyphae and microsclerotia, in plant roots. The DSE fungi have been found worldwide and coexist often with different mycorrhizal fungi [44]. They have been reported from more than 600 plant species, including plants that are considered non-mycorrhizal [44, 64].

AM fungi of the Glomeromycota are the most common soil microorganisms in natural and agricultural soils [3, 67]. Approximately 160 AM fungal taxa of the order *Glomales* (Glomeromycota) have been described to date. Based on the morphological and molecular identification methods, the AM fungi were divided into 12 genera (*Acaulospora*, *Ambispora*, *Archaeospora*, *Diversispora*, *Entrophospora*, *Kuklospora*, *Geosiphon*, *Gigaspora*, *Glomus*, *Intraspora*, *Paraglomus*, *Scutellospora*) with the *Glomus* species as dominant [60, 68–70]. AM fungi are ubiquitous and form symbiotic associations with the majority of terrestrial plants [71, 72]. Consequently, they represent an important part of the soil microbiome and provide their hosts with benefits including increased access to nutrients through the enlargement of soil volume that can be assessed by roots/mycelia [3, 40], especially phosphorus; they also improve water acquisition and reinforce pathogen resistance [73, 74]. Therefore, mycorrhizal plants invest less energy into the extension of the root system than non-mycorrhizal plants and are more resistant to drought stress and pathogen attacks [31].

Mycorrhizal fungi keep the number of pathogens low through acidification of the rhizosphere and/or increased production of antibiotics [31, 75]. Mycorrhizal fungi can also increase plant's resistance at a physiological level. It has been shown that AMF can lead to greater resistance to herbivores through the action of jasmonic acid [31, 75]. Moreover, mycorrhizal associations could promote a faster closure of the leaf stomata, preventing plant wilting [31, 75], and boost plant osmolyte levels such as proline [31, 76]. Studies carried out by Sarwat et al. [77] showed that AM fungi inoculation in mustard (*Brassica juncea*) raised osmolyte content as proline and mitigate overall symptoms of drought stress caused by sodium chloride.

Mycorrhiza can assist in phytoremediation either by making metal(loid)s more bioavailable for uptake by plants or by reducing metal toxicity in their host plants [12, 31, 37, 78]. However, the phytoremediation efficiency is extremely dependent on fungal species and ecotype [31, 37, 78–80]. The selection of the fungal isolate to use in phytoremediation determines which plant species can be chosen for the application. Obviously, to be able to exert a beneficial effect on phytoremediation effectiveness, mycorrhizal fungi must first establish a mycorrhizal symbiosis. Therefore, the ability of the fungus to survive in metal(loid)s contaminated areas is a prerequisite for its use in phytoremediation applications.

To ensure their survival in metal(loid)s-contaminated soils, mycorrhizal fungi can use different extracellular and intracellular defense mechanisms. Extracellular mechanisms, such as chelation and cell wall binding or biosorption, may be used to prevent metal uptake. Intracellular mechanisms, including binding to nonprotein thiols and transport into intracellular compartments, can reduce the concentration in the cytosol [31, 37]. Intracellular mechanisms depend on transporter proteins and intracellular chelation (e.g., by metallothioneins, glutathione, organic acids, amino acids, and compound-specific chaperones) [31, 81]. Metal transporter proteins can alleviate metal stress by subcellular compartmentation via transporters into the vacuole or other internal cell compartments and/or vacuolar compartmentation of a complex (e.g., the GSH-M complex). Once chelated, these metal complexes can be transported as well. Furthermore, uptake/efflux of metals via specific transporter systems located in the plasma membrane can be downregulated [31, 81].

Additionally, antioxidative defense processes to detoxify reactive oxygen species (ROS) and mechanisms that focus on the repair of metal-damaged biomolecules can be present [31, 37]. Chelation is a first defense mechanism of mycorrhizal fungi against high metal(loid)s concentrations, which is both metal- and species-dependent. In accordance with the literature, a large range of different chelating agents, excreted by fungi, can be able to chelate metals, but there are also a number of studies reporting the opposite, metal solubilization from metal-containing chelates due to the exudation of organic compounds by mycorrhizal fungi [31, 37, 39, 82, 83]. Mutualistic fungi can also play a role in the protection of roots from metal(loid)s toxicity by mediating the interactions between the metals and the plant roots. The ability of mycorrhizal associations to attenuate metal toxicity for higher plants has been demonstrated [60, 71, 72, 78, 84, 85]. Several mechanisms explain why AM fungi can alleviate the stress of metal(loid)s. Mycorrhizal plants have larger biomass that can dilute the metal concentration [3, 86], and the metal(loid)s can be immobilized and compartmentalized in AM hyphal cells [3, 86, 87]. In particular, some native mycorrhizae surviving at contaminated sites may cause precipitation of metal oxalates in the intracellular spaces of the fungi or the host plant and thus restrict apoplastic transport by the Casparian strip [60, 88]. Alternatively, mycorrhizal fungi can directly protect the plant from the buildup of phytotoxic concentrations of certain pollutants by secreting specific detoxifying compounds (e.g., organic acids) or by binding the pollutants into fungal tissues associated with the roots, thus creating a physical barrier against toxic metal translocation [60, 89].

In addition, AM fungi can produce fungal polyphosphates, metallothioneins, and glycoproteins as glomalin, which have high binding capacities for metal(loid)s [3, 90]. Glomalin is a component of spore and hyphal cell wall of AM fungi and has the ability to sequester metal(loid)s. Driver et al. [91] found that glomalin is tightly bound in AM fungi hyphal and spore walls, and small amounts (<20%) of glomalin are released by AM fungi into the soil environment. Glomalin, though still not biochemically defined, is an N-linked glycoprotein composed of 3–5% N, 36–59% C, 4–6% hydrogen, 33–49% oxygen, and 0.03–0.1% P, and it also contains 0.8–8.8% Fe, which may be responsible for the reddish color of glomalin [92]. Apart from the Glomeromycota, no other fungal group produces this glycoprotein in significant amounts [92].

A number of studies have reported the contributions of glomalin to phytoremediation [92]. While examining the roles of glomalin in metal(loid)s sequestration of two polluted soils, González-Chávez et al. [93] stated the potential of glomalin to reduce availability and toxicity of “potentially toxic elements” such as Cu, Cd, and Pb. Furthermore, Cornejo et al. [94] reported that glomalin-related soil proteins (GRSP) bound to about 28% of the Cu and 6% of the Zn present in a soil highly polluted by these metals. From their study, it appears that the higher the concentration of the pollutants, the higher the ability of GRSP to bind them and make the pollutants unavailable.

Although the mechanisms by which metal(loid)s are sequestrated by glomalin are not clear at all, Malekzadeh et al. [92] proposed that mycelium of AM fungi play a major role in the sequestration of metals. As a result, glomalin may be involved in metal(loid)s sequestration due to its presence on the cell wall of the hyphae. González-Chávez et al. [95] showed that the hyphae of AM fungi sequester Cu as previously illustrated by using transmission electron microscopy and scanning electron microscopy linked to an energy dispersive X-ray spectrometer. This sequestration occurs not only in the mucilaginous outer hyphal wall zone and the cell wall but also inside the hyphal cytoplasm. In conclusion glomalin may reduce toxic elements bioavailability via their stabilization and may decrease their toxicity risk to microorganisms and plants in metal(loid)s-polluted sites [92].

AM fungi may often lower metal(loid) mobility and toxicity either by increasing soil pH [20, 21, 96, 97] or by sequestering inside extraradical mycelium [21, 98]. The phytotoxicity of metal(loid)s and level of plant tolerance are closely related to the stored forms of metal(loid)s and their mobility in plant tissues [20, 21, 99, 100]. Therefore, the distribution of chemical forms could be one of the most important metal(loid)s detoxification mechanisms in plants. It has been reported that AM fungi might enhance the tolerance to Cd of *Medicago sativa* L. by altering Cd chemical forms in different plant tissues [20, 21, 96, 101]. AM fungi can also alter the gene expression that relates to metal tolerance of host plants [3]. AM fungi have also been frequently reported in hyperaccumulators growing in metal-polluted soils indicating that these fungi have evolved a heavy metal tolerance and that they may play important roles in the phytoremediation of these sites [14, 80, 86, 102, 103].

Recently, it has been demonstrated that AM fungi can increase the metal(loid) translocation factor, biomass, and trace element concentration of hyperaccumulators [104–106]. Hyperaccumulators combined with AMF have advantages over the independent use of hyperaccumulators and have been proposed as one of the most promising green remediation techniques [36, 104, 105]. However, it is important to note that reductions [104, 105, 107, 108], increases [104, 105, 109, 110], or no changes of metal(loid)s concentrations in plants following mycorrhizal inoculation have all been observed, depending on the fungal-plant association [104, 105, 111–113].

Sheikh-Assadi et al. [114] reported a higher accumulation of Pb and Cd in the roots of inoculated *Limonium sinuatum* in a pot experiment. Plants inoculated with a mixture of *G. mosseae* and *G. intraradices* and exposed to different Cd and Pb concentrations accumulated Cd and Pb in the roots and translocated very little to the shoots. Total Cd and Pb accumulated in the roots was nearly two to three times higher in AM fungi-inoculated plants compared to non-mycorrhized plants. Furthermore, it was observed that mycorrhized plants had a higher metal tolerance. Therefore, it was concluded that mycorrhization alleviated metal toxicity in the plants and that inoculated *L. sinuatum* could be useful as a Cd or Pb controlling agent for phytoremediation. A good understanding of AM fungal communities under natural metal(loid)s stress can contribute greatly to the recognition of interactions between fungi, hosts, and metal(loid)s and further their rational utilization in metal(loid)s-polluted sites. Compared with AM fungi, endophytic fungi are ubiquitous and comprise a diverse group of fungi also showing potential to enhance phytoremediation [46, 47, 115].

Endophytic microorganisms (including bacteria and fungi) are likely to interact closely with their hosts and are more protected from adverse changes in the environment. Exploiting endophytic microorganisms to reduce metal(loid) toxicity to plants has been investigated to improve phytoremediation efficiencies. Therefore, endophyte symbiosis can counteract metal(loid) stress that exerts negative effects on plant growth.

The endophytic fungi could increase resistance of host plants to multimetal contamination. They possess suitable metal sequestration or chelation systems to increase their tolerance to metal(loid)s, and their higher biomass is also suitable for bioremediation [44, 116]. Furthermore, the presence of metal-resistant endophytic microorganisms may be valuable for host plants because they can enhance both nutrient assimilation and metal bioavailability, through the exuded metabolites in the plant rhizosphere, improving the phytoextraction processes [44, 54]. The metabolic processes operated by endophytes make them relevant resources for phyto(bio)remediation, helping on the phyto(bio)remediation of pollutants and biotransformation of recalcitrant organic compounds through their own degradative capabilities (phytostimulation or rhizodegradation) [44, 48, 117, 118].

Endophytic yeasts *Cryptococcus* sp. CBSB78 and *Rhodotorula* sp. CBSB79 have been isolated from canola roots (*B. chinensis*) in multimetal-contaminated soils and show resistance to Cd, Pb, Zn, and Cu [44, 119, 120]. Multiple HMs hyperaccumulating plants of the genus *Portulaca* contain endophytic fungi such as *Trichoderma*, *Fusarium*, *Aspergillus*, *Paecilomyces*, *Penicillium*, *Paecilomyces*, *Cladosporium*, and *Lasiodiplodia* [44, 46, 47]. *Penicillium* spp. and *Trichoderma* spp. were the most frequently isolated fungal taxa that can counteract HMs stress [44, 121–123]. Recent studies highlight the possible role of fungal endophytes harbored inside S. *nigrum*, which are able to promote host plant growth and enhanced metal extraction, improving the efficiency of phytoremediation in the cleanup of Cd-contaminated soils [56, 57]. In fact, the inoculation of Cd-resistant endophytic fungi with *S. nigrum* increased the plant's tolerance to the high concentrations of Cd, and the parameters related to the biosorption of Cd, including translocation factor, bioconcentration factor, and Cd tolerance index, were significantly enhanced.

In addition, some filamentous fungi such as *Aspergillus niger*, *Mucor rouxii*, and *Rhizopus arrhizus* can be used as sorbents because of their capacity to sorb metal ions such as $Cu^{2+}$, $Co^{2+}$, $Cd^{2+}$, $Zn^{2+}$, and $Pb^{2+}$ [23].

Table 2.2 summarizes the published studies on microbial effects on plants under metal stress.

**Table 2.2** Microbial-mediated metal(loid)s stress tolerance

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Glomerales* species *Rhizophagus* (25.4%), *Funneliformis* (19.6%), *Claroideoglomus* (10.7%) | Sb | *Lactuca sativa*<br>*Daucus carota* | Significant increase on its accumulation in carrots (all organs) with higher accumulation in roots<br>In lettuce, accumulation appeared to be dependent on the Sb chemical species<br>Moreover, it was observed for the first time that AM fungi changed the human bioaccessible fraction of Sb in edible organs | Pierart et al. [124] |
| *Funneliformis mosseae*, *Rhizophagus irregularis*, *Claroideoglomus lamellosum* | Cr(III), Cr(VI) | *Ricinus communis* | Decreased Cr(VI) concentration in soils | Gil-Cardeza et al. [125] |
| AM fungi | Pb, As, Cd | *S. melongena* | AM fungi application improved growth, biomass, and antioxidative defense response of plants against metal(loid)s stress<br>The biomass and metal(loid)s uptake increased with AM fungi inoculation | Chaturvedi et al. [73] |
| *Funneliformis mosseae*, *Rhizophagus intraradices* | Cd | *S. nigrum* | Significantly enhanced shoot biomass and Cd shoot concentration | Li et al. [65] |
| *Rhizophagus intraradices* | Cd | *Oryza sativa* | Significant effects on root biomass, straw, and root Cd concentration | Luo et al. [21] |
| *G. intraradices* BEG140, *G. mosseae* BEG95, *G. etunicatum* BEG92, *G. claroideum* BEG96, *G. microaggregatum* BEG56, *G. geosporum* BEG199 | Hg | *Zea mays* L. | Played an important role in the biogeochemical cycle of Hg in terrestrial ecosystems, indicating that AM fungi can alter Hg ligand environment and Hg soil to root mobility | Kodre et al. [126] |
| *Rhizophagus irregularis* | Cd | *Phragmites australis* | AM fungi improved Cd tolerance by promoting growth and changes in the distributions of elements in the treated plants<br>For the first time, this study determined that *P. australis* inoculated by AM fungi could be a Cd-tolerant species | Huang et al. [127] |

(continued)

**Table 2.2** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Glomerella truncata* PDL-1, *Phomopsis fukushii* PDL-10 | Cd | *Solanum nigrum* | Significantly improved shoot and root length, chlorophyll content, and dry weight. The results of this study highlight the possible role of fungal endophytes harbored inside *S. nigrum*, which have the potential to improve the efficiency of phytoremediation or phytostabilization in the cleanup of Cd-contaminated soils | Khan et al. [57] |
| *Funneliformis mosseae* | Sb | *Cynodon dactylon* | Plant biomass was significantly increased by the symbiosis. Compared to uninoculated controls, mycorrhizal colonization significantly increased shoot and root Sb concentrations under all Sb treatment levels. Bioconcentration and translocation factors were elevated by mycorrhizal colonization. The fungus served an important role in Sb transport and fate in soil-plant systems | Wei et al. [128] |
| *Claroideoglomus claroideum*, *Funneliformis mosseae* | Cd, Pb | *Calendula officinalis* L. | Stimulated accumulation of important secondary metabolites (total phenols, flavonoids, carotenoids) in marigold flowers and, therefore, enhanced the antioxidant capacity. The highest b-carotene values and lycopene were found in the marigold-*F. mosseae* association | Hristozkova et al. [129] |
| *Rhizophagus irregularis* | Pb | *Trifolium repens* L. | Significant increase in glomalin production at all levels of Pb. Fungal symbiont seems to change the distribution pattern of Pb in organs including extraradical hyphae and roots | Malekzadeh et al. [92] |

(continued)

**Table 2.2** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Acaulospora*, *Glomus*, *Rhizophagus* | Pb | *Vetiveria zizanioides Ricinus communis* | Higher tendency for Pb absorbed by the roots to be transported to the shoots | Schneider et al. [63] |
| *Glomus fasciculatum* | Cd, Zn | *Helianthus annuus* L. | Increased the dry biomass of the plant and accumulation of Zn and Cd in roots and shoots | Mani et al. [130] |
| *Claroideoglomus claroideum, Funneliformis mosseae* | Cd, Pb | *Calendula officinalis* L. | Accumulation of secondary metabolites (phenols, flavonoids, carotenoids) and enhanced antioxidant capacity | Hristozkova et al. [129] |
| *Glomus viscosum*, *Glomus constrictum*, *Glomus intraradices*, *Rhizophagus intraradices* | Mn | *Phytolacca americana* | Accumulated much higher concentrations of Mn, but no obvious correlations with AM fungi | Wei et al. [105] |
| *Glomus etunicatum* | Pb | *Calopogonium mucunoides* | Promoting plant nutrient (P, S, and Fe) acquisition, attenuating the negative effects of Pb on membranes, and contributing to the reduction of ROS generation | De Souza et al. [131] |
| *Glomus mosseae* | Cd, Pb | *Cajanus cajan* (L.) Millsp. | Reduced metal translocation from root to shoot. Exposure to Cd and Pb significantly increased the levels of PCs and GSH. The metal contents were higher in roots and nodules when compared with that in shoots. The results indicated that PCs and GSH might function as potential biomarkers for metal toxicity, and microbial inoculation showed bioremediation potential by helping pigeon pea to grow in multimetal-contaminated soils | Garg and Aggarwal [132] |

(continued)

**Table 2.2** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Aspergillus niger*, *Penicillium bilaiae*, *Penicillium* sp. | Ni, Cu, Zn, Pb | NA | Main organic acids identified were oxalic acid (*A. niger*) and citric acid (*P. bilaiae*). Exudation rates of oxalate decreased in response to Pb exposure, while exudation rates of citrate were less affected<br>The release of metals was related to the production of chelating acids, but also to the pH decrease. This illustrates the potential to use fungi exudates in bioremediation of contaminated soil | Arwidsson et al. [133] |
| *Scleroderma citrinum*, *Amanita muscaria*, *Lactarius rufus* L. | Zn, Cd, Pb | *Pinus sylvestris* L. | Reduced translocation of Zn, Cd, or Pb from roots to shoots in pine seedlings | Krupa and Kozdrój [134] |
| *Beauveria caledonica* | Pb | NA | Solubilized Pb from pyromorphite and accumulated the highest water-soluble fraction and total Pb concentration in the mycelium | Fomina et al. [135] |
| *Glomus mosseae* | Cu, Pb, Cd | *Sorghum* | Immobilized Cu, Pb, and Cd and accumulated metals in a nontoxic form leading to increased plant growth | González-Chávez et al. [136] |
| *Oidiodendron maius* | Zn | NA | Mobilized insoluble inorganic zinc compounds (ZnO and $Zn_3(PO)_2$). Induction of organic acids (malate and citrate) by the metal compounds was at least in part responsible for metal solubilization | Martino et al. [137] |

*M* metal(loid)s, *NA* not applicable

## 2.3 Phytoremediation of Metals Assisted by Bacteria

Between the microorganisms involved in soil metal(loid)s phytoremediation, the rhizosphere bacteria deserve special attention because they can directly improve the phytoremediation process by changing the metal bioavailability via soil pH adjustment, release of chelators, induced redox changes, etc. [50, 54, 58, 61, 102, 138]. It was further demonstrated that some kinds of microorganisms are able to take up metal(loid)s from contaminated soils by reducing them to a lower redox state [23]. For example, some bacteria, such as *Bacillus arsenicoselenatis*, *Chrysiogenes arsenatis*, and *Sulfurospirillum arsenophilum*, have been found to have a large capacity for mobilizing As in mine tailings, wherein they can rapidly achieve microbial reduction of As(V) [23].

Combining increased rhizospheric plant bioavailability and reduced internal bioavailability of metal(oid)s should allow plants to bioaccumulate higher amounts of metal(oid)s, without increases on phytotoxicity [46, 47, 139]. Further, since the bacterial cells (approximately 1.0–1.5 mm$^3$) have an extremely high ratio of surface area to volume, they could sorb a greater quantity of metal(loid)s than inorganic soil components (e.g., kaolinite, vermiculite, mica) either by an independent passive metabolism or by a metabolism-dependent active process [50]. Several authors have pointed out that bacterial biosorption/bioaccumulation mechanisms, together with other plant growth-promoting features, accounted for improved plant growth in metal(loid)s-contaminated soils [50, 140, 141].

Plant growth-promoting (PGP) bacteria are so named due to their potential to enhance plant growth. These PGP bacteria may develop symbiotic/mutualistic associations with plants and may be found as free living rhizospheric or endophytic bacteria. Genera including *Gluconacetobacterium*, *Flavobacterium*, *Beijerinckia*, *Klebsiella*, *Erwinia*, *Enterobacter*, *Burkholderia*, *Pseudomonas*, *Serratia*, and *Bacillus* are among the beneficial PGP bacteria able to control plant growth [41, 142, 143]. Some studies have found that plant growth-promoting rhizobacteria (PGPR) have the capacity to alleviate metal-induced phytotoxicity and enhance biomass production of plants when grown in metal(loid)s-contaminated soils. Although the role of PGPR on plant growth and metal(loid)s phytoremediation potential in polluted soils has been studied extensively, the dynamics of plant-endophytic bacteria and their potential role in phytoremediation are only beginning to be described in the literature in recent years [25, 52, 144–147].

The metal-resistant bacteria on roots and in the rhizosphere can benefit from root exudates, but some bacteria enter the plant as endophytes that do not cause harm and can establish a mutualistic association [148, 149]. However, endophytic populations, as rhizospheric populations, can be restricted by biotic and abiotic factors [149–151]. The endophytic bacteria may be more protected from the effects caused by biotic and abiotic stresses than rhizospheric bacteria [149]. In accordance with their life strategies, endophytic bacteria can be classified as “obligate” or “facultative.” Obligate endophytes are strictly dependent on the host plant for their growth and survival, and their transmission to other plants occurs vertically or via vectors. Facultative endophytes have a stage in their life cycle in which they exist outside host plants. Endophytic bacteria enter plant tissues usually through the root zone; however, aerial parts of plants, such as flowers, stems, and cotyledons, may also be used for this entry [152]. The bacteria penetrate the plant tissues via germinating radicles [153], secondary roots [154], stomates [155], or as a result of foliar damage [156]. Endophytes, once inside the plant, may either become placed near the entrance area or spread throughout plant tissues. These microorganisms can be present within cells, in the intercellular spaces, or in the vascular system.

Although many reports attest the importance of PGPR in microbial-assisted phytoremediation, the bacterial endophytes offer several advantages over PGPR. For instance, colonization of the plant roots with PGPR is often problematic, because application of the inoculant should be at the exact time required for the plant. Moreover, being in natural biocenosis, they lose competitiveness over endemic bacteria [53]. The survival and colonization potential of PGPR depends also on various factors, such as intrinsic physiological characteristics of the organisms and abiotic and biotic soil factors [53]. The endophytic bacteria may derive significant competitive advantage over PGPR from their close contact with plants. In addition, reinoculation of endophytic bacteria does not affect the indigenous endophyte population in plants [53, 157]. Furthermore, localization within the plant may provide endophytic bacteria with the ability to recolonize the plant surface and tolerate some biotic and abiotic stress situations in the soil [53, 149].

Although this field of research is at an early stage, the available literature suggests that metal-resistant endophytic bacteria cannot only protect plants from metal(loid)s toxicity but also enhance the metal bioaccumulation in plant tissues with concurrent stimulation of plant growth. These beneficial effects exhibited by endophytic bacteria, together with the suggested interrelationship between microbial metal(loid)s tolerance and plant growth-promoting efficiency, indicate that inoculation with endophytic isolates might have significant potential to improve phytoremediation efficiency in polluted soils [53]. Endophytic bacteria reside in plant tissues beneath the epidermal cell layers, from where they can colonize the internal tissues and form a range of different lifestyles with their host including symbiotic, mutualistic, commensalistic, and trophobiotic [52, 158]. They are ubiquitous in a large diversity of plant species and can colonize a particular host with highest densities in root and less from stems to leaves [52, 159].

In general, most endophytes originate from the epiphytic bacterial communities in the rhizosphere or phyllosphere or other plant parts; however, some may be transmitted through the seed or damaged foliar tissues ([160, 161]). The long-term coevolution of plants and endophytic bacteria resulted in an intimate ecosystem, which helps plants to adapt/survive in both biotic and abiotic stress conditions (e.g., pathogen infection, drought, salinity, and contaminants) and enhance the ecological balance of the natural system [52, 162]. Although bacterial endophytes exist in plants variably and transiently [50, 163], they are often capable of triggering physiological changes that promote the growth and development of the plant [50, 164]. In general, the beneficial effects of endophytes are more extensive than those of many rhizobacteria [50].

PGP bacteria can also enhance plant development by acting as biofertilizers (increasing the availability of essential nutrients through, e.g., $N_2$ fixation and phosphate and iron solubilization), as organic contaminant biodegraders, as phytostimulants (producing plant growth regulators and hormones, such as indoleacetic acid—IAA; cytokinins; and other auxins), as stress controllers (by decreasing ethylene production through the synthesis of 1-aminocyclopropane-1-carboxylic acid deaminase—ACCD), and as plant defense inducers against phytopathogens (by producing siderophores, antibiotics, or fungicidal compounds) [25, 148, 165–170].

Moreover, a number of other beneficial effects on plant growth have been attributed to endophytes which include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals, and change on nitrogen accumulation and metabolism [148]. In recent years, phytoremediation assisted by bacterial endophytes has been highly recommended for cleaning up of metal(loid) s-polluted soils since endophytic bacteria may help host plants adapt to unfavorable soil conditions and enhance the efficiency of phytoremediation by promoting plant growth, alleviating metal stress, reducing metal phytotoxicity, and altering metal bioavailability in soil and metal translocation in plants [50, 52].

Endophytic bacteria improve plant growth in metal-polluted soils in two different ways: (1) directly by producing plant growth beneficial substances including solubilization and bioconversion of mineral nutrients (phosphorus, nitrogen, and potassium) and production of phytohormones, siderophores, and specific enzymes and (2) indirectly by controlling plant pathogens or by inducing a systemic resistance of plants against pathogens. Besides, they also change metal bioaccumulation ability in plants by excreting metal immobilizing extracellular polymeric substances (EPS), as well as metal mobilizing organic acids and biosurfactants [52]. Further, the extracellular polymeric substances secreted by endophytic bacteria, consisting mainly of polysaccharides, proteins, nucleic acids, and lipids, also play a significant role in metal complexation, thereby reducing their bioaccessibility and bioavailability [43, 52].

Joshi and Juwarkar [171] assessed the ability of *Azotobacter* spp. to produce EPS able to form complexes with Cd and Cr decreasing metal uptake by *Triticum aestivum*. Further, the biosurfactants produced by endophytic bacteria seemed to increase the bioavailability of poorly soluble metals and to improve phytoremediation rates [52, 160]. Biosurfactants are low-molecular-weight amphiphilic molecules consisting of a hydrophilic and a hydrophobic moiety, comprising a wide range of chemical structures, such as mycolic acid, glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, etc. [52, 172]. These molecules are able to decrease the surface tension, critical micelle concentration, and interfacial tension, thus affecting the distribution of the metals among the phases (Ma 2016). Endophytic bacteria produce the biosurfactants and release to the host as root exudates.

The secreted biosurfactants initially interact with complex insoluble metals on the interface of rhizosphere soil particles and then desorb metals from soil matrix, leading to the change of metal mobility and bioavailability in the soil solution [52, 53]. The interactions between endophytic bacteria, metals, and biosurfactants can be explained from a functional perspective, considering that the key role attributed to biosurfactants is their involvement in facilitating metal uptake [52, 160]. With regard to indirect effects on plant growth promotion, several plant growth-promoting endophytic (PGPE) bacteria are known to diminish the stress effects in plants by suppressing phytopathogen damage [52] either via biological control of pathogens or induced systemic resistance (ISR) of plants against pathogens.

The endophytic bacteria as natural biocontrol agents may have numerous competitive advantages over plant growth-promoting rhizobacteria [52, 53]. Some endophytic bacteria can produce substances that may effectively limit phytopathogens, such as antibiotics, siderophores, a variety of hydrolytic enzymes (such as chitinases, proteases, and glucanases), and antimicrobial volatile organic compounds [52, 173]. In many biocontrol systems, one or more antibiotics have been shown to play a role in disease suppression. For instance, the endophytic bacterial strains *Bacillus megaterium* BP 17 and *Curtobacterium luteum* TC 10 effectively suppressed the burrowing nematode (*Radopholus similis* Thorne) by the synthesis of antibiotics [52, 174]. Recently, Bacon et al. [175] also reported that a patented strain of *Bacillus mojavensis* produced the biosurfactant C-15 surfactin, which was able to control the maize mycotoxic fungus *Fusarium verticillioides*. Additionally, endophytic bacteria can also be effective as competitors of pathogens for colonization niches and bioavailable nutrients, indirectly promoting plant productivity ([176]; Ma 2016).

Induced systemic resistance (ISR) refers to the state of systemically enhanced resistance to a broad spectrum of pathogens [52]. The ISR to various diseases caused by chemicals and plant growth-promoting bacteria is highly beneficial in agroecosystem protection. ISR induced by endophytic bacteria has been demonstrated against various fungal, bacterial, and viral pathogens in many plant taxa ([176]; Ma 2016). Priming plants with bacterial endophytes induced a plant defense system, which pathogens must overcome to colonize the host [52]. Once the defense genes are expressed, ISR activates multiple potential defense mechanisms that include the increased activity of chitinases, β-1,3-glucanases, superoxide dismutase, guaiacol, catalase (CAT), and peroxidases (POS) [52, 177]. The activity of these enzymes is responsible for reactive oxygen species production, and protection of cell organelles against oxidative stress may change as a result of the activity of endophytic bacteria ([178]; Ma 2016).

In addition, Wan et al. [178] and Zhang et al. [179] found that endophytic bacteria could modulate the activity of plant antioxidant enzymes (such as POS, CAT, SOD, glutathione peroxidase, ascorbate peroxidase) and lipid peroxidation (malondialdehyde formation).

Recent experiments with hyperaccumulator plants revealed that the inoculation of soils/seeds/seedlings with metal-resistant endophytic bacteria improved plant growth and accelerated the phytoremediation process in naturally and/or artificially metal(loid)s-contaminated soils by enhancing nutrient acquisition, cell elongation, metal bioaccumulation or stabilization, and alleviation of metal stress in plants [52, 104, 105, 108, 145, 147, 180–183]. Similarly, the colonization and propagation of plant growth-promoting endophytic bacteria are also well known for their role in the enhancement of soil fertility and stimulation of host plant development by providing a plethora of growth regulators [52, 184] and essential nutrients [52] or by synthesizing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase [52, 185], as well as by reducing disease severity by suppressing pathogens [52, 174]. Further, bioaugmentation with such endophytic bacteria possessing multiple plant growth-promoting traits, including metal resistance/detoxification/accumulation/biotransformation/sequestration, can reduce phytotoxicity and change the bioavailability of metal(loid)s in contaminated soils, making them a perfect choice for microbial-assisted phytoremediation studies [50, 52, 53, 168, 169].

Although metal(loid)s negatively influence endophytic bacterial diversity in plants [52, 181], numerous studies have demonstrated that endophytic bacteria isolated from hyperaccumulator plants exhibit greater metal tolerance than those from non-hyperaccumulator plants [186]. This may be due to the adaptation strategy of endophytic bacteria to metal(loid)-containing environments [52, 187]. Numerous studies have demonstrated the effect of different metal(loid)s on endophyte diversity, biomass, and activity. However, it is well known that the bacteria isolated from polluted environments are tolerant to higher concentrations of metals than those isolated from unpolluted areas. Further, after the addition of metals, metal tolerance is increased in bacterial communities by the death of sensitive species and subsequent competition and adaptation of surviving bacteria [188]. Experimental results suggest that bacterial metal(loid) resistance can be a result of horizontal gene transfer (HGT) mediated by plasmids encoding metal resistance genes [52, 189]. For instance, some mercury-resistant endophytic bacteria express the *MerB* gene encoding organomercurial lyase that cleaves organomercurials into mercuric ion ($Hg^{2+}$) [52, 190] and *MerA* gene encoding mercuric reductase that converts the highly toxic ionic $Hg^{2+}$ into the less toxic and volatile $Hg^0$ [52, 191], thus alleviating metal toxicity and improving the efficiency of phytovolatilization. The genes specifying the various functions needed for biodegradation of organomercurials and reduction of $Hg^{2+}$ are organized in the mercury resistance (*Me*) operon, which is mostly found in gram-negative bacteria [52, 190].

Idris et al. [186] investigated the endophytic bacteria and rhizobacteria associated with the Ni hyperaccumulator plant *Thlaspi goesingense* using both cultivation-dependent and cultivation-independent techniques. The results showed that most of the endophytes were cultivation-independent and tolerated higher Ni concentrations than rhizobacteria. Furthermore, endophytic bacteria are thought to exhibit different multiple metal resistance through similar mechanisms described for rhizobacteria [50, 144]. Therefore, the beneficial endophytes have been proposed as potential natural resources to enhance phytoremediation of metal(loid)-contaminated soils, due to their biotechnological applications in metal bioremediation. The study of the diversity and structure of bacterial communities living in niches under metal stress is of paramount importance.

Like rhizobacteria, there are several mechanisms by which PGPE directly facilitate growth of their host plants. The mechanisms include nitrogen fixation, solubilization of minerals, and production of phytohormones, specific enzymes, and siderophores [52, 53, 192]. Bacteria that can fix nitrogen, i.e., convert stable atmospheric nitrogen gas into a biologically useful form, are known as diazotrophs. These organisms reduce dinitrogen to ammonia with the help of the enzyme nitrogenase [193]. Endophytic bacteria with strong associative nitrogen-fixing ability allow plants to survive in nitrogen-poor soil environments and play a major role in promoting plant health and growth, compared to other rhizospheric microbes [52, 194, 195]. Phosphorus (P) is one of the major essential plant nutrients as it plays a crucial role in the overall growth of plants by influencing various key metabolic processes such as cell division and development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis, root development, and respiration of plants [52, 196, 197]. Phosphorus in soil is immobilized or becomes less soluble by absorption and chemical precipitation, so that the amount of readily bioavailable phosphorus is very low, compared with the total amount of phosphorus present. Therefore, P is often regarded as a limiting nutrient in agricultural soils [197, 198]. Under metal(loid)s stress conditions, some metal-resistant endophytic bacteria were found to solubilize precipitated phosphates in soil by acidification, ion exchange, and release of chelating agents [52, 199] or to mineralize organic phosphorus in soil by exuding extracellular acid phosphatase [52], thereby enhancing P availability to plants. It is well known that endophytic bacteria assimilate soluble P and prevent its subsequent sorption [52, 200]. Hence, these endophytic bacteria may serve as a sink for phosphorus by rapid microbial phosphorus mobilization even under phosphate-limiting conditions. Subsequently, these endophytes become a source of phosphorus to the plant hosts upon its release from bacterial cells.

Phosphate solubilizing bacteria are common in the rhizosphere, and secretion of organic acids and phosphatases facilitates the conversion of insoluble forms of P to bioavailable forms. Endophytes such as *Pseudomonas citronellolis*, *Pseudomonas oryzihabitans*, *Enterobacter agglomerans*, *Pantoea* sp., *Enterobacteriaceae*, *Burkholderia*, *Ralstonia pickettii*, *Erwinia* sp., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Acinetobacter* sp., *Acinetobacter calcoaceticus*, *Enterobacter sakazakii*, *Agrobacterium* sp., and *Caulobacter/Asticcacaulis* have been defined as efficient P solubilizers [53]. Iron (Fe) is one of the important elements for life, and almost all organisms, with the exception of certain lactobacilli, need iron-containing proteins involved in physiological activities and a series of enzymatic reactions [52]. However, in the aerobic environment of soils, iron exists mainly as Fe(III) and tends to form insoluble hydroxides and oxyhydroxides, making it largely unavailable to microorganisms. To acquire sufficient iron, bacteria had to develop strategies to solubilize this metal before uptake. In addition to rhizosphere bacteria, metal-resistant endophytic bacteria that also produce siderophores have been isolated from many different plant species.

Siderophores are produced by a diverse group of microbes ranging from animal and plant pathogens to free-living and symbiotic nitrogen-fixing microorganisms. However, siderophore production is most common among plant growth-promoting rhizosphere bacteria, which exhibit their optimum growth and siderophore production activity at extreme environmental conditions, including scarcity of nutrients or the presence of elevated concentrations of metal(loid)s and thus may be particularly useful for phytoremediation purposes. Iron deficiencies induced by excess of metal(loid)s have been reported in various plant species as interveinal chlorosis in younger leaves [54]. Their low iron content generally inhibits both chloroplast development and chlorophyll biosynthesis, thus affecting plant growth [54]. Under such conditions, siderophore-producing bacteria (e.g., rhizosperic, endophytic) might offer a biological rescue system that is capable of scavenging $Fe^{3+}$ and making it available to iron-deficient plants.

In short, iron bioavailability to plant roots may be modified by the microbial production of chelating agents (e.g., siderophores), which can solubilize Fe under iron deficiency conditions. Siderophores are low-molecular-weight organic compounds (500–1500 Da) with an affinity for $Fe^{3+}$ ions, which can also bind other metal ions that can be uptaken by plants [52, 53]. Iron acquisition takes place by two basic strategies. The first is by the uptake of Fe in Fe-siderophore complexes after root-mediated biodegradation of the chelate [52, 53]. The second strategy involves the solubilization of unavailable forms of iron by the release of phytosiderophores. Since microbial siderophores typically have higher affinity for iron than phytosiderophores, plants growing in metal-contaminated soils are able to accumulate high amounts of iron with the help of siderophore-producing bacteria. Hence, bacterial siderophores are assumed to serve as major sources of phytoavailable Fe for plants under metal stress conditions [50, 52].

Mechanisms of iron acquisition in higher plants can be grouped into strategy I and strategy II [54]. Although strategy II plants (*Poaceae*), similar to microorganisms, release (phyto)siderophores (e.g., mugineic acid in barley and avenic acid in oat) to enhance their Fe uptake, in metal-contaminated soils, these plants are unable to accumulate sufficient amounts of iron, unless bacterial siderophores are also present due to their higher affinity for iron [54]. Unlike strategy II plants, strategy I plants (dicots and monocots, except *Poaceae*) do not produce phytosiderophores themselves. Rather, their iron acquisition is achieved by other means, such as an enhanced Fe(III)-reductase activity, the release of reducing agents such as phenolics, and acidification of the rhizosphere [54]. However, this strategy is considered to be less efficient than that of strategy II plants [54]. Therefore, inoculation of plants with bacteria that are able to produce siderophores could help to prevent them from becoming chlorotic when they are grown in metal-polluted soils.

Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. In addition to iron, siderophores can also form stable complexes with other metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb, and Zn, as well as with radionuclides including U and Np [54, 201]. Currently, almost 500 different siderophores have been identified [54, 202]. Although they differ widely in their overall structure, the functional groups that coordinate the iron atom are not as diverse. In their metal-binding sites, siderophores have either α-hydroxycarboxylic acid, catechol, or hydroxamic acid moieties and thus can be classified as hydroxycarboxylate-, catecholate-, or hydroxamate-type siderophores [54]. The biosynthetic pathways of siderophores are tightly connected to aerobic metabolism involving molecular oxygen activated by mono-, di-, and N-oxygenases and acids originating from the final oxidation of the citric acid cycle, such as citrate, succinate, and acetate. Moreover, many siderophores are polypeptides that are synthesized by members of the non-ribosomal peptide synthetase multienzyme family, which is also responsible for the synthesis of the majority of microbial peptide antibiotics [54]. However, many of the hydroxamate- and α-hydroxy acid-containing siderophores are not polypeptides but are assembled instead from alternating dicarboxylic acid and either diamine or amino alcohol building blocks (which are nevertheless derived from amino acids) that are linked by amide or ester bonds. Such siderophores are assembled by the non-ribosomal peptide synthetase-independent siderophore pathway, which is widely utilized in bacteria. Several recent reviews describe siderophore structure and biosynthesis mechanisms in more detail [54, 203].

Siderophores generally form 1:1 complexes with $Fe^{3+}$, which are then taken up by the cell membrane of bacteria, where the $Fe^{3+}$ is reduced to $Fe^{2+}$ and released from the siderophore into the cell. In some cases, the siderophore is destroyed during this reduction, but in other cases, it is recycled [54]. This mechanism of iron uptake from siderophores has already been identified in both gram-negative and gram-positive bacteria [54, 204]. Although siderophores are elicited primarily in response to iron deficiency, external pH is also important for the biosynthesis of microbial siderophores, as well as in their chemical stability in the environment [54]. Production of siderophores by different microorganisms appears to contradict the commonly acknowledged pH-dependence of Fe deficiency. Hydroxamate siderophores in general are prevalent in acidic soils and reflect the preference of low pH values for the synthesis of hydroxamate siderophores by microorganisms. In contrast, neutral to alkaline soils support the production of catecholate siderophores. In addition to pH, heavy metal concentrations in the surrounding environment could also influence microbial growth and siderophore biosynthesis.

Bacterial strains isolated from polluted environments were shown to be tolerant to higher concentrations of metals than those isolated from unpolluted areas [53, 54]. These metal-tolerant bacteria have evolved several mechanisms for survival under metal stress. Interestingly, several studies have found a stimulating effect of heavy metals on siderophore biosynthesis in various bacteria. Two different possible explanations have been suggested for the stimulating effect of heavy metals on siderophore production. Firstly, the heavy metal might be directly involved in the siderophore biosynthesis pathways or their regulation [54, 205]. Alternatively, the free siderophore concentration in the medium might be reduced by complex formation with metal ions. This process interferes with the complexation of siderophores with iron and thus decreases the soluble iron concentration. As iron deficiency stimulates siderophore production, more siderophores would then be produced [54, 206, 207].

Plants inoculated with siderophore-producing bacteria could then take up iron from siderophores via various mechanisms, such as chelate degradation and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction [54, 208, 209]. A variety of plant species were shown to acquire iron from Fe-siderophore complexes, including *Cucurbita pepo*, *B. juncea*, *Helianthus annuus*, *Medicago sativa*, and *Vigna unguiculata*, and, moreover, it was shown that this acquisition was the reason for their stimulated growth in metal-contaminated soils. Experiments with *Phaseolus vulgaris* revealed that the inoculation with the Pb- and Cd-resistant siderophore-producing bacteria strain *Pseudomonas putida* KNP9 significantly increased plant growth without showing any symptoms of lead and cadmium toxicity compared with the controls [54, 210]. This effect was attributed to the increased level of siderophores produced by the KNP9 strain that were able to provide iron to the plant in the presence of high levels of Pb and Cd.

Inoculation with other rhizobacteria, such as *Pseudomonas* sp. Ps29C and *Bacillus megaterium* Bm4C, which had been isolated from Ni-rich serpentine soils, has also been studied in detail [205, 211, 212]. These bacteria significantly reduced the toxicity of Ni in *B. juncea* and promoted plant growth. Although bacterial siderophores have the potential to sparingly mobilize soluble metals, the efficiency of siderophore-producing bacteria (SPB) to either mobilize or immobilize heavy metals from soils is dependent on several factors, such as the form of the heavy metals present, the charge of the siderophores, as well as the pH of the soil and its mineral composition and organic content [54, 205, 211, 212]. Endophytic bacteria are also able to supply essential vitamins and growth regulators (phytohormones) to plants, which can increase plant nutrient uptake through their effects on root growth dynamics [52, 213].

The phytohormone production by endophytes is believed to play an important role in plant-bacterial interactions and plant growth in metal-contaminated soils [214]. Recent studies investigating the role of phytohormones in protecting plants against metal(loid)s have demonstrated that the endophytic colonization often causes increases in nutrient uptake and plant biomass [52, 184, 213, 215]. These may suggest that metal(loid)s stress alleviation by endophytic bacteria results from a combination of nutritional and biochemical benefits. In general, the phytohormones comprise indole-3-acetic acid (IAA), cytokinins, gibberellins, abscisic acid, and ethylene, which may be either growth inhibitors or promoters depending upon the substance concentration [52, 213]. As a major auxin, IAA is involved in different physiological processes in plants, such as adjustment of plant development [52, 215], induction of plant defense systems [52], and as a cell-cell signaling molecule [52, 216]. IAA is transported downward causing a concentration gradient in different plant parts. Depending on its concentration, inhibition, or stimulation of growth, tissue differentiation may result [52, 217]. More recently, the amount of IAA released by endophytes is thought to play a vital role in modulating the plant-endophyte association and plant development in metal(loid)-contaminated soils [183].

Auxins and cytokinins were found to be produced by strains of *Pseudomonas*, *Enterobacter*, *Staphylococcus*, *Azotobacter*, and *Azospirillum*. These substances, together with gibberellins, may alter plant growth and development [53, 218]. For instance, *Azospirillum*-inoculated roots showed a stimulation of root cell membrane activity, as well as an increase in the levels of free IAA, indole-3-butyric acid, and the specific activities of both the tricarboxylic cycle and the glycolysis pathway [53, 219]. According to the IAA level, root elongation may change qualitatively. A low level of the phytohormone produced by bacteria promotes primary root elongation, whereas a high level of IAA stimulates lateral and adventitious root formation but inhibits primary root growth [220]. Thus, endophytes can facilitate plant growth by altering the plant hormonal balance. *P. fluorescens*, for instance, is generally regarded as being a rhizosphere bacterium that colonizes mainly the elongation and root hair zones of roots. Some *P. fluorescens* can also be endophytic, being found within the roots and stems of some hyperaccumulators [53, 221, 222]. The observed plant growth promotion, under Pb stress after inoculation of plant with *P. fluores-*

*cens*, is thought to be the consequence of bacterial IAA production and excretion [53, 222]. Therefore, any direct influence on phytohormone production by bacteria may in turn affect their phytostimulating efficiency.

Another way in which the endophytic bacteria might influence the host plant growth is the utilization of ACC as a sole N source. Endophytic bacteria that are generally beneficial to plants in situ, such as *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Staphylococcus*, etc., are known to be involved with production of phytohormones [52, 160]. Luo et al. [20] reported that the enhanced growth of the Cd hyperaccumulator S. *nigrum* L. induced by bacterial endophytes under cadmium stress might be the consequence of both bacterial IAA and ACC deaminase. Ethylene, as a ubiquitous plant hormone, plays a vital role in plant response (growth and survival) to abiotic and biotic stresses including root initiation and nodulation, cell elongation, leaf senescence, abscission and fruit ripening, as well as auxin transport [52, 223]. Ethylene is synthesized in higher plants via the following pathway [49, 52]: (1) the enzyme *S*-adenosyl-L-methionine (SAM) synthetase catalyzes the conversion of methionine and adenosine triphosphate (ATP) to SAM; (2) ACC synthase mediates the hydrolysis of SAM to ACC and 5′-methylthioadenosine (MTA); and (3) ACC is finally oxidized by ACC oxidase to form ethylene, carbon dioxide, and hydrogen cyanide. It has been reported that metal(loid) stress in plants induces ethylene production, which causes the inhibition of root elongation, lateral root growth, and root hair formation. Under such conditions, certain endophytic bacteria might alleviate the stress-mediated impact in plants by enzymatic hydrolysis of ACC.

Ethylene is implicated in virtually all aspects of plant growth and development, ranging from seed germination to shoot growth and leaf abscission [53, 224]. Therefore, production of ACC deaminase is likely an important and efficient way for endophytes to manipulate their plant hosts. The enzyme ACC deaminase may hydrolyze ACC into α-ketobutyrate and ammonia, which can readily metabolized by the bacteria as a source of nitrogen. Endophytic bacteria containing ACC deaminase are usually located inside plant roots in the apoplast. In this way, these bacteria act as a sink for ACC. By lowering ethylene levels, the bacteria increase the growth of plant roots and shoots and reduce the inhibitory effects of ethylene synthesis ([53, 225]; Ma 2016). A higher percentage of endophytes than of rhizosphere bacteria were able to utilize ACC as the sole N source. Experiments with *Methylobacterium oryzae* and *Burkholderia* sp. (isolated from rice tissue) showed the ability of these bacteria to reduce the level of ethylene, protecting tomato (*Lycopersicon esculentum*) from the toxicity of high concentrations of Ni and Cd [53].

The success of phytoremediation is widely dependent on the ability to overcome metal phytotoxicity. Therefore, different bacterial-mediated mechanisms are implicated on the endophyte-host coevolution process either by relieving metal toxicity or by enhancing plant metal tolerance [52, 53, 147]. Shin et al. [183] found that the endophytic bacterial strain *Bacillus* sp. MN3-4 evolved a better defined metal-resistant mechanism, e.g., active export via a P-type ATPase efflux pump, which can transport metal ions across biological membranes against the concentration gradient using energy released by ATP hydrolysis. Endophytic bacteria possessing specific and remarkable metal bioaccumulation abilities can be used in plant-endophyte mutualistic systems to facilitate detoxification of metal(loid)s and improve the efficiency of phytoremediation [52, 183, 226]. It is well known that the transfer of metal(loid)s from soils to plants depends primarily on metal bioavailability which can be affected by several factors, such as soil particle size, nutrients, soil pH, redox potential, organic matter content, and the presence of other ions [48, 52, 55]. Numerous studies have suggested that endophytic bacteria possessing a metal resistance/sequestration pathway (e.g., *ncc-nre*) can alleviate the phytotoxicity and enhance the bioavailability of metal(loid)s through the release of metal chelating agents (e.g., siderophores, biosurfactants, and organic acids), acidification of soils, redox activity, and phosphate solubilization [50, 52].

Currently, the interactions between endophytes and hyperaccumulator plants have attracted the attention of several investigators due to the biotechnological applications for bioremediation and to study the composition of bacterial communities living on naturally contaminated environments. In general, hyperaccumulating plants accumulate huge amounts of metal(loid)s and can therefore provide a specific environment for bacterial endophytes adapted to survive in high metal concentrations. For instance, metal-resistant endophytic bacteria have been isolated from various hyperaccumulating plants such as *Alyssum bertolonii*, *Thlaspi caerulescens*, *Thlaspi goesingense*, and *Nicotiana tabacum* [186, 227–230]. Attempts have been made to characterize the endophytic bacterial communities in plant tissues, but comparisons between studies are difficult to make. An interesting finding was that isolates from shoot and root displayed different tolerances, suggesting that different microbial communities exist in different compartments of the plant [53].

The beneficial effects of endophytes on their hyperaccumulators appear to occur through similar mechanisms described for PGPR. This makes sense because most of the bacterial endophytes isolated from various plants can be considered to be facultatively endophytic and are capable of living outside plant tissues as rhizospheric bacteria. Additionally, many endophytic bacterial taxa from hyperaccumulators were reported to be common soil bacteria [186, 222, 231, 232]. Metal biosorption by bacteria comprises two steps:

1. Passive biosorption of metals by living and dead/inactive cells that essentially take place in the cell wall due to a number of metabolism-independent processes [52, 233]. In this process, metal ions are sorbed rapidly to the cell surface by reactions between metals and functional groups on the cell surface, such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, amide, and phosphonate [50, 52]. Various metal-binding mechanisms such as ion exchange, complexation, coordination, sorption, chelation, electrostatic interaction, or microprecipitation may be synergistically or independently involved.
2. Active biosorption (bioaccumulation) referring to the uptake of metals (transport into cells). This only occurs in living cells through a slower active metabolism-dependent transport of metals into bacterial cells [52, 234]. Once the metals are inside living cells, they may be bound, precipitated, accumulated, sequestered

within specific intracellular organelles, or translocated to specific structures, depending upon the organism and element concerned [50, 52].

Particularly, in order to circumvent metal stress, endophytic bacteria have evolved several types of mechanisms, through which they alleviate the toxicity of metal ions, including the efflux of metal ions from cells and subsequent extracellular precipitation [52, 144], intracellular accumulation [52, 183], biotransformation of toxic metal ions into less or nontoxic forms [52, 108], sequestration of metals on the cell surface or in intracellular polymers, and precipitation, adsorption/desorption, or biomethylation [43, 52]. Binding of metals to extracellular material can immobilize the metal and prevent its entry into the cell. For example, different metal(loid)s can bind to anionic functional groups (e.g., sulfhydryl, carboxyl, hydroxyl, sulfonate, amine, and amide groups) present on cell surfaces. Similarly, microbial extracellular polymers, such as polysaccharides, proteins, and humic compounds, can effectively bind heavy metals. These substances thus detoxify metals simply by complex formation or by forming an effective barrier surrounding the cell [54, 235].

In addition, many bacteria mediate reactions or produce metabolites that result in crystallization and precipitation of metals [54, 206, 207, 236]. Furthermore, a great number of bacteria are known to possess transporters to promote metal(loid)s efflux from cells, in case of toxicity or excessive concentration, via ATPase pumps or chemiosmotic ion/proton pumps [54, 237]. These types of transporters are characterized by a high substrate affinity, and they are therefore able to reduce the metal load in the cytosol. Several bacteria have developed a cytosolic sequestration mechanism for protection from metal(loid)s. Once inside the cell, metal ions might also become compartmentalized or being converted into more innocuous forms. This process can constitute an effective detoxification mechanism, and the respective microbes might be able to accumulate higher intracellular concentrations [54, 237].

Examples include the synthesis of metal-binding proteins such as metallothioneins. These are low-molecular mass cysteine-rich proteins with high affinities for cadmium, copper, silver, lead, mercury, etc. Their production is induced by presence of metals, and their primary function is metal detoxification. In addition, certain bacteria use methylation as a metal resistance or detoxification mechanism. However, this process is considered to be metal-dependent as only some metals are methylated. It involves the transfer of methyl groups to metals and metalloids. Selenium is an example of metalloids that has reduced toxicity when methylated [54, 238]. In the last 15 years, several studies focused on the use of actinobacteria for cleaning up the environment [239]. Strategies such as bioaugmentation, biostimulation, cell immobilization, production of biosurfactants, design of defined mixed cultures, and the use of plant-microbe systems were developed to enhance the capabilities of actinobacteria in bioremediation.

Actinobacteria exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of a wide variety of secondary metabolites [239, 240]. This versatility in secondary metabolite production makes them important tools for pharmaceutical, medical, and biotechnological applications such as bioremediation. The quantitative analysis of soil microbial populations through total culturable numbers showed a marked decrease of the different microbial groups for contaminated soil samples, in comparison with uncontaminated samples. However, actinobacteria showed less sensitivity than other culturable heterotrophic bacteria and asymbiotic nitrogen fixers. Culture-dependent methods have allowed the isolation and characterization of over 35 genera of actinobacteria tolerant to heavy metals [239].

Finally, actinobacteria have demonstrated their potential as tools for bioremediation of several contaminants including oil, rubber, plastics, pesticides, and heavy metals, among others, based on their physiological and metabolic versatility. The real worldwide problem is co-contamination. Environments contaminated with inorganic and organic compounds are considered difficult to bioremediate since metal(loid)s would inhibit biodegradation. Nevertheless, recent works highlighted that actinobacteria strains are able to remove HMs and pesticides simultaneously [239]. *Streptomyces*, *Rhodococcus*, and *Amycolatopsis* are among the most studied genera, although their bioremediation skills were never supported by integrated omic approaches. The relevance of the use of omic tools relies on the fact that this information may be used to enhance bioremediation processes of actinobacteria through pathway engineering techniques. On the basis of the existence of co-contaminated environments, engineering bio-tools resistant to organic and inorganic toxic compounds could be necessary.

Plant-bacteria associations in several metal(loid)s phytoremediation studies are presented on Table 2.3.

## 2.4 Phytoextraction

Phytoextraction, also known as phytoaccumulation, phytoabsorption, or phytosequestration, is the uptake of contaminants from soil, water, wastewater, or sediments by plant roots and their translocation and accumulation in aboveground biomass, i.e., shoots or any other harvestable plant parts [1, 5]. Plants able to accumulate metal(loid)s are grown on contaminated sites, and the metal-rich aboveground biomass is harvested, resulting in the removal of a fraction of the contaminant. Phytoextraction is the main and most useful phytoremediation technique for removal of heavy metals and metalloids from polluted soils [1, 24]. It is also the most widespread and promising alternative of soil reclamation for commercial applications. Metal translocation to shoots is a crucial biochemical process desirable for an effective phytoextraction because the harvest of root biomass is generally not feasible.

Phytoextraction has important advantages:

- It does not damage/change the landscape.

**Table 2.3** Recent examples of bacterial-assisted phytoremediation studies

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Rhizobium* sp. strains E20-8 and NII-1 | Cd | *Pisum sativum* L. | Overcame Cd toxicity by extracellular immobilization, periplasmic allocation, cytoplasmic sequestration, and biotransformation of toxic products<br>Higher glutathione concentrations allowing cytosolic Cd complexation, reducing Cd deleterious effects, and rendering this strain more tolerant to Cd than the sensitive one<br>Promoted plant growth, thus rendering phytoremediation a more efficient technology | Cardoso et al. [241] |
| *F. tricinctum RSF-4L*, *A. alternata RSF-6L* | Cd | *Solanum nigrum* | Increased plant growth and chlorophyll content in inoculated RSF-6L plants in comparison to non-inoculated plants. RSF-6L inoculation decreased uptake of Cd in roots and aboveground parts. Inoculation protected the host as shown by low peroxidase and polyphenol peroxidase activities and high catalase activity | Khan et al. [56] |
| *Microbacterium* sp. NE1R5, *Curtobacterium* sp. NM1R1, and *Microbacterium* sp. NM3E9 | As, Cu, Pb, Zn | *Brassica nigra* | Facilitated root development and seed germination | Román-Ponce et al. [242] |

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Micrococcus* sp., *Pseudomonas* sp., *Arthrobacter* sp. | Cd | *Glycine max* L. | Cadmium accumulation in roots, stems, and leaves of *G. max* L. was significantly enhanced by *Arthrobacter* sp. with nutrient biostimulation. A combined use of *G. max* L. and *Arthrobacter* sp. with nutrient biostimulation accelerated cadmium phytoremediation. In addition, cadmium was retained in roots more than in stems and leaves, and *G. max* had the lowest translocation factor at all growth stages, suggesting that *G. max* can be a phytostabilizing plant | Rojjanateeranaj et al. [243] |
| *Thiobacillus thiooxidans* | Cd, Pb | *Gladiolus grandiflorus* L. | Promoted root length, plant height, dry biomass, and enhanced accumulation of Cd and Pb | Mani et al. [130] |
| *Bradyrhizobium japonicum* | Ni, Cu, Pb | *Lettuce* | Increased the shoot and root lengths | Seneviratne et al. [244] |
| *Pseudomonas putida* | Cd, Zn | *Helianthus annuus* L. | Promoted biomass of the plant, accumulation of Zn and Cd in roots and shoots | Mani et al. [130] |
| *Pseudomonas brassicacearum*, *Rhizobium leguminosarum* | Zn | *Brassica juncea* | Induced metal chelation and toxicity attenuation | Adediran et al. [245] |
| *Pseudomonas* spp. *Lk9* | Cd, Zn, Cu | *Solanum nigrum* L. | Improved soil Fe, P, and metal availability, shoot biomass, and uptake of Cd, Zn, and Cu. Production of siderophores and organic acids that induced growth and metal uptake | Chen et al. [145] |

(continued)

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Enterobacter* sp. *JYX7*, *Klebsiella* sp. *JYX10* | Cd, Pb, and Zn | *Polygonum pubescens* | Production of IAA, siderophores, ACC deaminase, and solubilized inorganic phosphate improved phytoremediation efficiency | Jing et al. [246] |
| *Bacillus thuringiensis GDB-1* | Cd, Ni, As, Cu, Pb, Zn | *Alnus firma* | Production of phytohormones, siderophore, ACC deaminase, and solubilization of phosphorus. Increased biomass, chlorophyll content, nodule number, and accumulation of metal(loid)s (As, Cu, Pb, Ni, and Zn) | Babu et al. [180] |
| *Rhodococcus* sp. *TS1*, *Delftia* sp. *TS33*, *Comamonas* sp. *TS37*, *Delftia* sp. *TS41*, *Streptomyces lividans* sp. *PSQ22* | As | *Pteris vittata* | Reduced As(VI) to As(III) and enhanced As uptake by *Pteris vittata* | Yang et al. [247] |
| *Serratia* sp. *MSMC541* | As, Cd, Cu | *Lupinus luteus* | Reduced translocation of As, Cd, and Cu from roots to shoots | Aafi et al. [248] |
| *Consortium of sulfur-oxidizing bacteria* | Cu | *Oryza sativa* | Increased bioavailability of Cu | Shi et al. [249] |
| *Pseudomonas* sp., *Sanguibacter* sp. | Cd, Zn | *Nicotiana tabacum* | Improved biomass production, as well as total plant Cd | Mastretta et al. [229] |
| *Arthrobacter* sp. *MT16*, *Microbacterium* sp. *JYC17*, *Pseudomonas chlororaphis SZY6*, *Azotobacter vinelandii GZC24*, *Microbacterium lactium YJ7 (EN)* | Cu | *Brassica napus* | Root length promotion, ACCD, siderophore, IAA, P solubilization | He et al. [250] |

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Sanguibacter* sp., *Enterobacter* sp., *Pseudomonas* sp. | Cd, Fe | *Nicotiana tabacum* | In several cases, inoculation with endophytes resulted in improved biomass production under conditions of Cd stress, as well as in higher plant Cd concentration and total plant Cd content compared to non-inoculated plants | Mastretta et al. [229] |
| *Cellulosimicrobium cellulans KUCr3* | Cr | Chilli plants | Reduced the mobile and toxic Cr(VI) to nontoxic and immobile Cr(III) and decreased Cr uptake by chilli plants | Chatterjee et al. [251] |
| *Streptomyces tendae* F4 | Cd | *Helianthus annuus* | Promoted plant growth, facilitated soil metal solubilization, enhanced Cd, and Fe uptake | Dimkpa et al. [209] |
| *Achromobacter xylosoxidans* Ax10 | Cu | *Brassica juncea* | Increased root and shoot length and biomass; ACC deaminase, IAA, and phosphate solubilization | Ma et al. [252] |
| *Enterobacter aerogenes*, *Rahnella aquatilis* | Ni, Cr | *Brassica juncea* | Stimulated plant biomass and enhanced phytoextraction of metals (Ni and Cr). Concurrent production of siderophores, ACC deaminase, IAA, and phosphate solubilization | Kumar et al. [140] |
| *Acidithiobacillus thiooxidans*, *A. ferrooxidans*, *Leptospirillum ferrooxidans* | Cu, Cd, Hg, Zn | NA | Increased the mobility of Cu, Cd, Hg, and Zn | Beolchini et al. [253] |
| *Streptomyces acidiscabies* E13 | Al, Cu, Fe, Mn, Ni, U | *Vigna unguiculata* | Protected plants from metal toxicity; enhanced uptake of Al, Cu, Fe, Mn, Ni, and U | Dimkpa et al. [208] |

(continued)

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Azotobacter* spp. | Cd, Cr | *Triticum aestivum* | Immobilized Cd and Cr and decreased their uptake by *Triticum aestivum* | Joshi and Juwarkar [171] |
| *Bacillus edaphicus* NBT | Pb | *Brassica juncea* L. Czern | Promoted plant growth (root and shoot biomass) and Pb uptake | Sheng et al. [222] |
| *Bacillus licheniformis*, *B. biosubtyl*, *B. thuringiensis* | Se, Cd, Cr | *Brassica juncea* | Increased metal uptake depending on specific metal-bacteria combinations | Hussein [254] |
| *Streptomyces acidiscabies* E13 | Ni | *Vigna unguiculata* | Simultaneous inhibition of Ni uptake and solubilization and supply of Fe to plants. Hydroxamate siderophores promoted growth under Ni contamination by binding Fe and Ni, thus playing a dual role of supplying Fe and protecting against Ni | Dimkpa et al. [206] |
| *P. putida HS-2* | Ni | *Brassica napus* | Increased seed germination and plant biomass, siderophores, IAA, ACC, deaminase | Rodriguez et al. [255] |
| *Pseudomonas* sp. *29C*, *Bacillus megaterium 4C* | Ni | *Brassica juncea* | Increased aboveground biomass, protected plants against the inhibitory effects of Ni, probably due to the production of IAA, siderophore, and solubilization of phosphate | Rajkumar and Freitas [211] |
| *Pseudomonas aeruginosa MKRh3* | Cd | *Vigna mungo* | Growth increment, ACCD, siderophore, auxin synthesis, P solubilization. Reduced Cd toxicity and uptake | Ganesan [256] |
| *Pseudomonas* sp., *Janthinobacterium lividum*, *Serratia marcescens*, *Flavobacterium* sp., *Streptomyces* sp., *Agromyces* sp. | Cd, Zn | *Salix caprea* | Increased plant leaf biomass, siderophore, IAA. Decreased Zn and Cd uptake | Kuffner et al. [257] |
| *Bacillus* sp. *J119* Biosurfactant-producing bacterial strain *Bacillus* sp. J119 | Cd | *Solanum lycopersicum*, *Zea mays*, *Brassica napus* | Increased Cd content in aboveground tissues of all plants | Sheng et al. [231] |

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *Gluconacetobacter diazotrophicus* PAl5 | Zn | NA | Solubilized ZnO<br>5-Ketogluconic acid, a derivative of gluconic acid was the major organic acid produced. This organic anion may be an important agent that helped in the solubilization of insoluble Zn compounds | Saravanan et al. [258] |
| *Methylobacterium oryzae CBMB20*<br>*Burkholderia* sp. CBMB40 | Ni, Cd | *Lycopersicon esculentum* | Reduced Ni and Cd uptake and their translocation to shoots<br>ACCD activity, phytohormone production | Madhaiyan et al. [141] |
| *Rhodococcus erythropolis MtCC* | Cr | *Pisum sativum* | Bacterial metal detoxification mechanism. Increased plant growth in the presence of Cr(VI) at low temperature | Trivedi et al. [259] |
| *Rhizobium* sp. *RP5* | Ni, Zn | *Pisum sativum* | Increased nitrogen fixation, growth promotion, and the ability to reduce the toxicity of Ni and Zn | Wani et al. [260] |
| *P. putida ARB86* | Ni | *Arabidopsis thaliana* | Increased biomass and chlorophyll content. Nickel influx into plants was decreased by bacterial sorption in the rhizosphere | Someya et al. [261] |
| *Pseudomonas aeruginosa BS2* | Cd, Pb | NA | Mobilized Cd and Pb | Juwarkar et al. [262] |
| *Pseudomonas* sp. *PsA4*, *Bacillus* sp. *Ba32* | Cr | *Brassica juncea* | Protected the plants against the inhibitory effects of Cr, probably due to the production of IAA, siderophores, and solubilization of phosphate | Rajkumar et al. [263] |
| *Pseudomonas fluorescens* | As | *Helianthus annuus* | Increased plant growth | Shilev et al. [264] |

(continued)

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| *P. putida HS-2* | Ni | *Brassica napus* | Increased biomass and total Ni per plant | Farwell et al. [265] |
| *Pseudomonas* sp. *RJ10*, *Bacillus* sp. *RJ16* | Cd | *Brassica napus* | Increased root and shoot weight and an increase in Cd content varying from 16% to 74%, compared to the non-inoculated control | Sheng and Xia [266] |
| *P. putida 06909* | Cd | *Helianthus annuus* | Increased Cd uptake and decreased toxicity; the bacterium expresses a metal-binding peptide | Wu et al. [267] |
| *Brevibacillus* sp. *B-I* | Zn | *Trifolium repens* | Decreased the concentration of Zn in shoot tissues | Vivas et al. [268] |
| *P. fluorescens*, *P. putida* | Ni | *Brassica napus* | Increased seed germination and plant growth | Ashour et al. [269] |
| *Pseudomonas putida KNP9* | Cd, Pb | *Phaseolus vulgaris* | Reduced Cd and Pb accumulation in *Phaseolus vulgaris* | Tripathi et al. [210] |
| *Pseudomonas asplenii* AC | Cu | *Brassica napus* | Increased biomass; IAA | Reed and Glick [270] |
| *Variovorax paradoxus*, *Rhodococcus* sp., *Flavobacterium* sp. | Cd | *Brassica juncea* | Increased root length, IAA, siderophores, and ACC deaminase | Belimov et al. [271] |
| *Pseudomonas putida KNP9* | Pb, Cd | *Vigna radiata* | Increased biomass and siderophore production. Decreased metal uptake | Tripathi et al. [210] |
| *Pseudomonas tolaasii* RP23, *Pseudomonas fluorescens* RS9 | Cd, Zn, Ni | *Graminaceae* | Have plant growth-promoting characteristics that can potentially support heavy metal uptake and reduce stress symptoms in plants | Dell'Amico et al. [272] |
| *Stenotrophomonas maltophilia* | Se | *Astragalus bisulcatus* | Reduced soluble and harmful Se(IV) to insoluble and unavailable Se(0) and thereby decreased plant Se uptake | Di Gregorio et al. [273] |

**Table 2.3** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress | References |
|---|---|---|---|---|
| Rhizosphere bacteria colonizing *T. goesingense* | Ni | *Thlaspi goesingense* | Increased siderophore production | Idris et al. [186] |
| *Pseudomonas fluorescens Avm*, *Rhizobium leguminosarum* bv. *phaseoli CPMex46* | Cu, Fe | *Medicago sativa* | Improved Cu and Fe translocation from roots to shoots | Carrillo-Castañeda et al. [274] |
| *Microbacterium arabinogalactanolyticum* | Ni | *Alyssum murale* | Increased Ni uptake | Abou-Shanab et al. [275] |
| *Brevibacillus* sp. | Pb | *Trifolium pratense* | Decreased Pb uptake; IAA | Vivas et al. [276] |
| *Enterobacter cloacae CAL2* | As | *Brassica napus* | Increased biomass; ACC deaminase | Nie et al. [277] |
| *Burkholderia cepacia with ncc-nre ncc-nre nickel resistance system Herbaspirillum seropedicae with ncc-nre nickel resistance system* | Ni | *Lupinus luteus* | Facilitated bioaccumulation of Ni in roots | Lodewyckx et al. [278] |
| *Microbacterium saperdae*, *Pseudomonas monteilii*, *Enterobacter cancerogenus* | Zn | *Thlaspi caerulescens* | Increased Zn uptake | Whiting et al. [279] |
| *Kluyvera ascorbata* SUD165, *SUD165/26* | Ni, Pb, Zn | *Brassica napus*, *Lycopersicon esculentum*, *Brassica juncea* | Increased biomass, ACC deaminase, siderophores | Burd et al. [280] |
| *Kluyvera ascorbata SUD165 Enterobacteriaceae* | Ni | *Brassica napus* | Increased biomass and ACC deaminase | Burd et al. [281] |

*M* metal(loid)s, *NA* not applicable

- It preserves the ecosystem.
- It is the main technique of phytoremediation for the removal of heavy metals from soil, sediments, and water.
- It is also considered as the most commercially promising technique because it is inexpensive.

However, although it presents several advantages, there are some factors that limit metal phytoextraction [13, 282]:

- A low bioavailability of metals in the rhizosphere
- A low uptake rate
- A low translocation rate confining the metals in the roots

The amount of metal removed depends on concentration in aboveground plant materials and the plant biomass produced [283, 284]. However, the small biomass and slow growth of many (hyper)accumulators, as well as a low soil metal bioavailability, can limit the effectiveness of phytoextraction [53, 284]. A large number of plant species have the ability to hyperaccumulate metal(oid)s in their tissues. Throughout the years a general increase in pollution and the necessity to find reliable methods for the restoration of contaminated sites have led to an increased interest on hyperaccumulation, phytostabilization, or phytoextraction. Pertinent problems arise when trying to define plants as either hyperaccumulators or as suitable for phytostabilization or phytoextraction events. Plant(part)/soil and plant part/plant part ratios have been described, and new terms have been created, i.e., a particular ratio has been defined by several different names and acronyms. So, in the bibliography different terms have been attributed to the same ratio, and this often represents an overlap in terminology. On the other hand, the same term corresponds to several different ratios, and this could create confusion and misinterpretation in data comparison. Recently, Buscaroli [285] reviewed the various factors, coefficients, and indexes developed so far to evaluate terrestrial plant performance in respect to phytoremediation.

Some important hyperaccumulator families are Brassicaceae, Fabaceae, Caryophyllaceae, Flacourtiaceae, Euphorbiaceae, Asteraceae, Lamiaceae, Poaceae, Violaceae, *and* Scrophulariaceae [1, 5, 193]. The hyperaccumulator species (e.g., *Thlaspi caerulescens*, *Alyssum bertolonii*, *Arabidopsis halleri*) are able to accumulate contaminants but produce little biomass, and therefore it is possible to use species that accumulate less but which produce more biomass like *Brassica* spp., *Arundo donax*, and *Typha*s spp. [1, 13, 286–289]. An ideal plant for trace element phytoextraction should possess the following characteristics: (a) tolerance to the trace element accumulated, (b) fast growth and highly effective trace element accumulating biomass, (c) accumulation of trace elements in the aboveground parts, and (d) easy to harvest [230].

A typical trace element phytoextraction protocol consists of the following steps: (a) cultivation of the appropriate plant/crop species on the contaminated soil, (b) removal of harvestable trace element-enriched biomass from the site, and (c) post-harvest treatments (i.e., composting, compacting, thermal treatments) to reduce volume and/or weight of biomass for disposal as a hazardous waste or for its recycling to reclaim the elements that may have an economic value. The storage, treatment, and placement of the contaminated plant biomass are of great concern. Compaction and composting of the plant biomass decrease its volume and transport costs, but increase leaching of dissolved metal organic compounds [10, 290]. One of the most economical approaches to deal with the contaminated biomass is its commercial use as a source of energy [10]. Gasification and combustion are important trends in the production of thermal and electric energies. Direct combustion should be accomplished under control with a reduction in the biomass to 3–6% and the correct placement of the ash [10]. Thermal processing or thermos chemical gasification of the plant biomass into biogas (gaseous fuel) is a complex and high-tech process proceeding at 800–1300 °C. The ash obtained can be used as biore, and this process is termed as phytomining [10, 291]. Another effective method is thermochemical processing of the plant biomass (pyrolysis), which is a process of thermal decomposition of organic compounds in an oxic condition at relatively low temperatures (500–800 °C). This process allows the collection of fluid oils (biofuel, pyrofuel, resins), gases, and coke. The coke retains the heavy metals and is useful as a fuel in industry [10, 292]. To apply phytoextraction techniques, metal(loid)s must be bioavailable and ready to be absorbed by roots. The speciation of metal(loid)s in soils can include [13, 293]:

1. Free metal ions
2. Oxides, hydroxides, and carbonates
3. Integration into the structure of silicate/aluminum minerals
4. Soluble metal complexes
5. Associations with soil organic matter

Metal speciation regulates metal uptake by plant roots and consequently phytoextraction efficiency. Indeed, it is known that low bioavailability of soil metal(loid)s may be a rate-limiting factor for metal uptake by plants. The major limitations of most metal phytoextraction processes are (1) the bioavailability of the target metal(loid)s and (2) the ability of various plants to bioaccumulate metals within their aboveground biomass [48, 294]. Metal bioavailability can be defined as the fraction of metal in the soil that can interact with a biological target. In the soil solution, elements are present as free uncomplexed ions, ion pairs, ions complexed with organic anions, and ions complexed with organic macromolecules and inorganic colloids. The most important metal pools in the solid phase include the metals complexed by organic matter, sorbed onto or occluded within oxides, and clay minerals coprecipitated with secondary pedogenic minerals (e.g., Al, Fe, Mn oxides, carbonates and phosphates, sulfides) or as part of the crystal lattices of primary minerals [44, 61].

Current phytoextraction practices employ either hyperaccumulators or fast-growing high biomass plants, and the phytoextraction process may be enhanced by addition of soil amendments that can increase trace element bioavailability in the soil [230]. When bioavailability of metal(loid)s in the soils is insufficient for active root sorption, transport in solution and mobilization can be promoted using chelating agents [295], such as organic and mineral acids, elemental sulfur, and ammonium fertilizers. This chelant-assisted accumulation of heavy metals by plants that do not concentrate them under normal conditions was termed as chelant-induced hyperaccumulation. The application of chelating agents, for instance, synthetic aminopolycarboxylic acids, is efficient. Hydroxyethylene diamine triacetic acids (HEDTA), ethylenediamine dihydroxyphenylacetic acid, and ethylenediaminetetraacetic acid (EDTA) are potential acidifying chelants [295]. These agents can enhance the phytoextraction of Cu, Cd, Pb, Zn, and Ni [296].

There are a few disadvantages when using chelating agents, i.e., adverse effects on soil microorganisms, possible contamination of groundwater, and slow (several weeks or months) decomposition of the synthetic organic acids [296–298]. Following the application of chelate-forming agents, the removal of metals may continue for a long time. There are some rapid decomposing natural organic acids which are considered alternatives to synthetic chelating agents, i.e., ethylenediamine-*N*,*N′*-disuccinic (EDDS) acids and nitrilotriacetic acid (NTA) [296, 297]. In addition, products of plant metabolism and low-molecular organic compounds, i.e., tartaric [299], acetic [297], and malic acids, putrescine (polyamine), and citric and oxalic acids [300], can also be used for the same purpose. In recent years, different biotechnological approaches have also been used to increase biomass and growth rate of hyperaccumulator plants through genetic engineering tools, i.e., synthesis of various metal-binding peptides [10, 301]. Along with engineering, metal(loid)s accumulation by plants can be enhanced using conventional agronomic practices, i.e., fertilization, irrigation, seed control, shorter growth cycle, and liming [12].

### *2.4.1 Assisted by Fungi*

There is increasing evidence that plant-microbe interactions/dynamics can define the efficiency of metal phytoextraction. Inoculation of the plant rhizosphere with microorganisms is an established route to improve phytoextraction efficiency. In general, microorganisms can improve phytoextraction by increasing the bioavailability of metal(loid)s to the plant and by increasing plant biomass [302].

A frequently utilized strategy to improve phytoextraction is the inoculation of beneficial microorganisms into the plant rhizosphere. Microorganisms may increase plant trace element uptake by three specific mechanisms: (1) they may increase root surface area and hair density, (2) increase element bioavailability, and/or (3) increase soluble element transfer from the rhizosphere to the plant [168]. Furthermore, enhanced plant biomass production can boost an increase on the efficiency of trace element phytoextraction [230]. Increased trace element uptake can be attributed to a microbial modification of the absorptive properties of the roots such as increased root length and surface area and numbers of root hairs or by increasing the bioavailability of trace elements in the rhizosphere and the subsequent translocation to shoots via beneficial effects on plant growth, trace element complexation, and alleviation of phytotoxicity [230].

Regardless of whether trace elements are mainly accumulated in roots or in shoots, internal tolerance mechanisms are the basis for efficient detoxification of the trace elements. These internal detoxification mechanisms are extensively described in the previous sections. Among various microbe-assisted strategies that have been proposed to date, AM fungi are known to benefit their host plants by affecting biological regulation of their phytoextraction efficiency [127, 303, 304]. AM fungi are thought to be one of the most important soil microbial groups that affect metal uptake by plants and metal immobilization in soils [74, 305] and are commonly introduced into soil for land reclamation [74, 127, 306]. Previous studies reported that both host plant biomass and metal concentrations could be increased via AM fungi inoculation [127, 307], thus resulting in increased uptake of metal(loid)s [127, 308]. Different host plants and fungal species as well as environmental conditions could potentially affect response strategies of plants ([88, 127]). The functional diversity is significantly reflected on plant growth, element uptake, and enzymatic activity [127, 309, 310]. In some cases, AM fungi contributed to a significant storage of metal(loid)s at the root level, instead of the aboveground tissues of the host plants (phytostabilization). However, in other cases AM fungi contributed to enhanced uptake and translocation to shoots promoting phytoextraction success [86, 127].

An important point about treating polluted soil with mycorrhizal plants is the selection of appropriate AM species [12]. The species selected from areas polluted with metal(loid)s are the most efficient species which have the ability to survive under metal stress conditions and hence may act more efficiently relative to other AM species [12, 14]. These communities are metabolically and taxonomically diverse, containing microorganisms that are preadapted to conditions in situ and are capable of performing metabolic activities that can alter metal(loid)s bioavailability and promote plant growth [252, 302, 311–314]. Generally, species of the genus *Glomus* are predominant in the rhizosphere of plants growing in metal(loid)s-contaminated soils [12, 315, 316]. AM fungi can colonize hyperaccumulator roots extensively in metal(loid)-contaminated soils forming symbiotic/mutualistic relationships with plants. In general, mycorrhizal hyperaccumulators produce considerably more biomass and grow faster than non-mycorrhizal plants, and because of the ability of AM fungi to survive in severely contaminated soils, they are able to play a role on phytoremediation processes enhancing the capacity of plants to withstand soil phytotoxicity [60, 71, 72]. AM fungi promote plant establishment in metal(loid)s-contaminated soils, strengthening plant defense systems, and promoting its growth [73, 127].

Several biological and physical mechanisms have been proposed to explain metal tolerance of AM fungi and their contribution to metal tolerance of host plants. These tolerance mechanisms are numerous, e.g., extracellular metal sequestration and precipitation, metal binding to the fungal cell walls, intracellular sequestration and complexation, compartmentation, and volatilization, and are broadly described in previous sections. Many fungi can survive and grow with high concentrations of toxic metals [44, 317]. The ability of mycorrhizal associations to decrease metal toxicity to higher plants has been shown for ericoid mycorrhizas, ectomycorrhizas, and arbuscular mycorrhizas, while some ectomycorrhizal fungi accelerate mineral weathering. The mycorrhizal mycelia provide an efficient system for the uptake and direct transport of mobilized essential nutrients to their host plants, which are large sinks [44, 318]. Mineral weathering can also increase the concentrations of phytotoxic aluminum in the soil solution. Some ectomycorrhizal fungi increase Al tolerance through an enhanced exudation of oxalate [44, 319]. Furthermore, AM fungi inoculation can improve plant performance under metal(oid)s stress due to a number of mechanisms involving antioxidant enzymes, lipid peroxidation, and soluble amino acid profile changes caused by the intimate relationship between fungi and the host plant [73, 320, 321]. In fact, when exposed to metal(loid)s such as Cu, Fe, Pb, Cd, Cr, As, Hg, Cr, and Zn, plants enhance their level of reactive oxygen species (ROS) [73, 322, 323].

The contribution of AM fungi, such as *Funneliformis mosseae*, to enhanced Cd and Zn uptake and translocation by *S. nigrum* in heavy metal-contaminated sites has been noted without deleterious effects on plant growth [65, 324–326]. Fiorentino et al. [287] observed increases on Cd phytoextraction by *Arundo donax* assisted by the fungal microorganism *Trichoderma harzianum* in both, leaves (+20%) and rhizomes (+30%), while Chen et al. [327], observed an increase on Pb uptake and consequent accumulation in *Kummerowia striata*, lxeris denticulate and Echinochloa crusgalli.

It has been discovered recently that adding indigenous AM fungi can boost the uptake and accumulation of As and the biomass of *P. vittata* [60]. It was also shown that plants from an As mine site, colonized by AM fungi, accumulated more As than the non-colonized plants. An increase on growth, promoted by the activation of the phosphate transport system in colonized plants, may justify these results.

The process of translocation may be assumed to vary with metal(loid)s concentrations in the substrate, even if they grow with the same AM inoculation. Consequently, whether AM fungi enhance the transport to shoots (phytoextraction) or immobilize in the roots (phytostabilization) depends on metal(loid)s stress in the substrate [127]. AM fungi play different roles to cope with Cd toxicity in *P. australis*. With a low Cd stress, AM enhanced plant growth, and this acted as an accumulator due to the growth dilution effect. However, with high Cd stress, the AM symbiosis leads the plant to act as an excluder [127]. Consequently, in the presence of AM fungi, phytoextraction played the predominant role at low Cd stress, while phytostabilization occurred with high Cd stress [127]. The AM *Glomus intraradices* was shown to enhance growth of *Helianthus annuus*, and as a result, the total Ni is extracted. It also increased the activity of glutamine synthetase, indicating an enhanced Ni tolerance [230, 328].

A stimulation on the biomass of *B. coddii* in mycorrhizal plants led to a higher total Ni content (and hence phytoextraction) [80]. The diverse endophytic fungi isolated from *S. nigrum* showed the symbiotic association of these microbes with the host plant and improved our understanding regarding plant fitness under extreme conditions [56, 57]. The inoculation of *S. nigrum* with both *Glomerella truncata* PDL-1 and *Phomopsis fukushii* PDL-10 increased its tolerance to high concentrations of Cd. As a result, the parameters related to the biosorption of Cd, including translocation, bioconcentration factors, and tolerance index, were significantly enhanced. The strongest evidence for the presence of endophytes was the apparent promotion of plant growth and enhanced biochemical content of chlorophyll as compared to those of non-inoculated control plants [56, 57]. Endophytic fungi may increase host fitness and competitive abilities by increasing successful germination and growth rate or enhancing the uptake nutritional elements by the host [44, 116]. Furthermore, due to possessing suitable degradation pathways, metal sequestration, or chelation systems, fungal endophytes are able to increase host plant tolerance to metal(loid)s and assist the host survival in contaminated soils [44, 116].

### *2.4.2 Assisted by Bacteria*

Plant-associated bacteria can potentially improve phytoremediation by altering the solubility, bioavailability, and transport of metal(loid)s and nutrients by altering soil pH, release of chelators (e.g., siderophores, organic acids, biosurfactants, glycoproteins), methylation, P solubilization, or redox changes [50, 54, 61, 138, 193, 284, 329]. Therefore, plant-associated bacteria can be exploited to improve the efficiency of the phytoextraction processes [48, 168, 169, 222, 230, 232, 257, 330–332]. Bacterial populations associated with plants growing in metalliferous soils have a high diversity. These communities might also have important functions in relation to plant growth under these adverse conditions as well as in improving uptake of trace elements [230]. Plant growth promotion plays a major role in the extraction and removal of trace elements since a simple improvement in biomass results in an increase in the overall trace element yield (phytoextracted trace elements) [230]. The Ni-resistant PGPB strain *Psychrobacter* sp. SRS8 originally isolated from the rhizosphere of the Ni hyperaccumulator *A. serpyllifolium* was found to effectively promote the growth and phytoextraction potential of the energy crops *Ricinus communis* and *Helianthus annuus* in artificially Ni-contaminated soils [50, 230].

The Ni hyperaccumulator *A. serpyllifolium* subsp. *lusitanicum* grown in an ultramafic soil showed a significantly higher translocation and shoot Ni concentration after inoculation with a Ni-resistant rhizosphere bacteria *Arthrobacter nitroguajacolicus* [333]. In both hydroponically and soil-grown plants, inoculating the Cd/Zn hyperaccumulator *S. alfredii* with the metal(loid)-tolerant rhizobacterial strains belonging to the genera *Burkholderia* improved plant tolerance, biomass production, and Cd (and Zn) uptake and extraction [334, 335]. Bacteria can acidify their environment by pumping protons to maintain the electrochemical gradient of membranes. These replace trace element cations at sorption sites and dissolve minerals such as phosphates. Acid-producing rhizosphere bacteria have been intensely studied due to their capacity to release phosphorus from insoluble phosphates. For the purpose, bacteria can produce and secrete an array of organic acids, such as gluconic, 2-ketogluconic, lactic, and acetic acids [302]. The associated decrease in soil pH can also increase the solubility of some metal(loid)s [336].

Recent studies investigating the role of endophytes in metal(loid)s uptake by plants have demonstrated that the production of specific bacterial organic acids (e.g., citric, oxalic, acetic acids, etc.) may result in increased nutrient and metal(loid)s uptake, probably as a result of the decrease in soil pH and their solubilizing power, improving the efficiency of phytoextraction processes [52, 145]. The different metabolites released by PGPB (e.g., siderophores, osmolytes, nitric oxide, antibiotics, biosurfactants, organic acids, and plant growth regulators) can change metal(loid)s uptake either directly through their effects on plant growth or indirectly through acidification, chelation, precipitation, immobilization, and oxidation-reduction reactions in the rhizosphere [50]. Organic chelators scavenge trace element ions from sorption sites and mineral lattices and protect them from resorption [138, 230]. To date two groups of bacterially produced natural chelators are known. These are carboxylic acid anions and siderophores. Among a large variety of carbon compounds, oxalic, malic, and citric acids are some of the most important organic acids identified in roots and in microbial exudates [230, 337].

As the first p*K*a values of most carboxylates are below 3.5 and the cytosolic pH of root cells typically ranges from 7.1 to 7.5, carboxylic acids are typically present in soil solution as fully or partially dissociated forms [162, 230]. In plant cells, complexation with carboxylic acids, particularly malate, citrate, but also with the basic amino acid histidine, is a powerful mechanism for trace element detoxification [230, 338]. In addition to plant growth promotion, bacteria were reported to have a beneficial effect on plant stress tolerance. This may be achieved by the enzyme ACC deaminase leading to a reduction of stress-induced ethylene levels in the plant [230, 280, 339].

For instance, experiments assessed by Sheng et al. [232] have shown the solubilizing potential of the Pb-resistant endophytic bacteria *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 on Pb uptake by *Brassica napus*. The results showed that both endophytes enhanced Pb bioavailability, thus increasing Pb accumulation in plant shoots from 76% to 131% (*P. fluorescens*) and from 59% to 80% (*Microbacterium* sp.), compared to the respective control. A possible explanation might be the production of siderophores or organic acids that induce solubilization of Pb.

These effects of inoculation were reported also by Mastretta et al. [229], who reported that the inoculation of *N. tabacum* with the Cd-resistant endophyte *Sanguibacter* sp. increased the concentration of Cd in shoot tissues and consequently the phytoextraction capacity of *N. tabacum*, by approximately threefold compared with the respective uninoculated control. These studies suggest that it should be possible to improve the metal phytoextraction potential of accumulating or hyperaccumulating plants, through the inoculation of seeds or rhizosphere soil with beneficial metal-resistant endophytic bacteria. Although several conditions, such as soil properties, environmental conditions, and microbial activity, must be optimized for any phytoextraction event to become effective, the bioavailability of metals in the rhizosphere is considered to be a critical requirement for metal uptake to take place [54]. Braud et al. [340] reported that inoculating soils with *P. aeruginosa* significantly increased the concentrations of bioavailable Cr and Pb compared with uninoculated controls. Furthermore, they also observed that *P. aeruginosa* significantly enhanced Cr and Pb accumulation in maize shoots by a factor of 4.3 and 3.4, respectively. In this case, metal(loid)s uptake could be correlated with the increased production of siderophores, in particular of pyoverdin and pyochelin [54]. These studies highlighted the potential of inoculating soils or plants with metal(loid)s-resistant siderophore-producing bacteria to further improve their phytoextraction potential.

Siderophores play a significant role in metal mobilization and accumulation, and siderophore-producing microbes are believed to play an important role in the phytoextraction of metal(loid)s [54, 209], as these compounds produced by PGPB may solubilize unavailable forms of metals but also form complexes with bivalent metal(loid)s ions that can be assimilated by root-mediated processes [50, 274, 340]. Siderophores are secreted, and Fe(III)-siderophore complexes are recognized and scavenged from the environment by membrane receptor proteins. They are too large to pass membrane porins [230]. All siderophores possess higher affinity for Fe(III) than for Fe(II) or any other trace element. However, complexes of lower stability are also formed with other trace elements [230, 341]. Divalent cations (e.g., $Fe^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Cd^{2+}$) form less stable complexes due to their reduced charge density (charge/size ratio). Addition of trace elements to bacterial cultures induces siderophore synthesis and leads to the formation of siderophore-metal complexes [206–208, 230]. Extracellular complexation by siderophores is considered to be a mechanism of bacterial trace element resistance [230, 332]. Siderophore synthesis was shown to simultaneously increase iron uptake and to reduce cadmium uptake in *Streptomycetes* [208, 209, 230]. In contrast, siderophore-mediated uptake of trivalent trace element cations ($Al^{3+}$) has been demonstrated in iron-depleted cultures [230]. Synthesis of several siderophores varying in trace element affinity, preferences, and inductivity may convey competitive advantage in trace element contaminated environments [208, 209, 230].

The biosurfactants produced by PGPB also enhance metal(loid)s mobilization and improve phytoextraction on contaminated soils [50, 342]. Biosurfactants can desorb metal(loid)s from the soil matrix and hence increase metal solubility and bioavailability [193, 231, 343]. Certain nitrogen-fixing bacteria produce molybdate-binding tetradentate catecholates, which also function as siderophores [230, 341]. The pigment melanin, which is produced by many fungi and *Streptomycetes*, can bind trace elements to its carboxylic groups and was shown to be involved in trace element sorption and trace element tolerance of *S. scabies* [230, 344]. In addition to beneficial effects on growth by improved plant nutrition, microorganisms can also enhance plant growth directly via the production of phytohormones, including IAA, cytokinins, and gibberellins. These compounds can stimulate germination, growth, and reproduction and protect plants against both biotic and abiotic stress [50, 345]. Indeed, the mechanism most often cited to explain the various direct effects of PGP on plants is the production of phytohormones, and most of the attention has focused on the role of the phytohormone auxin [48, 346, 347]. In addition to the well-characterized effects of microbial auxin and ethylene on plant growth, a number of plant growth-promoting bacteria synthesize cytokinins, which can stimulate the growth of different crops under both stressed and non-stressed conditions helping the phytoextraction processes [48, 348].

In the last years, it has been found that a number of PGPB contain the enzyme ACC deaminase, which can cleave the plant ethylene precursor ACC and thereby lower the level of the phytohormone ethylene in a developing or stressed plant [302].

After working on microbe-assisted Cd phytoextraction, Wei et al. [104, 105] reported that the endophytic bacterial strain *Rahnella* sp. JN27, isolated from roots of *Zea mays*, enhanced Cd bioavailability in the soil and solubilized phosphate, producing indole-3-acetic acid, siderophores, and ACC deaminase. Multiple reports showed that foliar application of IAA or other phytohormones can improve the phytoextraction of metals, including Ni, Pb, and Cd [302, 349, 350]. The proliferation of specific microorganism strains, able to aggressively colonize the root surface, can promote growth and healthier plants, improving root development, and/or enhance plant tolerance to different environmental stresses, strengthening the phytoextraction processes [48, 351]. Accordingly, metal phytoextraction (as well as plant growth) can be helped by soil microorganisms associated with plant roots [48, 351]. Finally, a few studies reported that plant growth and/or trace element accumulation has been improved using combinations of plant-associated microorganisms. In a hydroponic study, a combination of seven As-resistant rhizobacteria (identified as *Pseudomonas* sp., *Comamonas* sp., and *Stenotrophomonas* sp.) enhanced As uptake by the As hyperaccumulator *Pteris vittata*. Microbial exudation of pyochelin-type siderophores, together with root exudates, solubilized As from the growth media spiked with insoluble $FeAsO_4$ and $AlAsO_4$ minerals [352].

In soil-grown plants, inoculation of *Salix caprea* with *Streptomyces* sp. in combination with the fungus *Cadophora finlandica* led to an increase in phytoextraction of Cd and Zn (Table 2.4) [230, 365].

## 2.5 Conclusions and Future Prospects

Phytoremediation techniques, based on interactions between plants and microorganisms, have been proposed as eco-friendly methods to clean polluted soils. Soil microorganisms can improve pollutant mobilization and respective uptake by plants. The success of phytoextraction depends on several factors, including the concentration of soil pollutants, metal bioavailability for root uptake, and the capability of plants to intercept, sorb, and accumulate metal(loid)s in their tissues. Ultimately, the success of phytoextraction depends on interactions among soil, metals, and plants. However, low bioavailability of metals, low biomass of most hyperaccumulators, and restricted metal translocation to the shoots limit the efficiency of phytoextraction. In order to solve these restrictive factors, some strategies such as advanced agricultural practices, genetic engineering, and chelate treatments need to be adopted to improve phytoextraction performance. The use of natural chelators to

**Table 2.4** Results of phytoextraction studies assisted by plant-associated microbes

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress, phytoextraction | References |
|---|---|---|---|---|
| *Microbacterium*, *Variovorax, Micrococcus*, *Pseudomonas* | Zn | *Noccaea caerulescens, Rumex acetosa* | Increase in the growth of both plants, as well as higher values of Zn phytoextraction | Burges et al. [284] |
| *Bacillus pumilus* E2S2, *Bacillus* sp. E1S2 | Cd, Zn, Pb | *Sedum plumbizincicola* | *B. pumilus* E2S2 significantly increased root and shoot length, biomass, and plant Cd uptake, whereas *Bacillus* sp. E1S2 significantly enhanced the accumulation of Zn. Results demonstrated the potential to improve phytoextraction of soils contaminated with multiple heavy metals by inoculating metal hyperaccumulating plants with their own selected functional endophytic bacterial strains | Ma et al. [147] |
| *Streptomyces* sp., *Cadophora finlandica* | Cd, Zn | *Salix caprea* | Increased phytoextraction of Cd and Zn | Sessitsch et al. [230] |
| *Firmicutes* sp., *Actinobacteria* sp., *Proteobacteria* sp. | Cu | *Brassica napus* | Increased root and aboveground tissues weight and shoot Cu concentration | Sun et al. [353] |
| *Bacillus subtilis*, *B. cereus*, *Flavobacterium* sp., *Pseudomonas aeruginosa* | Zn | *Orychophragmus violaceus* | Increased root length, biomass of root, stems, and leaves, and Zn uptake | He et al. [354] |
| *Burkholderia cepacia* | Zn, Cd | *Sedum alfredii* | Higher ability to mobilize Cd and Zn and tolerate high concentrations of soluble Zn. Increased the soluble Zn concentration in the medium from insoluble zinc oxide and zinc carbonate. Oxalic, tartaric, formic, and acetic acids had a significant correlation with the concentrations of Cd and Zn being mobilized | Li et al. [355] |
| *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ16 | Cd, Pb | *Lycopersicon esculentum* | Increased root length, aboveground biomass and aboveground metal content, siderophores, IAA, ACC deaminase production | He et al. [356] |

(continued)

**Table 2.4** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress, phytoextraction | References |
|---|---|---|---|---|
| *Psychrobacter* sp. *SRA1 and SRA2*, *Bacillus cereus SRA10* | Ni | *Brassica juncea*, *Brassica oxyrrhina* | Significantly increased the accumulation of Ni in the root and shoot tissues reinforcing the efficiency of phytoextraction | Ma et al. [313] |
| *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia metallidurans* | Cr, Pb | *Zea mays* | Enhanced Cr and Pb uptake by plants through their mobilization | Braud et al. [340] |
| *Pseudomonas fluorescens G10*, *Microbacterium* sp. *G16* | Pb | *Brassica napus* | Increased plant weight, ACCD activity, IAA production, P accumulation, and Pb translocation to shoots | Sheng et al. [232] |
| *Bacillus edaphicus* NBT | Pb | *Brassica juncea* L. Czern | Promoted plant growth (root and shoot biomass) and Pb uptake | Sheng et al. [222] |
| *Pseudomonas tolaasii ACC23, P. fluorescens ACC9, Mycobacterium* sp. *ACC14* | Cd | *Brassica napus* | The strains did not influence Cd concentration in the root or shoot, but they increased plant biomass and consequently the total Cd extracted | Dell'Amico et al. [312] |
| *Enterobacter* sp. *NBRI K28* | Ni, Zn, Cr | *Brassica juncea* | Stimulated plant biomass and enhanced phytoextraction of Ni, Zn, and Cr<br>Production of siderophores, IAA, and phosphate solubilization. The strain also exhibited ACC deaminase activity | Kumar et al. [357] |
| *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas pseudoalcaligene*, *Brevibacterium halotolerans* | Cu, Cr, Pb, Zn | *Zea mays*, *Sorghum bicolor* | Increased metal availability in soil, thus enhancing Cr, Pb, Zn, and Cu accumulation by *Z. mays* and *S. bicolor* | Abou-Shanab et al. [358] |
| *Burkholderia* sp. *J62* | Pb, Cd | *Brassica juncea*, *Zea mays*, *Lycopersicon esculentum* | Increased biomass and metal uptake | Jiang et al. [359] |
| *B. weihenstephanensis SM3* | Cu, Zn | *Helianthus annuus* | Increased the plant biomass and the accumulation of Cu and Zn in the root and shoot systems | Rajkumar et al. [205] |

(continued)

**Table 2.4** (continued)

| Microorganisms | M | Test plant | Microbial effects on plants under metal stress, phytoextraction | References |
|---|---|---|---|---|
| *P. putida ARB86* | Ni | *Arabidopsis thaliana* | Increased biomass and chlorophyll content. Nickel influx into plants was decreased by bacterial sorption in the rhizosphere | Someya et al. [261] |
| *Burkholderia cepacia* | Cd, Zn | *Sedum alfredii* | Enhanced plant growth, metal uptake, and translocation of metals from root to shoot | Li et al. [335] |
| *P. marginalis Dp1*, *Rhodococcus* sp. *Fp2* | Cd | *Pisum sativum* | Increased Cd concentration in shoots | Safronova et al. [360] |
| *Pseudomonas monteilii* | Cd | *Sorghum bicolor* | Enhanced plant biomass, Cd uptake, and translocation to shoots | Duponnois et al. [361] |
| *Azotobacter chroococcum* HKN-5, *Bacillus megaterium* HKP-1, *Bacillus mucilaginosus* HKK-1 | Cd, Cu, Pb, Zn | *Brassica juncea* | Stimulated plant growth and protected the plant from metal toxicity<br>It did not influence metal concentrations in plant tissues, but led to greater aboveground biomass, thus resulting in much higher metal removal. It also influenced speciation of metals in the soil and consequently altered the bioavailability of metals | Wu et al. [362] |
| *Sinorhizobium* sp. Pb002 | Pb | *Brassica juncea* | Stimulated biomass production and, hence, phytoextraction of Pb | Di Gregorio et al. [363] |
| *B. subtilis SJ-101* | Ni | *Brassica juncea* | Exhibited the capability to produce IAA and to solubilize inorganic phosphate.<br>Promoted plant growth and decreased soluble soil Ni by biosorption and bioaccumulation | Zaidi et al. [364] |
| *Brevibacillus* sp. *B-I* | Zn | *Trifolium repens* | Decreased the concentration of Zn in shoot tissues | Vivas et al. [268] |

enhance metal bioavailability and inoculation of microorganisms, including bacteria and fungi, may facilitate the phytoextraction appliance at a commercial scale.

A number of bacteria and fungi have been studied by researchers which are able to enhance metal accumulation by plants and the metal(loid)s phytoextraction rate. Fungi are generally more tolerant to metal(loid)s than bacteria. Furthermore, AM fungi can efficiently explore the soil microsites that are not accessible for plant roots. In this context, a combination of plant-associated microorganisms could be more effective in enhancing reclamation of polluted soils than a single microorganism. It has been shown that endophytes (bacteria and fungi) can be a more reliable source of natural biocenosis because of their intimate association with plants. However, an understanding of the mechanisms enabling endophytic microbes to interact with host plants growing in metal-contaminated soils is essential to fully accomplish the biotechnological applications of efficient plant-microbe partnerships. Furthermore, there is ample experimental evidence that metal-resistant SPB are able to survive in adverse environmental conditions, where they carry out a variety of beneficial interactions that increase plant growth and metal(loid)s uptake. The beneficial effects exhibited by SPB indicate that the inoculation with metal-resistant strains may contribute to increase phytoextraction potential in metal-contaminated soils.

However, a detailed and accurate characterization of target metal(loid)-contaminated soils is needed before the inoculation of microbes, as well as adequate strategies to enhance inoculant performance by using efficient carrier materials. In addition, inoculation of mixtures of ecologically diverse microbes instead of single strains might represent a highly successful strategy. In this way, beneficial functions might be expressed more continually in a soil or rhizosphere system, even under ecologically distinct conditions. Furthermore, application of genetic engineering may enhance phytoextraction efficiency. After the identification of novel genes, transgenic plants may be produced with superior extracting capacity involving metal(loid)s hypertolerance, raised uptake and translocation to shoots, and highly efficient detoxification mechanisms. Genes for metal chelators production, metal homeostasis, transporters, biodegradative enzymes, metal uptake regulators, and biotic and abiotic stresses relievers are important candidates for making recombinant microbes.

The complexity and heterogeneity of soils contaminated with multiple metals and organic compounds requires the design of integrated phytoremediation systems that combine different processes and approaches. It is obvious that the complexity of interactions in the plant-microbe-soil-pollutant systems requires substantial further research efforts to improve our understanding of the rhizosphere processes involved.

Fortunately, modern biotechnology has opened up new possibilities concerning the application of beneficial microbes to improve plant growth, biological control, as well as metal(loid)s phytoremediation.

## References

1. Pinto AP, de Varennes A, Lopes ME, Teixeira DM (2016) Biological approaches for remediation of metal-contaminated sites. In: Ansari A, Gill S, Gill R, Lanza G, Newman L (eds) Phytoremediation. Management of environmental contaminants, vol 3. Springer, Wien, Austria, pp 65–112

2. Awad YM, Kim SC, Abd El-Azeem SAM, Kim KH, Kim KR, Kim K et al (2014) Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. Environ Earth Sci 71:1433–1440
3. He L, Yang H, Yu Z, Tang J, Xu L, Chen X (2014) Arbuscular mycorrhizal fungal phylogenetic groups differ in affecting host plants along heavy metal levels. J Environ Sci 26:2034–2040. https://doi.org/10.1016/j.jes.2014.07.013
4. Moreira H, Marques APGC, Rangel AOSS, Castro PML (2011) Heavy metal accumulation in plant species indigenous to a contaminated Portuguese site: prospects for phytoremediation. Water Air Soil Pollut 221:377–389
5. Pinto AP, Varennes A, Fonseca R, Teixeira DM (2015) Phytoremediation of soils contaminated with heavy metals: techniques and strategies. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Management of environmental contaminants, vol 1. Springer, Wien, Austria, pp 133–155
6. Song B, Zeng G, Gong J, Liang J, Xu P, Liu Z et al (2017) Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. Environ Int 105:43–55
7. Tang Z, Zhang L, Huang Q, Yang Y, Nie Z, Cheng J et al (2015) Contamination and risk of heavy metals in soils and sediments from a typical plastic waste recycling area in North China. Ecotoxicol Environ Saf 122:343–351
8. Teng Y, Wu J, Lu S, Wang Y, Jiao X, Song L (2014) Soil and soil environmental quality monitoring in China: a review. Environ Int 69:177–199
9. Zeng GM, Chen M, Zeng ZT (2013) Risks of neonicotinoid pesticides. Science 340(6139):1403. https://doi.org/10.1126/science.340.6139.1403-a
10. Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q et al (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121
11. Perez J (2012) The soil remediation industry in Europe: the recent past and future perspectives. Ernst and Young, London, pp 2–22
12. Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120
13. Cristaldi A, Conti GO, Jho EH, Zucarello P, Grass A, Copat C, Ferrante M (2017) Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. Environ Technol Innov 8:309–326
14. Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653
15. Shayler H, McBride M, Harrison E (2009) Sources and impacts of contaminants in soils. Department of Crop & Soil Sciences, Cornell Waste Management Institute, Ithaca, NY http://cwmi.css.cornell.edu
16. Conceição Gomes MA, Hauser-Davis RA, Nunes de Souza A, Vitória AP (2016) Metal phytoremediation: general strategies, genetically modified plants and applications in metal nanoparticle contamination. Ecotoxicol Environ Saf 134:133–147
17. Horta A, Malone B, Stockmann U, Minasny B, Bishop TFA, McBratney AB et al (2015) Potential of integrated field spectroscopy and spatial analysis for enhanced assessment of soil contamination: a prospective review. Geoderma 241–242:180–209
18. Cameselle C, Chirakkara RA, Reddy KR (2013) Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosphere 93:626–636
19. Heckenroth A, Rabier J, Dutoit T, Torre F, Prudent P, Laffont-Schwob I (2016) Selection of native plants with phytoremediation potential for highly contaminated Mediterranean soil restoration: tools for a non-destructive and integrative approach. J Environ Manage 183:850–863
20. Luo J, Cai L, Qi S, Wu J, Gu XS (2017) A multi-technique phytoremediation approach to purify metals contaminated soil from e-waste recycling site. J Environ Manage 204:17–22

21. Luo N, Li X, Chen AY, Zhang LJ, Zhao HM, Xiang L et al (2017) Does arbuscular mycorrhizal fungus affect cadmium uptake and chemical forms in rice at different growth stages? Sci Total Environ 599–600:1564–1572
22. Hazrat A, Ezzat K, Muhammad AS (2013) Phytoremediation of heavy metal–concepts and applications. Chemosphere 91:869–881
23. Wang L, Ji B, Hu Y, Liu R, Sun W (2017) A review on in situ phytoremediation of mine tailings. Chemosphere 184:594–600
24. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881
25. Balseiro-Romero M, Gkorezis P, Kidd PS, Van Hamme J, Weyens N, Monterroso C et al (2017) Use of plant growth promoting bacterial strains to improve *Cytisus striatus* and *Lupinus luteus* development for potential application in phytoremediation. Sci Total Environ 581–582:676–688
26. Mojiri A, Lou Z, Tajuddin RM, Farraji H, Alifar N (2016) Co-treatment of landfill leachate and municipal wastewater using the ZELIAC/zeolite constructed wetland system. J Environ Manage 166:124–130
27. Salam JA, Hatha MAA, Das N (2017) Microbial-enhanced lindane removal by sugarcane (*Saccharum officinarum*) in doped soil-applications in phytoremediation and bioaugmentation. J Environ Manage 193:394–399
28. Afzal M, Khan QM, Sessitsch A (2014) Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. Chemosphere 117:232–242
29. Jiang Y, Lei M, Duan L, Longhurst P (2015) Integrating phytoremediation with biomass valorisation and critical element recovery: a UK contaminated land perspective. Biomass Bioenergy 83:328–339
30. Tahir U, Yasmin A, Khan UH (2016) Phytoremediation: potential flora for synthetic dyestuff metabolism. J King Saud Univ Sci 28:119–130. https://doi.org/10.1016/j.jksus.2015.05.009
31. Coninx L, Martinova V, Rineau F (2017) Chapter Four - Mycorrhiza-assisted phytoremediation. Adv Bot Res 83:127–188
32. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A et al (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794
33. Gerhardt K, Gerwing P, Greenberg B (2017) Opinion: Taking phytoremediation from proven technology to accepted practice. Plant Sci 256:170–185
34. Mudhoo A, Sharma SK, Lin ZQ, Dhankher OP (2010) Phytoremediation of arsenic-contaminated environment an overview. In: Sharma A, Mudhoo SK (eds) Green chemistry for environmental sustainability, vol 127. Taylor and Francis Group, Boca Ratan
35. Sreelal G, Jayanthi R (2017) Review on phytoremediation technology for removal of soil contaminant. Indian J Sci Res 14:127–130
36. Alford ER, Pilon-Smits EAH, Paschke MW (2010) Metallophytes - a view from the rhizosphere. Plant Soil 337:33–50. https://doi.org/10.1007/s11104-010-0482-3
37. Cabral L, Soares CFRS, Giachini AJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31:1655–1664
38. Krznaric E, Wevers JHL, Cloquet C, Vangronsveld J, Vanhaecke F, Colpaert JV (2010) Zn pollution counteracts Cd toxicity in metal-tolerant ectomycorrhizal fungi and their host plant, *Pinus sylvestris*. Environ Microbiol 12(8):2133–2134
39. Sheoran V, Sheoran A, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41:168–214
40. Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A et al (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721

41. Ashraf MA, Hussain I, Rasheed R, Iqbal M, Riaz M, Arif MS (2017) Advances in microbe-assisted reclamation of heavy metal contaminated soils over the last decade: a review. J Environ Manage 198:132–143
42. Zubair M, Shakir M, Ali Q, Rani N, Fatima N, Farooq S et al (2016) Rhizobacteria and phytoremediation of heavy metals. Environ Technol Rev 5:112–119. https://doi.org/10.1080/21622515.2016.1259358
43. Rajkumar M, Prasad MNV, Sandhya S, Freitas H (2013) Climate change driven plant-metal-microbe interactions. Environ Int 53:74–86
44. Deng Z, Cao L (2017) Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere 168:1100–1106
45. Deng Z, Cao L, Huang H, Jiang X, Wang W, Shi Y et al (2011) Characterization of Cd- and Pb-resistant fungal endophyte Mucor sp. CBRF59 isolated from rapes (*Brassica chinensis*) in a metal-contaminated soil. J Hazard Mater 185:717–724
46. Deng Z, Zhang R, Shi Y, Hu L, Tan H, Cao L (2013) Enhancement of phytoremediation of Cd- and Pb-contaminated soils by self-fusion of protoplasts from endophytic fungus Mucor sp. CBRF59. Chemosphere 91:41–47
47. Deng Z, Zhang R, Shi Y, Hu L, Tan H, Cao L (2013) Characterization of Cd-, Pb-, Zn-resistant endophytic *Lasiodiplodia* sp. MXSF31 from metal accumulating *Portulaca oleracea* and its potential in promoting the growth of rape in metal-contaminated soils. Environ Sci Pollut Res 21:2346–2357
48. Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28(3):367–374
49. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242
50. Ma Y, Prasad M, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258
51. Ma Y, Rajkumar M, Rocha I, Oliveira RS, Freitas H (2015) Serpentine bacteria influence metal translocation and bioconcentration of *Brassica juncea* and *Ricinus communis* grown in multi-metal polluted soils. Front Plant Sci 5:757
52. Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manage 174:14–25
53. Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160
54. Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149
55. Lebeau T, Braud A, Jequel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522
56. Khan A, Ullah I, Waqas M, Park G, Khan A, Hong S et al (2017) Host plant growth promotion and cadmium detoxification in *Solanum nigrum*, mediated by endophytic fungi. Ecotoxicol Environ Saf 136:180–188
57. Khan AR, Waqas M, Ullah I, Khan AL, Khan MA, Lee IJ, Shin JH (2017) Culturable endophytic fungal diversity in the cadmium hyperaccumulator *Solanum nigrum* L. and their role in enhancing phytoremediation. Environ Exp Bot 135:126–135. https://doi.org/10.1016/j.envexpbot.2016.03.005
58. Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408
59. Cao XF, Liu LP (2015) Using microorganisms to facilitate phytoremediation in mine tailings with multi heavy metals. Adv Mater Res 1094:437–440. https://doi.org/10.4028/www.scientific.net/AMR.1094.437
60. Leung HM, Wang ZW, Ye ZH, Yung KL, Peng XL, Cheung KC (2013) Interactions between arbuscular mycorrhizae and plants in phytoremediation of metal-contaminated soils: a review. Pedosphere 23:549–563

61. Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S et al (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259
62. Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–189
63. Schneider J, Bundschuh J, Nascimento C (2016) Arbuscular mycorrhizal fungi-assisted phytoremediation of a lead-contaminated site. Sci Total Environ 572:86–97. https://doi.org/10.1016/j.scitotenv.2016.07.185
64. Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud Mycol 53:173–189
65. Li H, Li X, Xiang L, Zhao HM, Li YW, Cai QY et al (2018) Phytoremediation of soil co-contaminated with Cd and BDE-209 using hyperaccumulator enhanced by AM fungi and surfactant. Sci Total Environ 613–614:447–455
66. Lu YF, Lu M (2015) Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms. J Hazard Mater 285:535–541
67. Mohammad A, Mittra B (2013) Effects of inoculation with stress-adapted arbuscular mycorrhizal fungus *Glomus deserticola on* growth of *Solanum melongena L.* and *Sorghum sudanense* Staph. seedlings under salinity and heavy metal stress conditions. Arch Agron Soil Sci 59:173–183
68. Morton JB, Redecker D (2001) Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera Archaeospora and Paraglomus, based on concordant molecular and morphological characters. Mycologia 93:181–195
69. Sieverding E, Oehl F (2006) Revision of Entrophospora and description of Kuklospora and Intraspora, two new genera in the arbuscular mycorrhizal Glomeromycetes. J Appl Bot Food Qual 80:69–81
70. Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H et al (2007) Molecular phylogeny and new taxa in the Archaeosporales (Glomeromycota): *Ambispora fennica* gen. sp. nov., Ambisporaceae fam. nov., and emendation of Archaeospora and Archaeosporaceae. Mycol Res 111:137–153
71. 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 Phytoremediation 12:384–403
72. Leung HM, Wu FY, Cheung KC, Ye ZH, Wong MH (2010) Synergistic effects of arbuscular mycorrhizal fungi and phosphate rock on heavy metal uptake and accumulation by an arsenic hyperaccumulator. J Hazard Mater 181:497–507
73. Chaturvedi R, Favas P, Pratas J, Varun M, Paul MS (2018) Assessment of edibility and effect of arbuscular mycorrhizal fungi on T *Solanum melongena* L. grown under heavy metal(loid) contaminated soil. Ecotoxicol Environ Saf 148:318–326. https://doi.org/10.1016/j.ecoenv.2017.10.048
74. Qiao Y, Crowley D, Wang K, Zhang H, Li H (2015) Effects of biochar and Arbuscular mycorrhizae on bioavailability of potentially toxic elements in an aged contaminated soil. Environ Pollut 206:636–643
75. Fortin JA, Plenchette C, Piché Y (2009) Mycorrhizas: the new green revolution. Québec City, CA, Editions MultiMondes
76. Garg N, Chandel S (2012) Role of arbuscular mycorrhizal (am) fungi on growth, cadmium uptake, osmolyte, and phytochelatin synthesis in *Cajanus cajan* (l.) millsp. under NaCl and Cd stresses. J Plant Growth Regul 31:292–308
77. Sarwat M, Hashem A, Ahanger MA, Abd Allah EF, Alqarawi AA, Alyemeni MN et al (2016) Mitigation of NaCl stress by arbuscular mycorrhizal fungi through the modulation of osmolytes, antioxidants and secondary metabolites in mustard (*Brassica juncea* L.) plants. Front Plant Sci 7:869
78. Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Ann For Sci 68:17–24

79. Krznaric E, Verbruggen N, Vangronsveld J, Colpaert JV (2009) Cd-tolerant Suillus luteus: a fungal insurance for pines exposed to Cd. Environ Pollut 157:1581–1585
80. Orłowska E, Przybyłowicz W, Orlowski D, Turnau K, Mesjasz-Przybyłowicz J (2011) The effect of mycorrhiza on the growth and elemental composition of Ni-hyperaccumulating plant Berkheya coddii Roessler. Environ Pollut 159:3730–3738
81. Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181
82. Meier S, Borie F, Bolan N, Cornejo P (2012) Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Crit Rev Environ Sci Technol 42:741–777
83. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8:1–17
84. Tian W, Zhang CQ, Qiao P, Milne R (2011) Diversity of culturable ericoid mycorrhizal fungi of *Rhododendron decorum* in Yunnan. China Mycol 103:703–709
85. Wu FY, Ye ZH, Wu SC, Wong MH (2007) Metal accumulation and arbuscular mycorrhizal status in metallicolous and nonmetallicolous populations of *Pteris vittata* L. and *Sedum alfredii* Hance. Planta 226:1363–1378
86. Göhre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122
87. Andrade SAL, Silveira APD, 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 408:5381–5391
88. Leyval C, Joner E, Del Val C, Haselwandter K (2001) Potential of arbuscular mycorrhiza for bioremediation. Mycorrhiza 7:308–317
89. Vosátka M, Rydlová J, Sudová R, Vohník M (2006) Mycorrhizal fungi as helping agents in phytoremediation of degraded and contaminated soils. In: Mackova M, Dowling D, Macek T (eds) Phytoremediation rhizoremediation. Springer, Heidelberg, pp 237–257
90. Vodnik D, Grčman H, Maček I, van Elteren JT, Kovačevič M (2008) The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. Sci Total Environ 392:130–136
91. Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi, Soil Biol. Biochem. 37:101–106
92. Malekzadeh E, Aliasgharzad N, Majidi J, Abdolalizadeh J, Aghebati-Maleki L (2016) Contribution of glomalin to Pb sequestration by arbuscular mycorrhizal fungus in a sand culture system with clover plant. Eur J Soil Biol 74:45–51. https://doi.org/10.1016/j.ejsobi.2016.03.003
93. González-Chávez C, D'Haen J, Vangronsveld J, Dodd JC (2002) Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240:287–297
94. Cornejo P, Meier S, Borie G, Rillig MC, Borie F (2008) Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci Total Environ 406:154–160
95. González-Chávez C, Harris PJ, Dodd J, Meharg AA (2002) Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. New Phytol 155:163–171
96. Luo J, Cai L, Qi S, Wu J, Gu XS (2017) Improvement effects of cytokinin on EDTA assisted phytoremediation and the associated environmental risks. Chemosphere 180:386–393
97. Shen H, Christie P, Li X (2006) Uptake of zinc, cadmium and phosphorus by arbuscular mycorrhizal maize (*Zea mays* L.) from a low available phosphorus calcareous soil spiked with zinc and cadmium. Environ Geochem Health 28:111–119
98. Janoušková M, Pavlíková D (2010) Cadmium immobilization in the rhizosphere of arbuscular mycorrhizal plants by the fungal extraradical mycelium. Plant Soil 332:511–520

99. Yu H, Xiang Z, Zhu Y, Wang J, Yang Z, Yang PZ (2012) Subcellular and molecular distribution of cadmium in two rice genotypes with different levels of cadmium accumulation. J Plant Nutr 35:71–84
100. Zeng F, Zhou W, Qiu B, Ali S, Wu F, Zhang G (2011) Subcellular distribution and chemical forms of chromium in rice plants suffering from different levels of chromium toxicity. J Plant Nutr Soil Sci 174:249–256
101. Wang Y, Jing H, Gao Y (2012) Arbuscular mycorrhizal colonization alters subcellular distribution and chemical forms of cadmium in *Medicago sativa* L. and resists cadmium toxicity. PLoS One 7:3161–3164
102. Rajkumar M, Sandhya S, Prasad M, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574
103. Zarei M, Wubet T, Schäfer SH, Savaghebi GR, Jouzani GS, Nekouei MK et al (2010) Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environ Pollut 158:2757–2765
104. Wei Y, Hou H, Xian Y, Guan S, Li JN, Li FS (2014) Genetic diversity of endophytic bacteria of the manganese-hyperaccumulating plant *Phytolacca americana* growing at a manganese mine. Eur J Soil Biol 62:15–21
105. Wei Y, Hou H, Li JN, ShangGuan YX, Xu YF, Zhang J et al (2014) Molecular diversity of arbuscular mycorrhizal fungi associated with an Mn hyperaccumulator—*Phytolacca americana*, in Mn mining area. Appl Soil Ecol 82:11–17. https://doi.org/10.1016/j.apsoil.2014.05.005
106. Wu FY, Ye ZH, Wong MH (2009) Intraspecific differences of arbuscular mycorrhizal fungi in their impacts on arsenic accumulation by *Pteris vittata* L. Chemosphere 76:1258–1264
107. Lin AJ, Zhang XH, Wong MH, Ye ZH, Lou LQ, Wang YS et al (2007) Increase of multi-metal tolerance of three leguminous plants by arbuscular mycorrhizal fungi colonization. Environ Geochem Health 29:473–481
108. Zhu LJ, Guan DX, Luo J, Rathinasabapathi B, Ma LQ (2014) Characterization of arsenic-resistant endophytic bacteria from hyperaccumuators *Pteris vittata* and *Pteris multifida*. Chemosphere 113:9–16
109. 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
110. Lingua G, Franchin C, Todeschini V, Castiglione S, Biondi S, Burlando B et al (2008) Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. Environ Pollut 153:137–147
111. Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514
112. Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50:847–853
113. 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
114. Sheikh-Assadi M, Khandam-Mirkohi A, Alemardan A, Moreno-Jiménez E (2015) Mycorrhizal *Limonium sinuatum* (l.) Mill. enhances accumulation of lead and cadmium. Int J Phytoremediation 17:556–562
115. Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41:1–16
116. Aly A, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90:1829–1845
117. Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant microbe interaction. Mol Plant Microbe Interact 17:6–15
118. Stepniewska Z, Kuzniar A (2013) Endophytic microorganisms—promising applications in bioremediation of greenhouse gases. Appl Microbiol Biotechnol 97:9589–9596

119. Deng Z, Wang W, Tan H, Cao L (2012) Characterization of heavy metal-resistant endophytic yeast Cryptococcus sp. CBSB78 from rapes (*Brassica chinensis*) and its potential in promoting the growth of Brassica spp. in metal-contaminated soils. Water Air Soil Pollut 223:5321–5329
120. Wang W, Deng Z, Tan H, Cao L (2013) Effects of Cd, Pb, Zn, Cu-resistant endophytic *Enterobacter sp.* CBSB1 and *Rhodotorula sp.* CBSB79 on the growth and phytoextraction of Brassica plants in multimetal contaminated soils. Int J Phytoremediation 15:488–497
121. Babu AG, Shim J, Shea PJ, Oh BT (2014) *Penicillium aculeatum* PDR-4 and *Tri-choderma* sp. PDR-16 promote phytoremediation of mine tailing soil and bio-energy production with Sorghum sudan grass. Ecol Eng 69:186–191
122. Babu AG, Shim J, Bang KS, Shea PJ, Oh BT (2014) *Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. J Environ Manage 132:129–134
123. Khan AL, Waqas M, Hussain J, Al-Harrasi A, Lee IJ (2014) Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe). Biol Fertil Soils 50:75–85
124. Pierart A, Dumat C, Maes AQM, Sejalon-Delmas N (2018) Influence of arbuscular mycorrhizal fungi on antimony phyto-uptake and compartmentation in vegetables cultivated in urban gardens. Chemosphere 191:272–279
125. Gil-Cardeza ML, Müller DR, Amaya-Martin SM, Viassolo R, Gómez E (2018) Differential responses to high soil chromium of two arbuscular mycorrhizal fungi communities isolated from Cr-polluted and non-polluted rhizospheres of *Ricinus communis*. Sci Total Environ 625:1113–1121
126. Kodre A, Arcon I, Vogel-Mikuš K, Debeljak M, Potisek M, Likar M (2017) Arbuscular mycorrhizal fungi alter Hg root uptake and ligand environment as studied by X-ray absorption fine structure. Environ Exp Bot 133:12–23
127. Huang X, Ho SH, Zhu S, Ma F, Wu J, Yang J et al (2017) Adaptive response of arbuscular mycorrhizal symbiosis to accumulation of elements and translocation in Phragmites australis affected by cadmium stress. J Environ Manage 197:448–455. https://doi.org/10.1016/j.jenvman.2017.04.014
128. Wei Y, Su Q, Sun ZJ, Shen YQ, Li JN, Zhu XL et al (2016) The role of arbuscular mycorrhizal fungi in plant uptake, fractions, and speciation of antimony. Appl Soil Ecol 107:244–250
129. Hristozkova M, Geneva M, Stancheva I, Boychinova M, Djonovab E (2016) Contribution of arbuscular mycorrhizal fungi in attenuation of heavy metal impact on *Calendula officinalis* development. Appl Soil Ecol 101:57–63
130. Mani D, Kumar C, Patel NK (2016) Integrated micro-biochemical approach for phytoremediation of cadmium and lead contaminated soils using *Gladiolus grandiflorus* L. cut flower. Ecotoxicol Environ Saf 124:435–446
131. De Souza LA, Andrade SAL, De Souza SCR, Schiavinato MA (2012) Arbuscular mycorrhiza confers Pb tolerance in *Calopogonium mucunoides*. Acta Physiol Plant 34:523–531
132. Garg N, Aggarwal N (2011) Effects of interactions 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
133. Arwidsson Z, Johansson E, von Kronhelm T, Allard B, van Hees P (2010) Remediation of metal contaminated soil by organic metabolites from fungi—production of organic acids. Water Air Soil Pollut 205:215–226
134. Krupa P, Kozdrój J (2007) Ectomycorrhizal fungi and associated bacteria provide protection against heavy metals in inoculated pine (*Pinus sylvestris* L.) seedlings. Water Air Soil Pollut 182:83–90
135. Fomina MA, Alexander IJ, Hillier S, Gadd GM (2004) Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. Geomicrobiol J 21:351–366

136. González-Chávez 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
137. Martino E, Perotto S, Parsons R, Gadd GM (2003) Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biol Biochem 35:133–141
138. Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119
139. Weyens N, Croes S, Dupae J, Newman L, van der Lelie D, Carleer R et al (2010) Endophytic bacteria improve phytoremediation of Ni and TCE co-contamination. Environ Pollut 158:2422–2427
140. 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
141. 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
142. Dardanelli MS, Manyani H, Gonzalez-Barroso S, Rodríguez-Carvajal MA, Gil-Serrano AM, Espuny MR et al (2010) Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant Soil 328:483–493. https://doi.org/10.1007/s11104-009-0127-6
143. Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am J 74:533–542. https://doi.org/10.2136/sssaj2008.0240
144. Babu AG, Shea PJ, Sudhakar D, Jung IB, Oh BT (2015) Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil. J Environ Manage 151:160–166
145. Chen L, Luo SL, Li XJ, Wan Y, Chen JL, Liu CB (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
146. He H, Ye Z, Yang D, Yan J, Xiao L, Zhong T et al (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 90:196–195
147. Ma Y, Oliveira RS, Nai FJ, Rajkumar M, Luo YM, Rocha I et al (2015) The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J Environ Manage 156:62–69
148. Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678. https://doi.org/10.1016/j.soilbio.2009.11.024
149. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914
150. Hallmann J, Rodriguez-Kaban R, Kloepper J (1999) Chitin mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biol Biochem 31:551–560
151. Seghers D, Wittebolle L, Top EM, Verstraete W, Siciliano SD (2004) Impact of agricultural practices on the *Zea mays* L. endophytic community. Appl Environ Microbiol 70:1475–1482
152. Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF (eds) Microbial endophytes. Marcel Dekker, New York, pp 199–236
153. Gagne S, Richard C, Rousseau H, Antoun H (1987) Xylem-residing bacteria in alfalfa roots. Can J Microbiol 33:996–1000
154. Agarwal S, Shende ST (1987) Tetrazolium reducing microorganisms inside the root of Brassica species. Curr Sci 56:187–188

155. Roos IMM, Hattingh MJ (1983) Scanning electron microscopy of Pseudomonas syringae pv. morsprunorum on sweet cherry leaves. Phytopathol Z 108:18–25
156. Leben C, Daft GC, Schmitthenner AF (1968) Bacterial blight of soybeans: population levels of *Pseudomonas glycinea* in relation to symptom development. Phytopathology 58:1143–1146
157. Conn VM, Franco CMM (2004) Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism. Appl Environ Microbiol 70:6407–6413
158. Schulz B, Boyle C (2006) What are endophytes? In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, Viena, pp 1–15
159. Porteous-Moore F, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D et al (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29:539–556
160. Bacon CW, Hinton DM (2011) In planta reduction of maize seedling stalk lesions by the bacterial endophyte *Bacillus mojavensis*. Can J Microbiol 57:1–8
161. Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. JEnviron Manage. 174:14–25
162. Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–560
163. van Overbeek L, van Elsas JD (2008) Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). FEMS Microbiol Ecol 64:283–296
164. Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab G, Mauch F et al (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071
165. Becerra-Castro C, Kidd PS, Rodríguez-Garrido B, Monterroso C, Santos-Ucha P, Prieto-Fernández Á (2013) Phytoremediation of hexachlorocyclohexane (HCH)-contaminated soils using bacterial inoculants in soils with distinct organic matter content. Environ Pollut 178:202–210. https://doi.org/10.1016/j..2013.03.027
166. Becerra-Castro C, Prieto-Fernández Á, Kidd PS, Weyens N, Rodríguez-Garrido B, Touceda-González M et al (2013) Improving performance of *Cytisus striatus* on substrates contaminated with hexachlorocyclohexane (HCH) isomers using bacterial inoculants: developing a phytoremediation strategy. Plant Soil 362:247–260. https://doi.org/10.1007/s11104-012-1276-6
167. Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918
168. Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant–microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598. https://doi.org/10.1016/j.tibtech.2009.07.006
169. Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254. https://doi.org/10.1016/j.copbio.2009.02.012
170. Zafar M, Abbasi MK, Khan MA, Khaliq A, Sultan T, Aslam M (2012) Effect of plant growth-promoting rhizobacteria on growth, nodulation and nutrient accumulation of lentil under controlled conditions. Pedosphere 22:848–859. https://doi.org/10.1016/S1002-0160(12)60071-X
171. Joshi P, Juwarkar A (2009) In vivo studies to elucidate the role of extracellular polymeric substances from Azotobacter in immobilization of heavy metals. Environ Sci Technol 43:5884–5889
172. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L et al (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444
173. Sheoran N, Valiya Nadakkakath A, Munjal V, Kundu A, Subaharan K, Venugopal V et al (2015) Genetic analysis of plant endophytic Pseudomonas putida BP25 and chemo-profiling of its antimicrobial volatile organic compounds. Microbiol Res 173:66–78

174. Aravind R, Eapen SJ, Kumar A, Ramana KV (2010) Screening of endophytic bacteria and evaluation of selected isolates for suppression of burrowing nematode *Radopholus similis Thorne* using three varieties of black pepper (*Piper nigrum* L.). Crop Prot 29:318–324
175. Bacon CW, Hinton DM, Mitchell TR, Snook ME, Olubajo B (2012) Characterization of endophytic strains of *Bacillus mojavensis* and their production of surfactin isomers. Biol Control 62:1–9
176. Alvin A, Miller KI, Neilan BA (2014) Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol Res 169:483–495
177. Gechev TS, van Breusegem F, Stone JM, Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bioessays 28:1091–1101
178. Wan Y, Luo S, Chen J, Xiao X, Chen L, Zeng G, Liu C, He Y (2012) Effect of endophyte-infection on growth parameters and Cd-induced phytotoxicity of Cd-hyperaccumulator *Solanum nigrum* L. Chemosphere 89:743–750
179. Zhang XX, Li CJ, Nan ZB (2010) Effects of cadmium stress on growth and antioxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*. J Hazard Mater 175:703–709
180. Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater 250–251:477–483
181. Luo SL, Chen L, Chen JL, Xiao X, Xu TY, Wan Y et al (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
182. Luo SL, Wan Y, Xiao X, Guo H, Chen L, Xi Q et al (2011) Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum L.* and its potential for remediation. Appl Microbiol Biotechnol 89:1637–1644
183. Shin M, Shim J, You Y, Myung H, Bang KS, Cho M et al (2012) Characterization of lead resistant endophytic Bacillus sp. MN3-4 and its potential for promoting lead accumulation in metal hyperaccumulator *Alnus firma*. J Hazard Mater 199–200:314–320
184. Phetcharat P, Duangpaeng A (2012) Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. Proc Eng 32:177–183
185. Zhang YF, He LY, Chen ZJ, Zhang WH, Wang QY, Qian M et al (2011) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria and their potential in promoting lead accumulation of rape. J Hazard Mater 186:1720–1725
186. 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
187. Idris R, Kuffner M, Bodrossy L, Puschenreiter M, Monchy S, Wenzel WW et al (2006) Characterization of Ni-tolerant methylobacteria associated with the hyperaccumulating plant *Thlaspi goesingense* and description of *Methylobacterium goesingense sp.* nov. Syst Appl Microbiol 29:634–644
188. Diaz-Ravina M, Baath E (1996) Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. Appl Environ Microbiol 62:2970–2977
189. Shoeb E, Badar U, Akhter J, Shams H, Sultana M, Ansari MA (2012) Horizontal gene transfer of stress resistance genes through plasmid transport. World J Microbiol Biotechnol 28:1021–1025
190. Brown NL, Stoyanov JV, Kidd SP, Hobman JL (2003) The MerR family of transcriptional regulators. FEMS Microbiol Rev 27:145–163
191. Cursino L, Mattos SV, Azevedo V, Galarza F, Bucker DH, Chartone-Souza E et al (2000) Capacity of mercury volatilization by mer (from *Escherichia coli*) and glutathione S-transferase (from *Schistosoma mansoni*) genes cloned in *Escherichia coli*. Sci Total Environ 261:109–113

192. Pereira SIA, Castro PML (2014) Diversity and characterization of culturable bacterial endophytes from *Zea mays* and their potential as plant growth-promoting agents in metal-degraded soils. Environ Sci Pollut Res 21:14110–14123
193. Ullah A, Mushtaq H, Ali H, Munis MFH, Javed MT, Chaudhary HJ (2015) Diazotrophs-assisted phytoremediation of heavy metals: a novel approach. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-014-3699-5
194. Gupta G, Panwar J, Jha PN (2013) Natural occurrence of *Pseudomonas aeruginosa*, a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.). R. Br. Appl Soil Ecol 64:252–261
195. Hurek T, Reinhold-Hurek B (2003) *Azoarcus sp*. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178
196. Ahemad M (2015) Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: a review. 3 Biotech 5:111–121. https://doi.org/10.1007/s13205-014-0206-0
197. Kumar S (2016) Characterization of phosphate solubilising and mineralizing bacteria from grain legumes. Dissertation, Final thesis, Punjab Agricultural University, Ludhiana, India
198. Guiñazú LB, Andrés JA, Del MFP, Pistorio M, Rosas SB (2010) Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. Biol Fertil Soil 46:185–190
199. Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R, Verma D (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol Lett 182:291–296
200. Khan KS, Joergensen RG (2009) Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Bioresour Technol 100:303–309
201. Neubauer U, Furrer G, Kayser A, Schulin R (2000) Siderophores, NTA, and citrate: potential soil amendments to enhance heavy metal mobility in phytoremediation. Int J Phytoremediation 2:353–368. https://doi.org/10.1080/15226510008500044
202. Boukhalfa H, Crumbliss AL (2002) Chemical aspects of siderophore mediated iron transport. Biometals 15:325–339
203. Barry SM, Challis GL (2009) Recent advances in siderophore biosynthesis. Curr Opin Chem Biol 13:1–11
204. Krewulak KD, Vogel HJ (2008) Structural biology of bacterial iron uptake. Biochim Biophys Acta 1778:1781–1804
205. Rajkumar M, Ma Y, Freitas H (2008) Characterization of metal-resistant plant-growth promoting Bacillus weihenstephanensis isolated from serpentine soil in Portugal. J Basic Microbiol 48:1–9
206. Dimkpa C, Svatos A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172
207. Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces spp*. Chemosphere 74:19–25
208. Dimkpa CO, 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
209. 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
210. Tripathi M, Munot H, Shouche Y, Meyer JM, Goel R (2005) Isolation and functional characterization of siderophore-producing lead- and cadmium-resistant *Pseudomonas putida* KNP9. Curr Microbiol 50:233–237
211. Rajkumar M, Freitas H (2008) Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99:3491–3498

212. Rajkumar M, Freitas H (2008) Influence of metal resistant-plant growth promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842
213. Shi YW, Lou K, Li C (2009) Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol Fertil Soils 45:645–653
214. Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1245
215. Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977
216. Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448
217. Persello-Cartieaux F, Nussaumev L, Robaglia C (2003) Tales from the under-ground: molecular plant–rhizobacteria interactions. Plant Cell Environ 26:189–199
218. Bashan Y, Holguin G (1997) Azospirillum–plant relationships: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121
219. Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with Azospirillum. In: Okon Y (ed) Azospirillum/plant associations. CRC Press, London, pp 77–86
220. Xie H, Pasternak JJ, Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR 12-2 that over produce indole acetic acid. Curr Microbiol 32:67–71
221. Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 2006(19):827–837
222. Sheng XF, Jiang CY, He LY (2008) Characterization of plant growth-promoting inoculated *Bacillus edaphicus* NBT and its effect on lead uptake by Indian mustard in a lead-amended soil. Can J Microbiol 54:417–422
223. Sun Q, Rost TL, Matthews MA (2006) Pruning-induced tylose development in stems of current-year shoots of *Vitis vinifera* (Vitaceae). Am J Bot 93:1567–1576
224. Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot London 95:707–735
225. Viterbo A, Landau U, Kim S, Chernin L, Chet I (2010) Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. FEMS Microbiol Lett 305:42–48
226. Guo HJ, Luo SL, Chen L, Xiao X, Xi Q, Wei WZ et al (2010) Bioremediation of heavy metals by growing hyper-accumulator endophytic bacterium *Bacillus sp.* L14. Bioresour Technol 101:8599–8605
227. Barzanti R, Ozino F, Bazzicalupo M, Gabbrielli R, Galardi F, Gonnelli C et al (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. Microb Ecol 53:306–316
228. Lodewyckx C, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D (2002) Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator *Thlaspi caerulescens* subsp. calaminaria. Int J Phytoremediation 4:101–115
229. Mastretta C, Taghavi S, van der Lelie D, Mengoni A, Galardi F, Gonnelli C et al (2009) Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. Int J Phytoremediation 11:251–267
230. Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K et al (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194. https://doi.org/10.1016/j.soilbio.2013.01.012

231. Sheng XF, He LY, Wang Q, Ye H, 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
232. Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy-metal resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170
233. Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291
234. Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–262
235. Pulsawat W, Leksawasdi N, Rogers PL, Foster LJR (2003) Anions effects on biosorption of Mn(II) by extracellular polymeric substance (EPS) from *Rhizobium etli*. Biotechnol Lett 25:1267–1270
236. Diels L, Spaans PH, Van Roy S, Hooyberghs L, Ryngaert A, Wouters H et al (2003) Heavy metals removal by sand filters inoculated with metal sorbing and precipitating bacteria. Hydrometallurgy 71:235–241
237. Haferburg G, Kothe E (2007) Microbes and metals: interactions in the environment. J Basic Microbiol 47:453–467. https://doi.org/10.1002/jobm.200700275
238. Ranjard L, Nazaret S, Cournoyer B (2003) Freshwater bacteria can methylate selenium through the thiopurine methyltransferase pathway. Appl Environ Microbiol 69:3784–3790
239. Alvarez A, Saez JM, Costa JSD, Colin VL, Fuentes MS, Cuozzo SA et al (2017) Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166:41–62
240. Goodfellow M, Kampfer P, Busse HJ, Trujillo ME, Suzuki KI et al (2012) Bergey's manual of systematic bacteriology. Springer, New York
241. Cardoso P, Corticeiro S, Freitas R, Figueira E (2018) Different efficiencies of the same mechanisms result in distinct Cd tolerance T within *Rhizobium*. Ecotoxicol Environ Saf 150:260–269
242. Román-Ponce B, Reza-Vazquez DM, Gutierrez-Paredes S, De Haro-Cruz MJ, Maldonado-Hernandez J, Bahena-Osorio Y et al (2017) Plant growth-promoting traits in rhizobacteria of heavy metal-resistant plants and their effects on *Brassica nigra* seed germination. Pedosphere 27:511–526
243. Rojjanateeranaj P, Sangthong C, Prapagdee B (2017) Enhanced cadmium phytoremediation of *Glycine max* L. through bioaugmentation of cadmium-resistant bacteria assisted by biostimulation. Chemosphere 185:764–771
244. Seneviratne M, Gunaratnea S, Bandara T, Weerasundara L, Rajakaruna N, Seneviratne G et al (2016) Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. South Afr J Bot 105:19–24
245. Adediran GA, Ngwenyaa BT, Mosselmans JFW, Heal KV, Harvie BA (2015) Mechanisms behind bacteria induced plant growth promotion and Zn accumulation in *Brassica juncea*. J Hazard Mater 283:490–499
246. Jing YX, Yan JL, He HD, Yang DJ, Xiao L, Zhong T et al (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*. Int J Phytoremediation 16:321–333
247. 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 Phytoremediation 14:89–99
248. 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 Phytoremediation 14:261–274
249. Shi JY, Lin HR, Yuan XF, Chen XC, Shen CF, Chen YX (2011) Enhancement of copper availability and microbial community changes in rice rhizospheres affected by sulfur. Molecules 16:1409–1417

250. He LY, Zhang YF, Ma HY, Su LN, Chen ZJ, Wang QY et al (2010) Characterization of copper resistant bacteria and assessment of bacterial communities in rhizosphere soils of copper-tolerant plants. Appl Soil Ecol 44:49–55
251. Chatterjee S, Sau GB, Mukherjee SK (2009) Plant growth promotion by a hexavalent chromium reducing bacterial strain, *Cellulosimicrobium cellulans* KUCr3. World J Microbiol Biotechnol 25:1829–1836
252. 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
253. Beolchini F, Dell'Anno A, Propris LD, Ubaldini S, Cerrone F, Danovaro R (2009) Auto- and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. Chemosphere 74:1321–1326
254. Hussein HS (2008) Optimization of plant-bacteria complex for phytoremediation of contaminated soils. Int J Bot 4:437–443
255. Rodriguez H, Vessely S, Shah S, Glick BR (2008) Isolation and characterization of nickel resistant Pseudomonas strains and their effect on the growth of non-transformed and transgenic canola plants. Curr Microbiol 57:170–174
256. Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr Microbiol 56:403–407
257. Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant Soil 304:35–44
258. Saravanan VS, Madhaiyan M, Thangaraju M (2007) Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus. Chemosphere 66:1794–1798
259. Trivedi P, Pandey A, Sa T (2007) Chromate reducing and plant growth promoting activities of psychrotrophic *Rhodococcus erythropolis* MtCC 7905. J Basic Microbiol 47:513–517
260. Wani PA, Khan MS, Zaidi A (2007) 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
261. Someya N, Sato Y, Yamaguchi I, Hamamoto H, Ichiman Y, Akutsu K et al (2007) Alleviation of nickel toxicity in plants by a rhizobacterium strain is not dependent on its siderophore production. Commun Soil Sci Plant Anal 38:1155–1162
262. Juwarkar AA, Nair A, Dubey KV, Singh SK, Devotta S (2007) Biosurfactant technology for remediation of cadmium and lead contaminated soils. Chemosphere 10:1996–2002
263. Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and $Cr^{6+}$ on the growth of Indian mustard. Chemosphere 62:741–748
264. Shilev S, Fernandez A, Benlloch M, Sancho ED (2006) Sunflower growth and tolerance to arsenic is increased by the rhizospheric bacteria *Pseudomonas fluorescens*. In: Morel J-L, Echevarria G, Goncharova N (eds) Phytoremediation of metal-contaminated soils. Springer, Netherlands, pp 315–326
265. Farwell AJ, Vesely S, Nero V, Rodriguez H, Shah S, Dixon DG et al (2006) The use of transgenic canola (Brassica napus) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. Plant Soil 288:309–318
266. Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042
267. Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72:1129–1134
268. Vivas A, Biro B, Ruíz-Lozanoa JM, Azcon R (2006) Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn toxicity. Chemosphere 52:1523–1533
269. Ashour EH, El-Mergawi RA, Radwan SMA (2006) Efficiency of Pseudomonas to phytoremediate nickel by canola (*Brassica napus* L.). J Appl Sci Res 2:375–382

270. 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
271. Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S et al (2005) Cadmium-tolerant plant growth-promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol Biochem 37:241–250
272. Dell'Amico E, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. FEMS Microbiol Ecol 52:153–162. https://doi.org/10.1016/j.femsec.2004.11.005
273. Di Gregorio S, Lampis S, Vallini G (2005) Selenite precipitation by a rhizospheric strain of *Stenotrophomonas* sp. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective. Environ Int 31:233–241
274. Carrillo-Castañeda G, Munoz JJ, Peralta-Videa JR, Gomez E, Gardea-Torresdey JL (2003) Plant growth-promoting bacteria promote copper and iron translocation from root to shoot in alfalfa seedlings. J Plant Nutr 26:1801–1814
275. Abou-Shanab RA, Angle JS, Delorme TA, Chaney RL, van Berkum P, Moawad H et al (2003) Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. New Phytol 158:219–222
276. Vivas A, Azcón R, Biró B, Barea JM, Ruiz-Lozano JM (2003) Influence of bacterial strains isolated from lead-polluted oil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pratense* L. under lead toxicity. Can J Microbiol 49:577–588
277. Nie L, Shah S, Burd GI, Dixon DG, Glick BR (2002) Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol Biochem 40:355–361
278. Lodewyckx C, Taghavi S, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D (2001) The effect of recombinant heavy metal resistant endophytic bacteria in heavy metal uptake by their host plant. Int J Phytoremediation 3:173–187
279. Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ Sci Technol 35:3144–3150
280. Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245
281. Burd GI, Dixon DG, Glick BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668
282. Sharma P, Pandey S (2014) Status of phytoremediation in world scenario. Int J Environ Bioremediat Biodegrad 2:178–191
283. Barrutia O, Epelde L, García-Plazaola JI, Garbisu C, Becerril JM (2009) Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous and non-metalliferous sites: effect of fertilization. Chemosphere 74:259–264
284. Burges A, Epelde L, Blanco F, Becerril J, Garbisu C (2017) Ecosystem services and plant physiological status during endophyte-assisted phytoremediation of metal contaminated soil. Sci Total Environ 584–585:329–338
285. Buscaroli A (2017) An overview of indexes to evaluate terrestrial plants for phytoremediation purposes (Review). Ecol Indicators 82:367–380
286. Barceló J, Poschenrieder C (2003) Phytoremediation: principles and perspectives. Contrib Sci 2:333–344 Institut d'Estudis Catalans, Barcelona
287. Fiorentino N, Fagnano M, Adamo P, Impagliazzo A, Mori M, Pepe O et al (2013) Assisted phytoextraction of heavy metals: compost and trichoderma effects on giant reed (*Arundo donax* L) uptake and soil N-cycle microflora. Ital J Agron 8:244–250
288. Gou ZH, Miao XF (2010) Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L,) incontaminated soil with arsenic, cadmium and lead. J Cent South Univ Technol 17:770–777
289. Shiri M, Rabhi M, Abdelly C, El Amrani A (2015) The halophytic model plant *Thellungiella salsuginea* exhibited increased tolerance to phenanthrene-induced stress in comparison

with the glycophitic one *Arabidopsis thaliana*: application for phytoremediation. Ecol Eng 74:125–134

290. Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18
291. Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1999) Phytomining. Trends Plant Sci 1:359–362
292. Bridgwater AV, Meier M, Radlein D (1999) An over view of fast pyrolysis of biomass. Org Geochem 30:1479–1493
293. Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res 2:1–25
294. Raskin I, Ensley BD (2000) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley-Interscience, New York
295. Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants: biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:275–321
296. Evangelou MW, 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
297. Alkorta I, Hernández-Allica J, Becerril JM, Amezaga I, Albizu I, Onaindia M et al (2004) Chelate-enhanced phytoremediation of soils polluted with heavy metals. Rev Environ Sci Biotechnol 3:55–70
298. 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
299. Kos B, Leštan D (2004) Soil washing of Pb, Zn and Cd using biodegradable chelator and permeable barriers and induced phytoextraction by *Cannabis sativa*. Plant Soil 263:43–51
300. Wu LH, Luo YM, Christie P, Wong MH (2003) Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil. Chemosphere 50:819–822
301. Postrigan BN, Knyazev AV, Kuluev BRO, Yakhin I, Chemeris AV (2012) The activity of synthetic pseudophytochelating gene in tobacco plants. Fiziol Rastenii 59:303–308
302. Wood JL, Tang C, Franks AE (2016) Microbial associated plant growth and heavy metal accumulation to improve phytoextraction of contaminated soils. Soil Biol Biochem 103:131–137. https://doi.org/10.1016/j.soilbio.2016.08.021
303. Ali N, Masood S, Mukhtar T, Kamran MA, Rafique M, Munis MFH et al (2015) Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of *Linum usitatissimum* in association with *Glomus intraradices*. Environ Monit Assess 187:1–11
304. Wu Q-S, Zou Y-N, He X-H (2010) Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. Acta Physiol Plant 32:297–304
305. Piotrowski JS, Rillig MC (2008) Succession of arbuscular mycorrhizal fungi: patterns, causes, and considerations for organic agriculture. Adv Agron 97:111–130
306. Renker C, Blanke V, Rstler B, Heinrichs J, Buscot FO (2004) Diversity of cryptococcus and dioszegia yeasts (*Basidiomycota*) inhabiting arbuscular mycorrhizal roots or spores. FEMS Yeast Res 4:597–603
307. Shahabivand S, Maivan HZ, Goltapeh EM, Sharifi M, Aliloo AA (2012) The effects of root endophyte and arbuscular mycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. Plant Physiol Biochem 60:53–58
308. Wang FY, Lin XG, Yin R (2007) Effect of arbuscular mycorrhizal fungal inoculation on heavy metal accumulation of maize grown in a naturally contaminated soil. Int J Phytoremediation 9:345–353
309. Li H, Ye ZH, Chan WF, Chen XW, Wu FX, Wu SC, Wong MH (2011) Can arbuscular mycorrhizal fungi improve grain yield, as uptake and tolerance of rice grown under aerobic conditions? Environ Pollut 159:2537–2545

310. Li T, Liu MJ, Zhang XT, Zhang HB, Sha T, Zhao ZW (2011) Improved tolerance of maize (*Zea mays* L.) to heavy metals by colonization of a dark septate endophyte (DSE) Exophiala pisciphila. Sci Total Environ 409:1069–1074
311. Bai Y, Liang J, Liu R, Hu C, Qu J (2014) Metagenomic analysis reveals microbial diversity and function in the rhizosphere soil of a constructed wetland. Environ Technol 35:2521–2527
312. Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil Biol Biochem 40:74–84
313. Ma Y, Rajkumar M, Freitas H (2009) Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. J Hazard Mater 166:1154–1161
314. Sumi H, Kunito T, Ishikawa Y, Sato T, Park HD, Nagaoka K, Aikawa Y (2015) Plant roots influence microbial activities as well as cadmium and zinc fractions in metal-contaminated soil. Chem Ecol 31:105–110
315. Bedini S, Turrini A, Rigo C, Argese E, Giovannetti M (2010) Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. Soil Biol Biochem 42:758–765
316. Khade HW, Adholeya A (2009) Arbuscular mycorrhizal association in plants growing on metal-contaminated and noncontaminated soils adjoining Kanpur tanneries, Uttar Pradesh, India. Water Air Soil Pollut 202:45–56
317. Fomina MA, Alexander IJ, Colpaert JV, Gadd GM (2005) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biol Biochem 37:851–866
318. Finlay R, Wallander H, Smits M, Holmstrom S, van Hees P, Lian B et al (2009) The role of fungi in biogenic weathering in boreal forest soils. Fungal Biol Rev 23:101–106
319. Van Schöll L, Kuyper TW, Smits MM, Landeweert R, Holffland E, van Breemen N (2008) Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. Plant and Soil 303:35–47
320. Achakzai AK, Liasu MO, Popoola OJ (2012) Effect of mycorrhizal inoculation on the growth and phytoextraction of heavy metals by maize grown in oil contaminated soil. Pak J Bot 44:221–230
321. Punamiya P, Datta R, Sarkar D, Barber S, Patel M, Das P (2010) Symbiotic role of *Glomus mosseae* in phytoextraction of lead in vetiver grass [*Chrysopogon zizanioides* (L.)]. J Hazard Mater 177:465–474. https://doi.org/10.1016/j.jhazmat.2009.12.056
322. Körpe DA, Aras S (2011) Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. Mutat Res 719:29–34
323. Márquez-García B, Horemans N, Cuypers A, Guisez Y, Córdoba F (2011) Antioxidants in Erica andevalensis: a comparative study between wild plants and cadmium-exposed plants under controlled conditions. Plant Physiol Biochem 49:110–115
324. Liu H, Yuan M, Tan S, Yang X, Lan Z, Jiang Q et al (2015) Enhancement of arbuscular mycorrhizal fungus (*Glomus versiforme*) on the growth and Cd uptake by Cd-hyperaccumulator *Solanum nigrum*. Appl Soil Ecol 89:44–49
325. Marques AP, Oliveira RS, Rangel AO, Castro PM (2006) Zinc accumulation in *Solanum nigrum* is enhanced by different arbuscular mycorrhizal fungi. Chemosphere 65:1256–1263
326. Marques AP, Oliveira RS, Rangel AO, Castro PM (2008) Application of manure and compost to contaminated soils and its effect on zinc accumulation by Solanum nigrum inoculated with arbuscular mycorrhizal fungi. Environ Pollut 151:608–620
327. Chen BD, 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
328. Ker K, Charest C (2010) Nickel remediation by AM-colonized sunflower. Mycorrhiza 20:399–406
329. Sheoran V, Sheoran A, Poonia P (2016) Factors affecting phytoextraction: a review. Pedosphere 26:148–166

330. Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, Gorfer M et al (2010) Bacteria associated with Zn and Cd-accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. J Appl Microbiol 108:1471–1484
331. Sessitsch A, Puschenreiter M (2008) Endophytes and rhizosphere bacteria of plants growing in heavy metal contaminated soil. In: Dion P, Nautiyal CS (eds) Microbiology of extreme soils. Springer, Berlin
332. van der Lelie D, Corbisier P, Diels L, Gilis A, Lodewyckx C, Mergeay M et al (2000) The role of bacteria in the phytoremediation of heavy metals. In: Terry N, Bañuelos GS (eds) Phytoremediation of contaminated soil and water. Lewis Publishers (CRC Press), Boca Raton, FL, pp 265–281
333. Cabello-Conejo MI, Becerra-Castro C, Monterroso C, Prieto-Fernández A, Mench M, Kidd PS 2011 Effects of rhizobacterial inoculation on biomass and nickel concentration in Alyssum pintodasilvae. In: Proceedings of the 11th international conference on the biogeochemistry of trace elements (ICOBTE), 2011 July 4–7, Florence, Italy
334. Guo J, Tang S, Ju X, Ding Y, Liao S, Song N (2011) Effects of inoculation of a plant growth promoting rhizobacterium *Burkholderia sp*. D54 on plant growth and metal uptake by a hyperaccumulator *Sedum alfredii* Hance grown on multiple metal contaminated soil. World J Microbiol Biotechnol 27:2835–2844
335. 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
336. Kim JO, Lee YW, Chung J (2013) The role of organic acids in the mobilization of heavy metals from soil. KSCE J Civil Eng 17:1596–1602
337. Li W, Ye Z, Wong M (2009) Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii*. Plant Soil 326:453–467
338. Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (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
339. Glick BR (2004) Teamwork in phytoremediation. Nat Biotechnol 22:526–527
340. 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
341. Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657
342. Braud A, Jézéquel K, Vieille E, Tritter A, Lebeau T (2006) Changes in extractability of Cr and Pb in a polycontaminated soil after bioaugmentation with microbial producers of biosurfactants, organic acids and siderophores. Water Air Soil Pollut 6:3–4
343. Venkatesh NM, Vedaraman N (2012) Remediation of soil contaminated with copper using rhamnolipids produced from *Pseudomonas aeruginosa* MTCC 2297 using waste frying rice bran oil. Ann Microbiol 62:85–91
344. Haferburg G, Kothe E (2010) Metallomics: lessons for metalliferous soil remediation. Appl Microbiol Biotechnol 87:1271–1280
345. Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N et al (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar. Appl Environ Microbiol 75:748–757
346. Garcia de Salamone IE, Hynes RK, Nelson LM (2005) Role of cytokinins in plant growth promotion by rhizosphere bacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, The Netherlands, pp 173–195
347. Patten CL, Glick BR (2002) Role of Pseudomonas putida indole acetic acid in development of host plant root system. Appl Environ Microbiol 48:3795–3801
348. Ortiz Castro R, Valencia Cantero E, Lopez Bucio J (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signal Behav 3:263–265

349. Cabello-Conejo MI, Prieto-Fernandez T, Kidd PS (2014) Exogenous treatments with phytohormones can improve growth and nickel yield of hyperaccumulating plants. Sci Total Environ 494–495:1–8
350. Hadi F, Bano A, Fuller MP (2010) The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere 80:457–462
351. Shilev SI, Ruso J, Puig A, Benlloch M, Jorrin J, Sancho E (2001) Rhizospheric bacteria promote sunflower (*Helianthus annus* L.) plant growth and tolerance to heavy metals. Minerva Biotechnol 13:37–39
352. Ghosh P, Rathinasabapathi B, Ma LQ (2011) Arsenic-resistant bacteria solubilized arsenic in the growth media and increased growth of arsenic hyperaccumulator *Pteris vittata* L. Bioresour Technol 102:8756–8761
353. Sun LN, Zhang YF, He LY, Chen ZJ, Wang QY, Qian M et al (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper tolerant plant species on copper mine wasteland. Bioresour Technol 101:501–509
354. He CQ, Tan GE, Liang X, Du W, Chen YL, Zhi GY et al (2010) Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. Appl Soil Ecol 44:1–5
355. Li WC, Ye ZH, Wong MH (2010) Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant *Sedum alfredii*. Plant Soil 326:453–467
356. He LY, Chen Z-J, Ren G-D, Zhang Y-F, Qian M, Sheng X-F (2009) Increased cadmium and lead uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. Exotoxicol Environ Saf 72:1343–1348
357. Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere 72:678–683
358. Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008) The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol 24:253–262
359. Jiang C-Y, Sheng X-F, Qian M, Wang Q-Y (2008) Isolation and characterization of a heavy metal-resistant *Burkholderia sp*. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72:157–164
360. 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
361. Duponnois R, Kisa M, Assigbetse K, Prin Y, Thioulouse J, Issartel M et al (2006) Fluorescent pseudomonads occurring in Macrotermes subhyalinusmound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Sci Total Environ 370:391–400
362. Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ Pollut 140:124–135. https://doi.org/10.1016/j.envpol.2005.06.023
363. Di Gregorio S, Barbafieri M, Lampis S, Sanangelantoni AM, Tassi E, Vallini G (2006) Combined application of Triton X-100 and *Sinorhizobium sp* Pb002 inoculum for the improvement of lead phytoextraction by *Brassica juncea* in EDTA amended soil. Chemosphere 63:293–299
364. 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
365. De Maria S, Rivelli AR, Kuffner M, Sessitsch A, Wenzel WW, Gorfer M et al (2011) Interactions between accumulation of trace elements and major nutrients in *Salix caprea* after inoculation with rhizosphere microorganisms. Chemosphere 84:1256–1261

# Part II

# Phytoremediation Applications for Contaminated Soils

# Chapter 3
# Sorption: Release Processes in Soil—The Basis of Phytoremediation Efficiency

**G. Petruzzelli, M. Grifoni, M. Barbafieri, I. Rosellini, and F. Pedron**

## 3.1 Introduction

"Phytoremediation" is a broad term that includes a series of technologies based on the use of plants to remediate both organic and inorganic contaminants in soil and other environmental matrices (sediments, water) [1, 2]. The interest in these phytotechnologies is high given that compared to traditional remediation technologies it is inexpensive, simple to use, and environmentally friendly [3].

However, phytoremediation has its own limitations: the long growth required by the plants and the fact that contamination needs to affect above all the layers of soil explored by the roots. Moreover, in highly contaminated soils, this technology is not always applicable since plant growth can be hindered due to the onset of phytotoxicity phenomena. The limitations of phytoremediation technology are essentially related to plant growth cycles, yet sometimes these drawbacks are not taken into consideration by the "stakeholders," who are impressed by the theoretical, environmental, and economic aspects of the use of plants [4, 5].

In the case of soil contaminated by organic compounds, positive results have often been obtained, since the plants also act as a support for the microbial activity of the soil in pollutant degradation [6]. However, in most cases of heavy metal pollution, optimal results are still a long way off. Results from the field application of the technology in the last 20 years highlight the gap between the scientific publications and the results obtained in real-scale remediation [7]. The difficulties to overcome derive above all from the impossibility of exactly replicating an intervention in the field with the same methods tested in the laboratory or in the greenhouse.

Among the phytoremediation technologies, heavy metal phytoextraction in particular has encountered numerous obstacles at the field scale, highlighting the need

---

G. Petruzzelli (✉) · M. Grifoni · M. Barbafieri · I. Rosellini · F. Pedron
Institute of Ecosystem Study, CNR, Pisa, Italy
e-mail: gianniantonio.petruzzelli@ise.cnr.it; petruzzelli@ise.cnr.it; martina.grifoni@ise.cnr.it; meri.barbafieri@ise.cnr.it; irene.rosellini@ise.cnr.it; francesca.pedron@ise.cnr.it

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_3

to understand much more about the interactions between the contaminants, the soil, and the plant before this technology can become competitive [8, 9]. Phytoextraction technology originated just from studies on plant species that can hyperaccumulate heavy metals, which not only grow in soils that are rich in metals but can also accumulate very high quantities in the epigeal parts [10]. Although these plants grow in soils that are rich in metals and also accumulate very high quantities in their epigeal parts, the biomass production typical of most of these species is low.

Alternatives have been developed to overcome this limitation, such as the use of species with a high biomass production and lower accumulation capacity. However, in this case further interventions are necessary that modify the chemical environment of the metals in the soil in order to enhance the metal uptake by plants—this is known as assisted phytoextraction [11, 12]. For example, additives can be applied to the soil that promote the release of metals from the solid phase to the liquid phase (mobilizing agents), with a consequent increase in metal bioavailability and absorption by the plants [13–16].

The success of phytoextraction strictly depends on the ability of plants to uptake metals from the soil through the roots and to translocate and accumulate them in the aerial parts. Only metals present in soluble forms in a soil solution can be absorbed by plants, and generally they are a small fraction of the metals present in a contaminated soil. However, this aspect is scarcely considered in the planning of many full-scale technologies [17]. Generally, this bioavailable fraction is also the only one that creates a risk to human health and to the environment, unlike the other metals that remain linked on the soil surface in nonmobile bioavailable forms [18].

The bioavailability of elements in the soil is the result of a series of complex mass transfer and absorption processes which are determined by the properties of the substances, the characteristics of the soil, and the biology of the organisms involved, in this case the plants [19, 20]. Soil is a heterogeneous system consisting of three phases, solid, liquid, and gaseous, together with a fourth fundamental phase, the "living phase." This last phase consists of the innumerable organisms that live in the soil and which influence all the processes that take place in the soil. When a metal is present in soil, it is distributed among these soil phases. The linkage with the solid phase can occur through sorption processes both on the mineral matrix (clays, oxides, hydroxides) and on the organic matrix (humic substances). Bonds of various types and strength are formed between the heavy metals and soil surfaces [21].

Bioavailability processes are therefore key to evaluating and planning plant-based phytoremediation strategies. For phytoextraction technology, it is essential to analyze how an element from the solid phase, in which it is basically unavailable for any environmental process, moves to the liquid phase in which it is potentially available for the uptake by plants [22]. This step is governed by the specific characteristics both of the soil and the contaminant. The release of a contaminant from the soil surface into the liquid phase occurs in response to changes in the chemical environment of the soil solution. Once released into the liquid phase, a contaminant can move freely to the plants as a result of transport processes (diffusion, dispersion, etc.), which bring it into contact with the roots. During the transport phase, the contaminants may be subject to further reactions (oxidation-reduction, hydrolysis, photolysis, degradation, etc.) which can modify both their toxicity and bioavailability [23].

Since heavy metals are the most persistent contaminants in soil, in this chapter we focus on them in order to understand how the specific characteristics of each type of soil influence the bioavailability mechanisms in the light of possible phytoremediation. In particular, the parameters that determine the release from the solid phase and the sorption phenomena are examined. Sorption plays a key role in soil chemistry and is the most common form of retention responsible for the accumulation of heavy metals in soil. For this reason, the study of adsorption processes is very important for an efficient phytoextraction technology implementation, since the bioavailable metal amount for plants depends on them. Following the sorption phenomena, the bioavailability is modified, because a certain amount of metals is retained by the solid phase and the desorption rate can greatly limit the uptake by the plants.

## 3.2 Soil Sorption and Bioavailability

All the processes that determine a mass exchange between the solid and liquid phases of the soil are generally defined as “sorption processes,” which can be in turn divided into absorption and adsorption reactions, according to the type of interaction between the molecules in the solution and solid phases. Through absorption the solute is incorporated into the solid phase of the soil and may even penetrate a few nanometers. On the other hand, adsorption is the process in which the solute adheres or binds physically to the solid phase surface alone. Adsorption, in turn, involves two different mechanisms for the metal removal from the soil solution, namely, ion exchange and specific adsorption. Ion exchange occurs by non-specific electrostatic attraction forces, which enable the process to be reversible. On the other hand, specific adsorption takes place when the covalent bondings predominate, in which the electrons are shared between the metals and the adsorbent surface [24]. Due to the numerous retention mechanisms, which are difficult to distinguish, the term “sorption” refers to all reactions involving solid solution interfaces in soil.

The fate and behavior of inorganic compounds in soil are largely controlled by the retention/release processes, which determine the amount of heavy metals in the soil liquid phase. These processes can delay metal leaching along the soil profile but can also reduce the bioavailability for plant uptake. The sorption/desorption reactions strictly depend on the specific characteristics of the soil; thus, they differ from one soil to another in relation to the amount and the kind of soil components, such as organic matter, clay minerals, iron, aluminum, and manganese oxides, and soil properties, such as cation exchange capacity (CEC), pH, and pE [25].

### 3.2.1 Soil Properties

Soil pH is key to regulating the concentrations of soluble and available metals for plants [26]. The solubility of metals, in fact, varies with the pH. For transition elements (Cu, Zn, Ni, etc.), it tends to increase at a lower pH, while for other elements such as Cr and As, it increases at basic pH values. Also specific sorption is dependent on pH. For heavy metals, however, the competition between $H^+$ and metal ions can be reduced even if the metal concentrations are relatively low, due to specific sorption [27]. The surfaces of the soil with variable charges regulate the sorption of metals in relation to the pH. Sorption can also be independent of pH, when soil surfaces with permanent charges are involved.

The association between the sorption process of a metal in the soil and the pH can be ascribed to the competition between the $H^+$ (and $Al^{3+}$) ions for the sorption sites with a consequent reduction in metal sorption. In alkaline conditions, the hydrolysis of the metal gives rise to a strong sorption of the hydroxy complexes of the metal. The pH also strongly impacts on the precipitation/dissolution reactions. For example, increasing pH from near neutral to basic values, various heavy metal salts (phosphates, carbonates) start to precipitate, while the same happens in acidic conditions for those elements such as As, Cr, and W, which are present in anionic forms. The concentrations of the elements in the soil solution, however, cannot be predicted with the solubility isotherms due to the extreme complexity of the soil-water natural system. The presence of different ligands and microorganisms creates conditions that are very different from those used to determine solubility products, i.e., solutions in equilibrium with pure compounds.

Depending on the pH values, sorption and precipitation are the most important processes that regulate the amount of metals in soil solution, and thus their uptake by plants can be used for remediation. Moreover, pH influences the uptake of metals in different ways for hyperaccumulator with respect to non-accumulator plants [28, 29].

Clay minerals are some of the main adsorbent surfaces of the soil and generally slow down the migration of metals along the soil profile. The significance of clay minerals in sorption has a notable effect on the metal bioavailability and phytoextraction efficiency [30]. The metal ion sorption process occurs both by ion exchange and the formation of stronger chemical linkages. The sorption of a metal on the surface of clay occurs following the sorption of a hydroxyl group on which the metal ion can be subsequently linked. Metal ions can also bind directly on the surface of clay following the removal of protons. Highly selective sorption always occurs at the mineral edges, but several clay minerals influence the sorption and therefore the solubility of the metals in different ways. The majority of phyllosilicate minerals, such as montmorillonites and vermiculites, have permanent surface negative charges due to an isomorphic (nonstoichiometric) substitution of cations within their structures: for example, $Al^{3+}$ substituted $Si^{4+}$ in the tetrahedral sheet. In these kinds of soils, the sorption capacity is due to a permanent charge, which is not dependent on pH.

The organic matter content in soils is often lower than that of clay; however, humic substances have a significant influence on the sorption of metals. Complexation and sorption are the mechanisms by which metals are retained by humic materials. These reactions can occur with the formation of inner-sphere surface complexes as well as ion exchange processes with the formation of outer-sphere complexes characterized by electrostatic bonds. The negatively charged functional groups of organic substances are involved in the sorption of metals. These functional groups are more abundant in well-humified materials, and their ionization increases as the pH increases. At alkaline pH, organometal complexes become more stable, and metals are less bioavailable. However, metals can be complexed with less humified organic components such as fulvic acids, forming compounds that are fairly soluble. This can be an important source of metals in soil solution. The formation of soluble complexes with organic substances leads to the solubilization of metals, which are available for plant uptake and transport through the soil as soluble organic complexes. The complexation of a metal is fundamental because under certain conditions, most of the metals in the soil liquid phase are in complexed forms. Organic ligands that form complexes with metals include low-weight organic acids such as citric, oxalic, or other complex acids derived from soluble humic substances.

Complexation is particularly important for certain metals (Cu, Zn, Pb, etc.), and depending on the type of organic substance present, the metal can remain in the soil solution as a complex with the soluble organic substance. Complexation reactions can also be carried out by inorganic ligands. The most important are hydroxide and chloride ions. For example, the chloride ion with its high affinity for the $Hg^{2+}$ can reduce the sorption of Hg due to competition with the negatively charged soil surface. The complexation of a metal cation has a great impact on the sorption, and the humic content in contaminated soil largely affects the phytoextraction efficiency [31].

The hydrated oxides of Fe, Al, and Mn can reduce the metal concentrations in the soil liquid phase by sorption reactions including specific sorption. Following sorption, metal cations can also enter the oxides within the structure of the mineral lattice or in micropores. It has been observed that in soils contaminated with metals, the interactions of these elements with iron and aluminum oxides/hydroxides are particularly high, in particular at pH levels between 6 and 7. Fe and Al oxides are generally more abundant in soils compared to those of Mn [32], but the latter may be particularly important in some metal retention such as Pb. The specific sorption of metals by the oxides in soil generally follows the order: $Pb > Cu \gg Zn > Cd$ [33, 34].

Fe, Al, and Mn oxides/hydroxides influence the solubility and bioavailability of metal elements in soil. The retention/release of the metals by the oxides/hydroxides is also influenced by the redox conditions of the soil, since their stability tends to decrease in a reducing environment. Under reduced conditions, the dissolution of oxides/hydroxides promotes the release of adsorbed metals, thus increasing phytoextraction efficiency [35].

The redox reactions in the soil derive from the activity of free electrons in the aqueous phase. High values of redox potential (pE or Eh) are found in well-aerated soils, whereas these values decrease in anoxic soils, which are submerged and/or very rich in organic matter [36]. The effects of redox conditions on the solubility of metals may depend on different environmental conditions and the type of soil. Reduction of the redox potential can lead to the dissolution of Fe-Mn oxides/hydroxides with the consequent release of the adsorbed metals, which therefore become more bioavailable for plant uptake. This effect can be exploited by phytoextraction in soils contaminated with arsenic and chromium, which are released by adsorbing surfaces with which they generally form special stable bonds. In the case of transition elements, with low Eh values, the precipitation of sulfides drastically reduces the bioavailability of these metals for plants. However, plants can promote the induced reduction of the redox potential, and low Eh values increase the solubility of some metals such as arsenic [37].

The cation exchange capacity depends on the density of negative charges on the soil surfaces. When an isomorphic substitution occurs in the clay minerals, the negative surface charges can be pH-dependent or permanent. The negative surface charge is balanced by an equal amount of cations in the soil solution in order to maintain electroneutrality. When the metal cations are linked to the soil surfaces by weak electrostatic bonds, they can be easily exchanged with other cations in the soil solution. One cation may also be preferentially adsorbed with respect to another on the surface of the solid phase of the soil, which directly depends on the valence of the metal and is inversely proportional to the hydrated radii.

The amount of metals adsorbed by a soil can be higher than predicted by CEC, due to a specific sorption process, which involves the formation of partially covalent bonds between the metal cations with the functional groups on the soil surface [32]. Specific sorption is strongly pH-dependent, involving both organic and inorganic surfaces, and often occurs even when metals in soil solution are present at low concentrations [36, 38]. The sorption sites are selective toward metal cations that have a specific affinity [38–41]. However, the specificity of the adsorbing surfaces toward some metals decreases with the increasing saturation of sorption sites [41].

When studying the plant uptake in contaminated sites, it is not sufficient just to examine the soil characteristics mentioned above. In fact, there may be several materials, waste, and industrial residues present in these sites, which increase the complexity of the chemical and biological interactions in which heavy metals are involved.

## 3.3 Sorption Processes in Soil

As mentioned above, the study of sorption processes is essential to evaluate the environmental consequences due to the accumulation of metals in soil and consequently to define the best phytoremediation techniques to apply. The mechanisms that influence the heavy metal sorption in soil can also be investigated through a quantitative description by sorption isotherms. Sorption processes in the soil are governed by attractive and repulsive forces between the solute-soil solution and the solid phase. Often several forces act simultaneously. Considering the main attractive forces, it is possible to distinguish physical sorption and chemical sorption. Physical sorption is characterized by long-range interactions between the solid phase and the solute due to electrostatic or van der Waals forces. This type of sorption is reversible and involves a relatively small level of sorption heat (1–10 kcal mol$^{-1}$). Since the attracting forces are quite weak, the adsorbed species can maintain coordinated water molecules and therefore cannot be brought closer to the surface at smaller distances than the radius of the solvation sphere. Chemical sorption is characterized by strong "short-range" interactions of a chemical nature, such as a hydrogen bond or interactions between orbitals. In the soil these processes are hard or very slow to reverse or completely irreversible. High sorption heat (10–100 kcal mol$^{-1}$) is involved in the process.

Since the attracting forces are relatively strong, the adsorbed species lose any coordinated water molecules, and, therefore, the minimum distance at which they can approach the surface depends on the ionic radius of the species. Chemical sorption takes place with a high degree of specificity.

The retention/release processes in soil can be described quantitatively by sorption isotherms. The isotherms describe the retention of a substance on the soil solid phase. They are an essential tool to evaluate the mobility of a substance in the soil and thus its bioavailability to plants.

The isotherms used in soil chemistry are generally divided into four types (S, L, H, C) according to Giles' classification which reflect the different mechanisms of sorption and the different solute-solvent surface adsorbent interactions.

The graphic representation of the isotherms is schematically reported in Fig. 3.1, where $C_{ads}$ is the concentration of the metal sorbed on the soil solid phase and $C_{eq}$ is the metal concentration in the liquid phase.

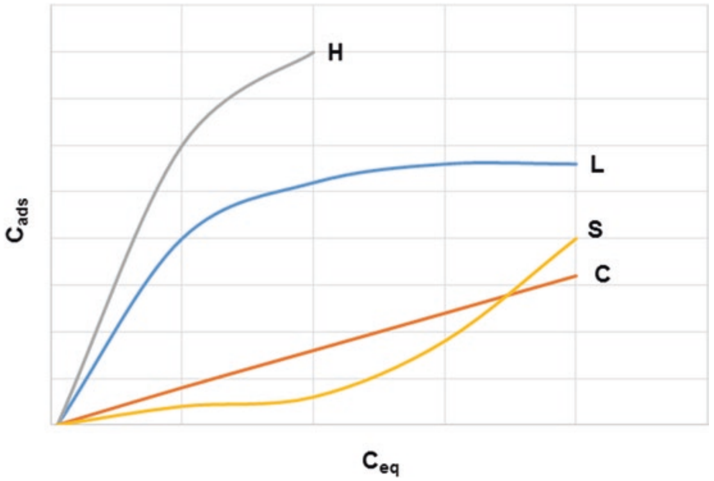


**Fig. 3.1** Pattern of sorption isotherms used in soil chemistry

The isotherm S (S is related to the shape of the curve) has an upward curvature and represents a situation in which the sorption is unfavorable at low metal concentrations in soil solution. This means that the interaction between the solid phase of the soil and the solute is less strong than that of solute-soil solution. This type of trend in the soil is rarely found for metal ions. The L isotherm (L stands for Langmuir) is commonly used in soil chemistry to describe sorption processes. Theoretically, on the soil surface, the number of adsorbent sites is constant; therefore, their availability decreases with increasing sorption. When the metal concentration in soil solution becomes very high, the isotherm presents a plateau, which represents the maximum adsorbent capacity of the soil. This isotherm suggests stronger soil surface-solute interactions than those between the solute and soil solution.

The H isotherm (H stands for "high" affinity) identifies cases when the affinity of the solute for the soil surface is extremely high. The initial slope of the curve, typical of this isotherm, indicates complete sorption at low concentrations and thus a high specificity of the adsorbing sites. The C isotherm (C stands for "constant" partition) is characterized by a rectilinear initial trend, which indicates a constant distribution of the solute between the soil solution and the solid phase, until saturation is reached. This sorption type does not depend on the concentration in solution, and the number of available sites remains constant as sorption increases. New sites are therefore created, while the available ones become occupied. This situation occurs only at low concentrations and when the solute-solid interactions are stronger than the solvent-solid interactions.

Considering the sorption processes of heavy metals, both ion exchange reactions and the formation of surface complexes can occur. The extent of the interaction of these elements in the soil solution and the solid phase depends on the characteristics of the soil and in particular on the surface charges and pH. On the adsorbing surfaces of the soil, two types of surface complexes can be formed: the outer-sphere and inner-sphere [42–44]. In outer-sphere complexes, the ions adsorbed in a nonspecific way maintain their own sphere of hydration; in the inner-sphere complexes, the ions are specifically adsorbed after having lost any hydration water molecules. The formation of outer-sphere complexes involves electrostatic forces, and the reactions are generally rapid and reversible. In the formation of inner-sphere complexes, covalent bonds can be also involved, and the reaction is slower and often considered irreversible. The formation of outer- and inner-sphere complexes can also take place simultaneously.

### 3.3.1 Modeling Soil Sorption

Many phenomenological and mechanistic models of heavy metal sorption in soils have been used to describe the sorption mechanism. Most investigations have shown a nonlinear isotherm pattern and a not fully reversible release [45, 46]. These features are of primary importance in contaminated sites where phytoremediation could be applied, since the composition of soil greatly influences solution in equilibrium with plant roots. Several equations are used to evaluate the retention/release process in soil. These sorption equations theoretically refer to a state of equilibrium; however, in soils most of the sorption processes are not fully reversible. Despite this limitation, it is common practice in soil chemistry to use sorption equations, which describe the phenomena very well and provide an insight into the bioavailability of contaminants.

#### 3.3.1.1 The Langmuir Equation

This equation was originally developed for the sorption of a gas on a solid material. It is based on two main assumptions: (1) the enthalpies and the sorption energy for each molecule are constant and independent of the degree of surface coverage (thus all sites on soil surfaces have the same affinity for the adsorbing substance), and (2) the sorption occurs at finite localized sites with no interactions and no steric hindrance among adsorbate molecules. Despite these assumptions not being fully valid in the soil environment, the Langmuir is one of the most common equations in soil chemistry, since it describes the process whenever the sorption approaches saturation at increasing adsorbate concentrations. The most common form of the Langmuir equation in soil chemistry is

$$q = \frac{q_{\max} \times KC_{\mathrm{e}}}{1 + KC_{\mathrm{e}}}$$

where $q$ = the amount of metal adsorbed per unit weight of soil (mmol kg$^{-1}$) and $C_{\mathrm{e}}$ = the equilibrium concentration (mmol L$^{-1}$). $q_{\max}$ (mmol kg$^{-1}$) and $K$ (L mmol$^{-1}$) are adjustable parameters linked to the maximum and to the sorption energy that can be obtained by statistical procedure from the experimental data. $q_{\max}$ defines the maximum sorption capacity of the soil, whereas the constant $K$ is related to the affinity of the compound for the soil solid surfaces. The equation is particularly useful for studying soils from contaminated sites, since it indicates the point beyond which the soil surfaces are saturated by the contaminants, and the soil is not able to retain them in the solid phase. Thus, any further contaminants added are potentially bioavailable. From the Langmuir equation, it is possible to derive the separation factor $R_{\mathrm{L}}$ [47, 48], defined by the equation:

$$R_{\mathrm{L}} = \frac{1}{1 + KC_{\mathrm{i}}}$$

where K is the Langmuir constant and $C_{\mathrm{i}}$ is the initial metal concentration (mmol L$^{-1}$).

This dimensionless constant describes the favorability of sorption, which can be defined as unfavorable ($R_{\mathrm{L}} > 1$), linear ($R_{\mathrm{L}} = 1$), favorable ($0 < R_{\mathrm{L}} < 1$), or irreversible ($R_L = 0$). The Langmuir equation can also be used to derive the distribution coefficient $K_d$, defined as the ratio between the amount of contaminant sorbed and that remaining in solution. The distribution coefficient is an index of the variation in metal content on the solid phase in relation to the metal concentration in solution. It thus describes the distribution of mobilizable forms of metals between solid and liquid phases of the contaminated soil. $K_d$ is one of the most useful parameters derived from isotherms since the soil solution concentration is key to defining an element's bioavailability. $K_d$ is an essential parameter in risk assessment procedures, since a high value of $K_d$ reflects the high ability of soil to retain the contaminants in the solid phases, thus reducing their mobility and bioavailability [49]. This means a reduction in the immediate risk for the targets of contamination; however, it also highlights the reduced bioavailability of the contaminants, which should be considered in planning phytoremediation as a cleanup strategy. The Langmuir isotherm is widely used in its simple form with good results. However, to take into account different kinds of sorption sites, a more complex Langmuir equation can be used:

$$q = \sum_{p}^{i=1} q_{\max,i} \frac{K_i C}{1 + K_i C}$$

where $K_i$ and $q_{\max,i}$ correspond to each type of site. This equation is generally used when the linearization of the graph $q/C$ versus $q$ shows a convex curve [50].

### 3.3.1.2 The Freundlich Equation

The Freundlich equation derives from the assumption that there is a linear relationship between the surface energy and the sites occupied. Although it does not have a thermodynamic basis, this equation has been widely applied in soil systems to describe sorption in soil.

The general form of the equation is

$$q_e = K_F C_e^{1/n}$$

The equation can be used in the linear form by taking the logarithm of both sides as

$$\log q_e = \log K_F + \frac{1}{n} \log C_e$$

where $q_e$ is the adsorbed concentration in soil, $C_e$ is the concentration in solution, and $K_F$ and $1/n$ are empirical parameters which are related to the amount of adsorbable metals and to the energy of linkages between metals and soil surfaces, respectively. A value of $1/n$ lower than 1 indicates that sorption proceeds via chemisorptions, whereas $1/n$ above 1 reveals a cooperative sorption.

The Freundlich equation describes the sorption on heterogeneous soil surfaces. The amount of adsorbed substance is different on various sorption soil sites, with the sites with the highest bonding energy being the first to be occupied. The Freundlich model can also be modified to take into account the competition of several metals for the same sites. Often the equation for a competitive metal species, “m,” is used in the form:

$$q_i = K_{\mathrm{F}_i} C_i \left( \sum_{m}^{j=1} a_{i,j} C_j \right)^{n_i - 1}$$

where $a_{\mathrm{i,j}}$ is a dimensionless coefficient which accounts for the competition of the species $i$ in the presence of species $j$ [50] and $K_{\mathrm{F}i}$ and $n_i$ are the coefficients of the Freundlich isotherm.

This equation can be applied for the sorption of both positive and negative metal ions. In terms of phytoremediation, it is important to calculate the amount of contaminants in the liquid phase and to know the sorption/release reactions. It is thus necessary to consider that often contaminants firstly form outer-sphere surface complexes through a fast kinetic sorption reaction. This is then followed by slow specific sorption reactions with the formation of more stable inner-sphere complexes with solid surfaces. These competitive reactions have an immediate effect on the amount of metals available for plants and therefore on the efficiency of phytoextraction.

#### 3.3.1.3 Further Equations

Over the years, many isotherm models have been formulated; however, it is beyond the scope of this review to explore the different equations and models generally used to describe the sorption processes in soil, given that they have already been covered in detail elsewhere [50, 51]. A brief synthesis of the sorption equations used in soil chemistry is reported in Table 3.1.

The choice of models and equations to describe the set of sorption phenomena must be based on a mechanistic knowledge of the interactions between metals and soil surfaces, in order to predict the mobility and bioavailability of these elements in the specific contaminated soil in which phytoremediation is going to be applied. Whichever equation is used, it is important to consider that sorption reactions are governed by several processes, which are characterized by great kinetic variability. In the processes of sorption and release, the competition between the two opposite reactions, characterized by the respective activation energies $E_{\mathrm{a}}$ and $E_{\mathrm{d}}$, gives rise to a thermodynamic equilibrium. The standard free energy of the sorption reaction is negative, since the system energy decreases moving toward a more stable state [44].

**Table 3.1** Some further isotherm models for soil or soil components

| Reference | Isotherm models | Adsorbate | Adsorbent |
|---|---|---|---|
| Benhammou et al. [52] | Dubinin–Radushkevich<br>Redlich–Peterson | Cd(II), Cu(II), Mn(II), Pb(II), Zn(II) | Moroccan stevensite |
| Fonseca et al. [53] | Dubinin–Radushkevich<br>Temkin<br>Khan<br>Redlich–Peterson<br>Toth<br>Sips | Cr(VI), Pb(II) | Loamy sand soil |
| Perić et al. [54] | Dubinin–Radushkevich<br>Redlich–Peterson<br>Toth | Zn, Cu, Pb | Natural zeolitic tuff |
| Günay et al. [55] | Dubinin–Radushkevich<br>Temkin<br>Khan<br>Redlich–Peterson<br>Toth<br>Sips | Pb | Clinoptilolite |
| Ho et al. [48] | Dubinin–Radushkevich<br>Temkin<br>Redlich–Peterson<br>Toth<br>Sips | Pb, Cu, Ni | Peat |

### *3.3.2 Desorption Processes*

In contaminated soils, desorption processes are as important as sorption processes in predicting the fate and transport of contaminants and in developing effective remediation strategies [56]. For example, a contaminant that is strongly linked to the solid phase, which is not desorbed or only partially desorbed, does not constitute a pollution risk to the water table. On the other hand, this behavior can impair the use of some remediation technologies such as phytoextraction. Conversely, when predominantly desorption occurs, contaminants are released into the soil solution, and biological technologies are more effective. Often desorption is more difficult than sorption, and the adsorbate part appears to be irreversibly adsorbed on the solid matrix.

This apparent irreversibility is often called hysteresis. In these cases, the sorption and desorption isotherms do not coincide. Hysteresis depends above all on the type of adsorbent, especially on the presence or absence of humic substances, and on the duration of the sorption (ageing). The ageing effect has been explained by supposing that the contaminant remains “trapped” in micropores of the soil, thus making the release very slow, if not impossible [57]. The process of desorption greatly changes the metal bioavailability following the ageing process. During ageing, metals move into the soil solid phase becoming entrapped in sites that are not involved in desorption reactions [57].

During the long-term reactions of historical contamination, sorption can also involve the diffusion of a metal into the solid phase, and the reactions of metals with the components of the solid phase are then responsible for the reduced reversibility of the process. There is thus a clear need to conduct studies on long-term contaminated soils since short-term laboratory studies cannot accurately predict the sorption-desorption behavior of contaminants. Heavy metal sorption in soils at contaminated sites involves several reactions, which are more complex when there are several contaminants present at the same time. Competitive sorption reactions are further complicated by the presence of plants, which influence the soil environment by the release of root exudates and the stimulation of microbial activity. Although the rhizosphere is a restricted area of soil, the processes in this limited zone are very important, because the interactions between roots, contaminants, and soil components greatly influence the sorption and bioavailability of metals and the number of metals that are absorbed from plants. Root exudates released into the rhizosphere have a great impact on the chemical form of metal cations and anions as well as their sorption/desorption processes at the soil-plant interface, which are the most important reactions that control their bioavailability by altering the level of soluble ions and molecules. Roots also release high quantities of organic compounds, which lead to an increase in the microbial population and activity [58]. These compounds are constituted by both low molecular weight substances such as carbohydrates, amino acids, and organic acids and high molecular weight compounds, such as polysaccharides and mucilage.

Organic substances released by root exudates drastically influence the desorption of heavy metals from soil surfaces, changing their availability which may be either increased or reduced according to different processes such as modification of surface charge and competition for adsorbing sites.

Due to their chelating properties, if the organic acid concentrations (e.g., citric, oxalic, succinic, tartaric) are increased, this promotes the formation of mobile organically bound metals which facilitate their uptake by plants. These organically complexed metals are generally more easily translocated in plants, from roots to shoots, than ionic forms. Organic ligands not only reduce the metal sorption on soil, thus enhancing their solubility in soil solution, but also decrease their toxicity to plants [3]. The metal-solubilizing ability of organic acids to reduce metal sorption depends on their metal-binding ability [59], which in turn is determined by acid dissociation constants. Carboxylic acids bind divalent cations by forming stable complexes, which are not prone to sorption [60].

The sorption of cations on negatively soil surfaces is decreased by organic acids due to the formation of negatively charged soluble complexes. This characteristic is exploited in the field of phytoextraction when complexing agents are used to increase the mobility and therefore the bioavailability of the metallic elements. Root exudates can influence the sorption processes on the mineral surfaces of variable charges (iron and aluminum oxides) in several ways. These minerals have positive charge at normal values of soil pH between 4.0 and 8.0; thus, sorption can be increased due to the formation of negatively charged complexes between a metal and organic acids. However, retention can be decreased both by metal complexation, with the consequent release of metals from the soil surfaces, and by the dissolution of variably charged minerals if a high concentration of chelating organic acids is released [61].

In many contaminated sites, metals in anionic forms, such as arsenate and chromate, are the main pollutants. The effects of root exudates on their sorption processes are of great importance, because the nature and amount of root exudates drastically influence the plant bioavailability of these anionic contaminants. Carboxylic acids from root exudates can hinder the arsenate sorption on variable charge minerals, thereby promoting the release of the metals in soil solution [62, 63]. In the process of plant uptake, the roots compete with soil surfaces for metal uptake; thus, the success of phytoextraction is determined by the plant characteristics and the soil properties. The metal uptake by the same plant in different soils is extremely variable, as well as the uptake of different plant species from the same soil. The plant uptakes a relatively higher amount of metals from soils with solid surfaces where metals have been sorbed with a lower energy.

## 3.4 A Case Study: The Relationship Between Desorption Parameters and Plant Uptake at a Pb Contaminated Site

### *3.4.1 The Site*

The soil used for this study was contaminated due to the uncontrolled disposal of sludge containing lead-based paints from a former ceramic industry located at a short distance from the sampling site. The contaminated area had originally been agricultural land. It was separated by various ditches without banks from the old abandoned factory, which had been shut down about 30 years ago. During frequent floods, the contaminated water from the ditches flooded this site, coloring some areas violet. The violet color derived from ceramic paints and was still visible in many areas of the contaminated site, despite the fact that many years had passed since the factory had been dismantled. In the area there were many perennial ryegrass (*Lolium perenne*) plants growing spontaneously; thus, phytoextraction was tested as a possible remediation strategy using this plant species.

### *3.4.2 Experimental Procedure*

For the feasibility field test, the total area under examination was subdivided into four Thiessen polygons named A, B, C, and D, in increasing order of Pb concentration. Six soil samples from each polygon were collected. These were air-dried and sieved with a 2-mm sieve. They were then homogenized and analyzed for physicochemical characteristics, Pb total, and extractable concentrations. Soil pH was determined using a glass electrode at a soil/water ratio of 1:2.5 [64], CEC was determined using barium chloride (pH = 8.1) [65], and texture was determined (sand, silt, and clay) by the pipette method [66]. Organic matter was determined by wet combustion [67]. Vegetative samples were also collected from each polygon, which were then transported to the laboratory in plastic bags and separated into tops and roots. The aerial parts of plants were washed with distilled water. The samples were air-dried at 50 °C and then crushed and ground to pass a 2-mm sieve. Only the aerial parts were analyzed.

Soil and vegetable samples were digested in aqua regia, and Pb content was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), with a Liberty AX Varian spectrometer. In the soil samples, the desorption of Pb was carried out by equilibration of the soil with a potassium nitrate ($KNO_3$) 1 M solution or an ethylenediaminetetraacetic acid (EDTA) 1% solution for 12 h with a ratio soil solvent of 1:5 [68]. The analyses were carried out in three replicates.

### *3.4.3 Results and Discussion*

A key issue of phytoremediation at a field scale is the heterogeneity of contamination within the same contaminated site. The main physical-chemical characteristics of the soil were the same throughout the whole area: 13.8% clay, 29.2% silt, 55.5% sand, 0.9% organic matter, 5.3 pH, and 14.4 $\mathrm{cmol_{(+)}kg^{-1}}$ CEC. The total and extractable concentrations of Pb are reported in Table 3.2.

The results showed that the soil contamination was not uniformly distributed, with zones of very different Pb concentrations from 40 to 5000 mg $\mathrm{kg^{-1}}$. Feasibility tests should therefore be based not only on experimental data on plant growth and the accumulation of contaminants in plant tissues but should also provide an uptake model that accounts for plant growth in areas with different concentrations at the same contaminated site. A study of Pb desorption by the contaminated soil was thus carried out to modeling and predicts the transfer of contaminants from soil to plants. The desorption processes thus evaluated the amount of Pb which is desorbed from the soil, related to the residual quantity of the metal adsorbed on the soil solid phase. For practical use at the field scale, a very simple model is needed, which can be applied immediately and which provides the essential data to plan the remediation procedure.

In this study some approximations were made:

**Table 3.2** Mean total and extractable Pb concentrations (mg $\mathrm{kg^{-1}}$)

| Polygons | Total | $KNO_3$ | EDTA |
|---|---|---|---|
| A | 46.8 ± 4.3 | 21.2 ± 2.3 | 14.0 ± 2.2 |
| B | 99.0 ± 11.2 | 21.0 ± 1.2 | 52.1 ± 4.7 |
| C | 654 ± 81.0 | 22.3 ± 1.6 | 295 ± 21.8 |
| D | 2510 ± 173 | 23.0 ± 1.4 | 932 ± 88.4 |

- In this case of aged-contaminated soil, the quantity adsorbed was considered as the total concentration.
- The desorbed quantity at equilibrium was obtained using an extraction with a suitable reagent.

In the case of lead, to examine the desorbed quantity, there are two possible extractions. The first uses a solution of an alkaline metal such as $KNO_3$, which identifies the bonds of an electrostatic nature with which the metal is bonded to the surfaces of the soil. The other uses a solution of a complexing agent (EDTA) that releases a metal from the surfaces of the soil, which is also linked with covalent bonds.

For this study, the desorption processes in each polygon were described by the Freundlich desorption equation, which for each of the six soil samples relates the Pb concentration remaining in the solid phase and the concentration of the desorbed Pb in the solution at desorption equilibrium:

$$C_S = K_{des} C_e^{\frac{1}{n}}$$

or in linear form:

$$\log C_S = \log K_{des} + \frac{1}{n} \log C_e$$

where:

$C_s$ is the Pb concentration remaining adsorbed in the soil at desorption equilibrium.
$K$ is the Freundlich desorption constant.
$1/n$ is the Freundlich parameter.
$C_e$ is the Pb concentration in solution at desorption equilibrium.

The equilibrium distribution of Pb concentrations showed (Table 3.2) that the sorption process was not fully reversible at any sampling point, notwithstanding the addition of a high concentration of EDTA. The partial irreversibility observed can be ascribed to the existence of sorption sites on which specific sorption occurred with stronger linkages than the Pb-EDTA complexes.

The coefficients of the Freundlich equation are shown in Table 3.3.

Concerning the plants, the biomass production was the same in all four polygons, indicating that the uptake of Pb did not influence the growth of the plants. Conversely, the Pb concentration in the aboveground parts of the *L. perenne* plants grown in the contaminated site ranged from about 7 mg $kg^{-1}$ to about 37 mg $kg^{-1}$, according to the concentration of Pb in the four polygons. In fact, the highest values of Pb uptake were recorded in soils with the highest Pb total and EDTA extractable concentrations. However, the concentration of Pb in the shoots of the plants grown in the different areas did not correlate well with the amount of Pb extracted by EDTA from the soil in the same areas (Fig. 3.2). Conversely, there was a high correlation between the Pb concentrations in the plants and the values of the parameter $K$ of the

**Table 3.3** Parameters of the desorption Freundlich equation for the various polygons of the contaminated site

| Polygons | *K* | 1/*n* | $R^2$ |
|---|---|---|---|
| A | 1.51 | 1.17 | 0.9880 |
| B | 1.22 | 1.05 | 0.9958 |
| C | 1.15 | 1.07 | 0.9963 |
| D | 1.05 | 1.01 | 0.9982 |

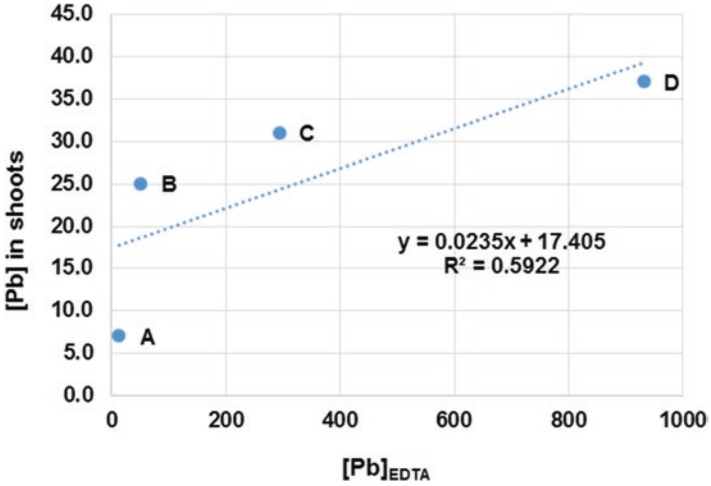


**Fig. 3.2** Relationship between Pb concentration in shoots and EDTA extractable Pb. All values are expressed as mg $kg^{-1}$

Freundlich desorption equation (Fig. 3.3). There was no correlation between plant content and $KNO_3$ extractable lead since this latter value was about the same in all the samples from the different areas.

The results showed a strong relationship between the desorption Freundlich constants (*K* and 1/*n* parameters) and the plant tissue Pb concentrations. The coefficient *K* of the Freundlich equation can be considered as a measure of the ability of the soil solid phase to retain the contaminant. A greater value of *K* therefore indicates a lower capacity of the soil to release Pb in soil solution in a form available for plant uptake. The utility of the Freundlich constants to predict substance concentrations in plant tissue has been reported elsewhere with specific emphasis on phosphorus uptake [69–71]. The results from this case study seem to indicate the possibility of extending the correlations between the parameters of the desorption processes and the quantity of elements absorbed by the plants, even in contaminated soils where phytoextraction is used as a remediation technology. However, further studies are necessary to understand the wider applicability of these coefficients to phytoremediation efficiency, using different contaminated soils and different plant species.

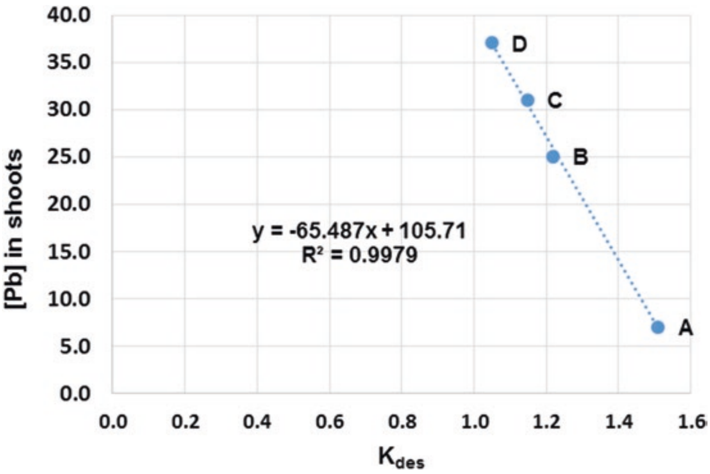


**Fig. 3.3** Relationship between Pb concentration (mg kg$^{-1}$) in shoots and the desorption Freundlich constant K

## 3.5 Concluding Remarks

The soil properties that regulate sorption/release processes are also critical in the plant uptake of heavy metals and are thus essential in determining phytoremediation efficiency. Plant uptake is strictly related to metal bioavailability, which is determined by the distribution of metals between the solid and liquid phases of soil. This distribution can be evaluated in terms of sorption isotherms, which describe the ability of soils to retain contaminants in the solid phase. When assessing phytoremediation technologies as a remediation procedure, it is essential to analyze the soil properties that influence contaminant bioavailability, which are mostly the result of retention/release processes. These processes are highly site specific, and the plant-based technologies should only be used after a site-specific feasibility test to investigate the mobile and bioavailable fractions of the contaminants in relation to the uptake ability of different plant species [72].

The retention/release of heavy metals in the soil solid phase primarily depends on soil characteristics such as pH, clay minerals, humic substances, iron oxides, and hydroxides. The distribution of heavy metals between the solid phase and the soil solution should be considered as key in planning phytoremediation. Phytoextraction aims to reduce the mobile and bioavailable fractions of heavy metals [73]. These technologies able to remove these fractions but not the total amount of metals present in polluted soils [9]. Ultimately there is a close relationship between the sorption and desorption processes of contaminants in soil and their bioavailability for plants. Knowledge of sorption processes is therefore the key to understanding how efficient a phytoremediation procedure will be in relation to the characteristics of the specific contaminated soil and those of the chosen plant species. The parameters describing the retention release processes can also be used to define the amount of metals that will be absorbed by the plants. This is of great importance when phytoremediation is employed in the field because it helps to overcome the difficulties in predicting the efficiency of remediation resulting from the extreme heterogeneity of soils in contaminated sites.

## References

1. Paz-Alberto AM, Sigua GC (2013) Phytoremediation: a green technology to remove environmental pollutants. Am J Clim Change 2(01):71
2. Pilon-Smits EAH (2005) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 56:15–39
3. Petruzzelli G, Pedron F, Rosellini I, Grifoni M, Barbafieri M (2016) Polycyclic aromatic hydrocarbons and heavy metal contaminated sites: phytoremediation as a strategy for addressing the complexity of pollution. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation. Springer, Cham, pp 61–90
4. Cundy AB, Bardos RP, Church A, Puschenreiter M, Friesl-Hanl W, Müller I et al (2013) Developing principles of sustainability and stakeholder engagement for "gentle" remediation approaches: the European context. J Environ Manag 129:283–291
5. Gerhardt KE, Gerwing PD, Greenberg BM (2017) Opinion: Taking phytoremediation from proven technology to accepted practice. Plant Sci 256:170–185
6. Franchi E, Agazzi G, Rolli E, Borin S, Marasco R, Chiaberge S et al (2016) Exploiting hydrocarbon-degrading indigenous bacteria for bioremediation and phytoremediation of a multicontaminated soil. Chem Eng Technol 39(9):1676–1684
7. Schwitzguébel JP (2017) Phytoremediation of soils contaminated by organic compounds: hype, hope and facts. J Soils Sediments 17(5):1492–1502
8. Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manag 105:103–120
9. Petruzzelli G, Pedron F, Rosellini I, Barbafieri M (2013) The bioavailability processes as a key to evaluate phytoremediation efficiency in metal-contaminated soils. In: Gupta DK (ed) Plant-based remediation processes. Springer, Berlin, pp 273–289
10. Minguzzi C, Vergnano O (1948) Il contenuto di nichel nelle ceneri di *Alyssum bertolonii*. Atti Soc Tosc Sci Nat 55:49–74 Italian
11. Evangelou MW, 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
12. Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res 2(1):5
13. Barbafieri M, Pedron F, Petruzzelli G, Rosellini I, Franchi E, Bagatin R et al (2017) Assisted phytoremediation of a multi-contaminated soil: investigation on arsenic and lead combined mobilization and removal. J Environ Manag 203:316–329
14. Doumett S, Fibbi D, Azzarello E, Mancuso S, Mugnai S, Petruzzelli G et al (2011) Influence of the application renewal of glutamate and tartrate on Cd, Cu, Pb and Zn distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study. Int J Phytoremediation 13(1):1–17
15. Pedron F, Petruzzelli G, Barbafieri M, Tassi E, Ambrosini P, Patata L (2011) Mercury mobilization in a contaminated industrial soil for phytoremediation. Commun Soil Sci Plant Anal 42(22):2767–2777
16. Pedron F, Petruzzelli G, Barbafieri M, Tassi E (2013) Remediation of a mercury-contaminated industrial soil using bioavailable contaminant stripping. Pedosphere 23(1):104–111

17. Petruzzelli G, Pedron F, Rosellini I, Barbafieri M (2015) The bioavailability processes as a key to evaluate phytoremediation efficiency. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation. Springer International Publishing, New York, pp 31–43
18. Van Gestel CA (2008) Physico-chemical and biological parameters determine metal bioavailability in soils. Sci Total Environ 406(3):385–395
19. Pedron F, Grifoni M, Barbafieri M, Petruzzelli G, Rosellini I, Franchi E et al (2017) Applicability of a Freundlich-like model for plant uptake at an industrial contaminated site with a high variable arsenic concentration. Environments 4(4):67
20. Petruzzelli G, Pedron F (2006) "Bioavailability" at heavy metal contaminated sites: a tool to select remediation strategies. Int Conf "Remediation of contaminated sites", Rome, pp 1–8
21. Petruzzelli G, Pedron F, Rosellini I (2014) Effects of thiosulfate on the adsorption of arsenate on hematite with a view to phytoextraction. Res J Environ Earth Sci 6(6):326–332
22. Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T et al (2014) Remediation of heavy metal(loid)s contaminated soils–to mobilize or to immobilize? J Hazard Mater 266:141–166
23. Robinson B, Bolan NS, Mahimairaja S, Clothier B (2006) Solubility, mobility and bioaccumulation of trace elements: abiotic processes in the rhizosphere. In: Prasad M, Sajwan K, Naidu R (eds) Trace elements in the environment: biogeochemistry, biotechnology and bioremediation. CRC Press, London, pp 97–110
24. Petruzzelli G (1997) Soil sorption of heavy metals. In: Cheremisinoff PN (ed) Ecological issues and environmental impact assessment. Gulf Publishing Company, London, pp 145–174
25. Kim RY, Yoon JK, Kim TS, Yang JE, Owens G, Kim KR (2015) Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review. Environ Geochem Health 37(6):1041–1061
26. Brallier S, Harrison RB, Henry CL, Dongsen X (1996) Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. Water Air Soil Pollut 86(1–4):195–206
27. McLaren RG, Swift RS, Williams JG (1981) The adsorption of copper by soil materials at low equilibrium solution concentrations. Eur J Soil Sci 32(2):247–256
28. Chaney RL, Angle JS, McIntosh MS, Reeves RD, Li YM, Brewerd EP et al (2005) Using hyperaccumulator plants to phytoextract soil Ni and Cd. Z Naturforsch 60(3–4):190–198
29. Li YM, Chaney R, Brewer E, Roseberg R, Angle JS, Baker A et al (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant Soil 249(1):107–115
30. Abdullah S, Sarem SM (2010) The potential of *Chrysanthemum* and *Pelargonium* for phytoextraction of lead - contaminated soils. J Civil Eng 4:409–416
31. Wang Q, Li Z, Cheng S, Wu Z (2010) Effects of humic acids on phytoextraction of Cu and Cd from sediment by *Elodea nuttallii*. Chemosphere 78(5):604–608
32. Alloway BJ (1995) Soil processes and the behaviour of metals. In: Alloway BJ (ed) Heavy metals in soils. Blackie, Glasgow
33. Brümmer GW (1986) Heavy metal species, mobility and availability. In: Bernhard M, Brinckman FE, Sadler PJ (eds) The importance of chemical 'speciation' in environmental processes. Dahlem Conference; 1984 Sept 2–7. Springer, Berlin, pp 169–192
34. McKenzie RM (1980) The adsorption of lead and other heavy metals on oxides of manganese and iron. Aust J Soil Res 18(1):61–73
35. Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremediation. J Biotechnol 99(3):259–278
36. Evans LJ (1989) Chemistry of metal retention by soils. Environ Sci Technol 23(9):1046–1056
37. Cherlatchka R, Cambier P (2000) Influence of reducing conditions on solubility of trace metals in contaminated soils. Water Air Soil Pollut 118(1–2):143–167
38. Jarvis SC, Jones LHP (1980) The contents and sorption of cadmium in some agricultural soils of England and Wales. Eur J Soil Sci 31(3):469–479

39. Basta NT, Tabatabai MA (1992) Effect of cropping systems on adsorption of metals by soils: II. Effect of pH. Soil Sci 153:195–204
40. Forbes EA, Posner AM, Quirk JP (1976) The specific adsorption of divalent Cd, Co, Cu, Pb and Zn on goethite. Eur J Soil Sci 27(2):154–166
41. Tiller KG, Gerth J, Brümmer G (1984) The sorption of Cd, Zn and Ni by soil clay fractions: procedures for partition of bound forms and their interpretation. Geoderma 34(1):1–16
42. Borda MJ, Sparks DL (2008) Kinetics and mechanisms of sorption/desorption in soils: a multiscale assessment. In: Violante A, Huang PM, Gadd GM (eds) Biophysico-chemical processes of metals and metalloids in soil environments. Wiley, Hoboken, NJ, pp 97–168
43. Bradl HB (2004) Adsorption of heavy metal ions on soils and soils constituents. J Colloid Interface Sci 277(1):1–18
44. Sparks DL (2003) Environmental soil chemistry. Academic, San Diego, CA
45. Selim HM, Zhao K, Liao L, Xu J (2010) Adsorption/desorption kinetics of Zn in soils: influence of phosphate. In: Xu J, Huang PM (eds) Molecular environmental soil science at the interfaces in the earth's critical zone. Springer, Berlin, pp 88–90
46. Zhang H, Selim HM (2005) Kinetics of arsenate adsorption-desorption in soils. Environ Sci Technol 39(16):6101–6108
47. Hall KR, Eagleton LC, Acrivos A, Vermeulen T (1966) Pore- and solid-diffusion kinetics in fixed-bed adsorption under constant-pattern conditions. Ind Eng Chem Fundam 5(2):212–223
48. Ho YS, Porter JF, McKay G (2002) Equilibrium isotherm studies for the sorption of divalent metal ions onto peat: copper, nickel and lead single component systems. Water Air Soil Pollut 141(1–4):1–33
49. Shaheen SM, Tsadilas CD, Rinklebe J (2013) A review of the distribution coefficients of trace elements in soils: influence of sorption system, element characteristics, and soil colloidal properties. Adv Colloid Interf Sci 201:43–56
50. Limousin G, Gaudet JP, Charlet L, Szenknect S, Barthes V, Krimissa M (2007) Sorption isotherms: a review on physical bases, modeling and measurement. Appl Geochem 22(2):249–275
51. Pignatello JJ (2000) The measurement and interpretation of sorption and desorption rates for organic compounds in soil media. Adv Agron 69:1–73
52. Benhammou A, Yaacoubi A, Nibou L, Tanouti B (2005) Adsorption of metal ions onto Moroccan stevensite: kinetic and isotherm studies. J Colloid Interface Sci 282(2):320–326
53. Fonseca B, Maio H, Quintelas C, Teixeira A, Tavares T (2009) Retention of Cr (VI) and Pb (II) on a loamy sand soil: kinetics, equilibria and breakthrough. Chem Eng J 152(1):212–219
54. Perić J, Trgo M, Medvidović NV (2004) Removal of zinc, copper and lead by natural zeolite—a comparison of adsorption isotherms. Water Res 38(7):1893–1899
55. Günay A, Arslankaya E, Tosun I (2007) Lead removal from aqueous solution by natural and pretreated clinoptilolite: adsorption equilibrium and kinetics. J Hazard Mater 146(1–2):362–371
56. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol. https://doi.org/10.5402/2011/402647
57. Alexander M (2000) Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ Sci Technol 34(20):4259–4265
58. Hinsinger P (2011) Biogeochemical, biophysical, and biological processes in the rhizosphere. In: Huang PM, Li Y, Summer ME (eds) Handbook of soil science resource of management and environmental impacts, 2nd edn. CRC Press, Taylor & Francis, Boca Raton, FL, pp 1–30
59. Mench M, Martin E (1991) Mobilization of cadmium and other metals by roots exudates of *Zea mays* L. *Nicotiana tabacum* L. and *Nicotiana rustica* L. Plant Soil 132(2):187–196
60. Nigam R, Srivastava S, Prakash S, Srivastava MM (2001) Cadmium mobilisation and plant availability – the impact of organic acids commonly exuded from roots. Plant Soil 230(1):107–113
61. Violante A, Caporale AG (2015) Biogeochemical processes at soil-root interface. J Soil Sci Plant Nutr 15(2):422–448

62. Zhu J, Pigna M, Cozzolino V, Caporale AG, Violante A (2011) Sorption of arsenite and arsenate on ferrihydrite: effect of organic and inorganic ligands. J Hazard Mater 189(1–2):564–571
63. Zhu J, Pigna M, Cozzolino V, Caporale AG, Violante A (2013) Higher sorption of arsenate versus arsenite on amorphous Al-oxide, effect of ligands. Environ Chem Lett 11(3):289–294
64. Thomas GW (1996) Soil pH and soil acidity. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, Soil Science Society of America Book Series. Soil Science Society of America Inc., Madison, pp 475–490
65. Sumner ME, Miller WP (1996) Cation exchange capacity and exchange coefficients. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, Soil Science Society of America Book Series. Soil Science Society of America Inc., Madison, pp 1201–1230
66. Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) Methods of soil analysis. Part 1. Physical and mineralogical methods, Agronomy monograph No. 9, 2nd edn. American Society of Agronomy, Soil Science Society of America, Madison, pp 383–411
67. Nelson DW, Sommers LE (1996) Total carbon, organic carbon and organic matter. In: Sparks DL (ed) Methods of soil analysis. Part 3. Chemical methods, Soil Science Society of America Book Series. Soil Science Society of America Inc., Madison, pp 961–1010
68. Pedron F, Rosellini I, Petruzzelli G, Barbafieri M (2014) Chelant comparison for assisted phytoextraction of lead in two contaminated soils. Res Environ 4(5):209–214
69. Holford ICR (1982) The comparative significance and utility of the Freundlich and Langmuir parameters for characterizing sorption and plant availability of phosphate in soils. Aust J Soil Res 20(3):233–242
70. Shafqat MN, Pierzynski GM (2011) Bioavailable phosphorus in animal waste amended soils: using actual crop uptake and p mass balance approach. Environ Sci Technol 45(19):8217–8224
71. Shafqat MN, Pierzynski GM (2014) The Freundlich adsorption isotherm constants and prediction of phosphorus bioavailability as affected by different phosphorus sources in two Kansas soils. Chemosphere 99:72–80
72. Pedron F, Petruzzelli G, Barbafieri M, Tassi E (2009) Strategies to use phytoextraction in very acidic soil contaminated by heavy metals. Chemosphere 75(6):808–814
73. Barbafieri M, Japenga J, Romkens P, Petruzzelli G, Pedron F (2013) Protocols for applying phytotechnologies in metal-contaminated soils. In: Gupta D (ed) Plant-based remediation processes. Springer, Berlin, pp 19–37

# Chapter 4
# A Survey on the Metal(loid) Accumulation Ability of Spontaneous and Established Plants for the Phytomanagement of an Industrial Landfill in the Venice Lagoon

**Fabrizio Pietrini, Valentina Iori, Lucia Pietrosanti, Laura Passatore, Maria Clara Zuin, Rita Aromolo, Guido Capotorti, Angelo Massacci, and Massimo Zacchini**

## 4.1 Introduction

The reclamation of metal-contaminated sites originated by industrial activities during decades is a major environmental issue to be addressed worldwide. The request for the mitigation of metal pollution in soil and groundwater is based on the knowledge about the harmful effects exerted by metals on biota because of soil erosion, leaching, and transfer by plants to food chains. An eco-friendly approach to the management of such environmental concerns is highly demanded. In this context, plant communities can play a multifaceted role [1], globally defined as ecological services [2, 3]. In fact, they can take up metals and (hyper-) accumulate them in their organs, resulting in a reduction of the soil and water metal content [4], through several biological mechanisms currently exploited by the phytoremediation technology (phytoextraction). As a drawback, the metal enrichment of plant organs devoted to animal and human feeding represents a great concern because of the

---

F. Pietrini · V. Iori · L. Pietrosanti · L. Passatore · A. Massacci · M. Zacchini (✉)
Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Montelibretti, Monterotondo Scalo (RM), Italy
e-mail: massimo.zacchini@ibaf.cnr.it

M. C. Zuin
Istituto di Biologia Agro-ambientale e Forestale (CNR), Sezione di Legnaro, Agripolis, Legnaro (PD), Italy

R. Aromolo
Centro di ricerca Agricoltura e Ambiente, Rome, Italy

G. Capotorti
Syndial Servizi Ambientali, Rome, Italy

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_4

health problems connected to the metal toxicity [5]. For such reason, the use of non-food plants for phytoremediation purposes has been suggested [6]. Regardless of metal accumulation, the establishment of a vegetation cover can be extremely beneficial in a metal-contaminated site acting as a biological pump system regulating soil water content and blocking metals at root level, in both cases reducing metal leaching to the water table. Moreover, it can limit soil erosion by wind and water runoff, create an aerobic environment in the rhizosphere that favors microorganism colonization and activity, enhance soil organic matter and increase soil aggregation, generate renewable energy and material, and mitigate greenhouse gas [7, 8]. All these processes favored by the plant community in a contaminated site are at the base of the phytomanagement, as defined by Dominguéz et al. [9]. Phytomanagement is recognized as an effective approach to carry out a risk management strategy [7, 10], even if barriers or impediments related to technical issues and stakeholder perceptions are still limiting a wider application [11]. Phytomanagement is constituted by an array of gentle remediation options (GROs) technologies [7] that can be applied as a part of integrated, mixed, site risk management solutions, particularly suited for contaminated sites where the reclamation cannot be performed through the conventional technologies for technical or economical reasons. In this sense, the economical advantages provided by the phytotechnologies, estimating to be 50–80% less expensive than conventional applications, have been long discussed [12, 13]. At sustainable level, the phytomanagement improves up the phytoremediation approach, exploiting in a broader way the ecological benefits offered by plants.

As pointed out by Dominguéz et al. [9], the success in the phytomanagement of contaminated sites depends primarily on the appropriate choice of plant species that tolerate the local conditions, including the elevated concentrations of trace elements. For this purpose, the characterization of the plant species growing in a contaminated site for metal accumulation represents a basic step in order to successfully approach the phytomanagement strategy. In this work, results from a survey conducted in a particular industrial landfill, formed by an island, within the Venice Lagoon (Italy) are presented. This site has been claimed as an “Italian national interest site” (SIN) by the Italian Ministry of Environment, recognizing the high environmental risk and the urgent demand for reclamation [14, 15]. For this purpose, a study area was first characterized for soil and groundwater metal(loid) presence. Successively, an investigation on the most represented tree, shrub, and herbaceous species, spontaneous or established (individuals previously planted for revegetation), growing in the area was performed, describing their metal accumulation ability in the aboveground organs. The contribution of such outcomes for a phytomanagement approach of industrial contaminated sites is discussed.

## 4.2 Materials and Methods

### *4.2.1 Study Area*

Porto Marghera is one of the main chemical districts in Italy, located in the Venice Lagoon, a shallow transitional environment in the northern basin of the Adriatic Sea. The industrial zone was created in 1917 on the border of the Venice Lagoon, as an extension of the Venice Port, to sustain oil- and coal-related activities. Considerable industrial activities, in particular chemical production, oil refining and storage, shipbuilding, metal extraction and metallurgy, energy production and distribution, wastewater treatment, and hazardous waste incineration, caused the extensive contamination of air, soil, groundwater, and inner tidal canals. Moreover, industrial plants were built on marshlands, previously filled with contaminated materials. The industrial zone of Porto Marghera is classified since 1998 as one of the most important "contaminated sites of national interest" (SIN) in Italy and is considered an area of high environmental risk that needs to be reclaimed [14, 15].

Previous studies on the sediments of the Venice Lagoon have evidenced a diffuse contamination of both organic compounds and heavy metals, with a prevalence of Zn over Pb, As, and Cd, caused by activities involving the use of minerals, metals, and catalysts [16, 17]. The experimental site, 2700 square meters wide, is located in an island of 12 ha, until recently used as an industrial landfill. The site was chosen as representative for that regards the contamination characteristics and the vegetation community growing in the island. The site was completely fenced and the access strictly restricted to authorized persons. In this island, previous studies (https://www.regione.veneto.it/web/ambiente-e-territorio/database-indagini-ambientali-georeferenziate) have shown that 90% of soil contamination is caused by heavy metals, in particular As, Zn, and Cd, with concentrations higher than the Italian law limits in the soil depth range of 1–2 m.

### *4.2.2 Plant, Water, and Soil Sampling*

Plants, soil, and groundwater in the experimental site were sampled at the end of the vegetation phase, following a randomized scheme. Regarding plants, the survey was primarily conducted to recognize the most abundant tree, shrub, and herbaceous species growing on the site. A list of the identified plant species, utilized for the purpose of the present work, is shown in Table 4.1. Plant species were spontaneous or established (individuals previously planted for revegetation), and they were all characterized by a good adaptation to the site pedo-climatic conditions. For each species, at the end of the vegetative period, three to five representative individuals were sampled depending on their abundance. For herbaceous and shrub species, pooled leaves from each individual were taken. For three established woody species, due to their large interest for phytoremediation purposes, besides leaves,

**Table 4.1** Plant species recognized and sampled in the study area (landfill island, Venice Lagoon, Italy)

| Plant species | Family | Origin[a] | Type[b] |
|---|---|---|---|
| *Amaranthus retroflexus* L. | Amaranthaceae | S | H |
| *Blackstonia perfoliata* (L.) Hudson | Gentianaceae | S | H |
| *Cornus sanguinea* L. | Cornaceae | S | S |
| *Elaeagnus angustifolia* L. | Elaeagnaceae | S | S |
| *Ulmus minor* Miller | Ulmaceae | S | T |
| *Phragmites australis* (Cav.) Trin. | Poaceae | S | H |
| *Rubus caesius* L. | Rosaceae | S | S |
| *Verbena officinalis* L. | Verbenaceae | S | H |
| *Chrysopogon zizanioides* (L.) Roberty | Poaceae | E | H |
| *Salix matsudana* Koidz. | Salicaceae | E | T |
| *Salix alba* L. | Salicaceae | E | T |
| *Eucalyptus camaldulensis* Dehnh. | Myrtaceae | E | T |

[a] Origin—S, spontaneous; E, established
[b] Type—H, herbaceous; S, shrub; T, tree

stem samples were also cored from each individual by an increment borer. Following the aim of the survey, only renewable aboveground organs were sampled. Thirteen soil sampling points were cored in a randomized block design, covering the whole experimental area, and three samples for three depth layers (0–40 cm, 40–80 cm, and 80–100 cm) were collected. Groundwater samples were collected from 12 piezometers randomly distributed over the study area. Conductivity measurements on water samples were taken in situ by a Hydrolab Quanta G (Loveland, Colorado, USA) multiparameter probe.

### 4.2.3 Sample Preparation for Soil Characterization and Metal(loid) Analysis in the Matrices

Soil samples were oven-dried at 60 °C and passed through a 2 mm stainless steel mesh, separating the skeleton fraction. The sieved fraction was used for granulometry, pH, and carbon and nitrogen determination and for the analysis of heavy metals and metalloids. Groundwater samples were stored in polypropylene bottles in dark in a refrigerated room. All plant samples were oven-dried at 60 °C until a constant weight was reached. Before analysis, plant samples were ground into a fine powder and stocked.

### 4.2.4 *Soil and Groundwater Properties and Chemical Analysis*

To determine the soil granulometry and the consequent texture, the physical separation of soil particles was carried out. In particular, a wet sieving procedure was performed to determine the amount of sand. The finer fractions, i.e., silt and clay, were separated on the basis of the fall rate in a liquid, followed by the pipette method as described by Fontaine et al. [18]. For pH analysis (HI9321 pH meter, Hanna Instruments, Woonsocket, RI, USA), 10 g of sieved and oven-dried soil was placed into a beaker with 25 mL of 1 M KCl and stirred for 2 h. Carbon and nitrogen analysis was performed by an elemental analyzer (Carlo Erba CHNS 1108, Rodano, Italy). For this purpose, an oven-dried (60 °C) aliquot of sieved soil was ground and directly analyzed on a tin cup. Groundwater samples were filtered through a 0.45 μm filter (Polypropylene Filter Media, Whatman) and analyzed for pH determination. To preserve water characteristics, samples were acidified with $HNO_3$ to pH < 2.

### 4.2.5 *Heavy Metal and Metalloid Concentration Analysis*

Arsenic, cadmium, zinc, and lead concentrations were determined in the plant, soil, and water samples. Plant materials (0.5 g DW) were digested with 5 mL of 65% (v/v) $HNO_3$ and 2 mL of 60% (v/v) $HClO_4$, whereas soil samples (1 g DW) were digested with 3 mL of 65% (v/v) $HNO_3$, 9 mL of 37% (v/v) HCl, and 3 mL of 35% (v/v) $H_2O_2$ using for both matrices the TMD20 digesting system (VELP Scientifica, Milano, Italy). The metal and metalloid concentrations were determined in filtered extracts of soil samples by ICP-MS (Thermo Jarrell, Ash Iris Advantage, Thermo Electron Corp., Milford, MA, USA) and of plant and groundwater samples by Zeeman Graphite Tube Atomic Absorption Spectroscopy (Varian SpectrAA-800, Mulgrave, Victoria, Australia). Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis.

### 4.2.6 *Data Analyses*

Statistical analyses of the heavy metal concentrations in samples were performed using analysis of variance (ANOVA) and means were compared by Tukey's test (SPSSWIN software, Chicago, IL, USA). Results were evaluated on the basis of homogeneous groups at a given significance level ($p < 0.05$). Principal component analysis (PCA) was performed to highlight the relationship between two variables: the plant species and the metal accumulation. In this way, it was possible to graphically put in evidence the different correlation between plant metal concentration ability and the type of metal(loid), grouping the different plant species with similar properties. Only PCA data with eigenvalues >1, explaining more than a single parameter alone, were extracted. For the principal components, the Varimax rotation was applied on the obtained factor.

## 4.3 Results

### *4.3.1 Characterization of Soil and Groundwater*

Soil characterization (Table 4.2) showed a sandy clay loam type of soil, with an approximately neutral pH and low contents of total C and total N, delineating a poor soil characterized by slow biological and mineralization processes. The characteristics of the groundwater sampled in the piezometers of the experimental site are listed in Table 4.3. The water table depth varied from 86 to 150 cm and pH was approximately neutral. Electrical conductivity analysis allowed to evaluate the depth of seawater infiltration. Soil concentrations of As, Cd, Pb, and Zn of sampling sites at different depths are reported in Table 4.4. A large degree of heterogeneity in contamination at all depths was found. The levels of metal(loid)s were generally higher at greater depths (80–100 cm), considerably exceeding the Italian Guideline Values [19] for private green areas. Values up to around 5 times higher than the IGV were found for As, 13 times for Cd, 8 times for Zn, and 2 times for Pb. As concerns the mean values of metal(loid) concentrations in the experimental site, the values ranged approximatively from 1.5× (As), 2× (Pb), and 4.5× (Zn) to 18× (Cd) higher than the average concentration in Veneto Region soils [20]. Metal(loid) concentrations in groundwater are listed in Table 4.5. The mean concentration values were under the IGV threshold except for As. High scattered values of metal concentration were detected among piezometers, with the upper values ranging 2–3 times higher than the IGV threshold.

**Table 4.2** Soil characteristics in the study area (landfill island, Venice Lagoon, Italy)

| Parameters | Value |
|---|---|
| *Granulometry (%)* | |
| Skeleton | 0 |
| Sand | 48 |
| Silt | 23 |
| Clay | 29 |
| pH | 7.05 ± 0.05 |
| Total C (%) | 6.16 ± 0.14 |
| Total N (%) | 0.07 ± 0.02 |

Granulometry was assessed according to USDA classification. Data referred to mean value ± SE (*n* = 12)

**Table 4.3** Groundwater characteristics in the study area (landfill island, Venice Lagoon, Italy)

| Parameters | Value |
|---|---|
| Depth of water table (cm) | 121 ± 5.9 |
| pH | 7.3 ± 0.2 |
| Depth of mixing zone (fresh and salt water, cm) | 32.3 ± 8.1 |
| Conductivity (mS/cm) | |
| Above mixing border<br>Below mixing border | 8.7 ± 1.1<br>18.8 ± 1.5 |

Data referred to mean value ± SE ($n = 12$)

**Table 4.4** Concentration (range, mean ± SE, mg kg$^{-1}$ DW) of arsenic, cadmium, zinc, and lead in different soil depth layers of the study area (landfill island, Venice Lagoon, Italy)

| Heavy metal | Soil depth | Range (min-max) | Concentration (mean value) | SE | Mean concentration in Veneto Region soil (mg kg$^{-1}$)[a] | IGV[b] |
|---|---|---|---|---|---|---|
| As | Average | 3.75–105.15 | 20.05 | 4.88 | 15.1 | 20 |
| | 0–40 | 4.70–41.33 | 13.53 b | 2.76 | | |
| | 40–80 | 3.75–18.77 | 6.82 b | 1.01 | | |
| | 80–100 | 78.86–105.15 | 97.33 a | 6.24 | | |
| Cd | Average | 1.51–27.70 | 8.36 | 1.18 | 0.47 | 2 |
| | 0–40 | 1.84–26.80 | 7.73 a | 1.94 | | |
| | 40–80 | 1.51–27.70 | 9.17 a | 2.12 | | |
| | 80–100 | 3.93–13.35 | 8.26 a | 1.29 | | |
| Zn | Average | 123.6–1183.8 | 447.1 | 50.19 | 95.6 | 150 |
| | 0–40 | 133.4–859.1 | 363.9 b | 53.52 | | |
| | 40–80 | 123.6–812.1 | 345.9 b | 47.76 | | |
| | 80–100 | 795.3–1183.8 | 1050.3 a | 66.95 | | |
| Pb | Average | 22.15–199.5 | 75.60 | 7.75 | 35.7 | 100 |
| | 0–40 | 30.55–154.9 | 64.43 b | 9.34 | | |
| | 40–80 | 22.15–123.3 | 58.12 b | 7.31 | | |
| | 80–100 | 88.91–199.5 | 152.7 a | 16.84 | | |

The average data referred to all sampling points ($n = 78$; each depth layer $n = 26$). For comparison of means, ANOVA followed by Tukey's test ($P \leq 0.05$) was performed. Values in columns followed by different letters are significantly different among depth layers for each heavy metal analyzed

[a] Giandon et al. [20]

[b] IGV—Italian Guideline Values for soil of private green areas (D.Lgs 152/06) [19]

**Table 4.5** Concentration (range, mean ± SE, $n = 12$, μg L$^{-1}$) of arsenic, cadmium, zinc, and lead in groundwater samples of the study area (landfill island, Venice Lagoon, Italy)

| Heavy metal | Concentration | | | IGV[a] |
|---|---|---|---|---|
| | Range (min-max) | Mean | SE | |
| As | 12–20 | 15 | 1 | 10 |
| Cd | 0–14 | 4 | 2 | 5 |
| Zn | 70–3668 | 1500 | 659 | 3000 |
| Pb | 3–16 | 6 | 1 | 10 |

[a] IGV—Italian Guideline Values for groundwater (D.Lgs 152/06) [19]

### *4.3.2 Characterization of the Plant Community*

In the study area, the vegetation was represented mainly by herbaceous species with the presence of few shrub and tree species. The type of vegetation recognized in the site is not peculiar to coastal areas being the island the result of decades of buildup with industrial waste. Therefore, the type of dispersal has likely represented a very important key for the successful establishment of the plant species in this particular biosystem. The plant cover was not uniformly distributed over the site, probably due to different factors such as the transitory occurrence of flooded areas caused by rainfall and the patchiness of the toxic compound distribution both at area scale and through the soil layers. As reported in Table 4.1, the most represented spontaneous plant species chosen for the survey were eight, namely, *Amaranthus retroflexus* L., *Blackstonia perfoliata* (L.) Hudson, *Cornus sanguinea* L., *Elaeagnus angustifolia* L., *Ulmus minor* Miller, *Phragmites australis* (Cav.) Trin., *Rubus caesius* L., and *Verbena officinalis* L. Moreover, four established plant species such as *Chrysopogon zizanioides* (L.) Roberty, *Salix matsudana* Koidz., *Salix alba* L., and *Eucalyptus camaldulensis* Dehnh. were further selected.

Among herbaceous species, *Amaranthus retroflexus* L. (annual), *Blackstonia perfoliata* (L.) Hudson (annual), and *Verbena officinalis* L. (perennial) have the common characteristic of colonizing uncultivated environments and wastelands. *B. perfoliata* was localized in the wet soil showing its hydrophilic nature. The occurrence of these barocorous species was due to the impact of human activities, moving the industrial waste containing some seeds of these species. *Cornus sanguinea* L. and *Rubus caesius* L. are deciduous shrubs, very common in the natural hedges. *Elaeagnus angustifolia* L. is a deciduous shrub, with high salinity tolerance, commonly occurring in the vegetation of the lagoon, being used to consolidate the dunes. These plant species are zoocorous or more precisely ornithocorous. Their presence is therefore due to the birds that have flown over the site after having fed on fruits of these plants. *Phragmites australis* (Cav.) Trin. is a perennial rhizomatous invasive species typical of wetland environment, being tolerant to brackish water, and commonly used for wastewater phytomanagement. In the study site, about 30% of the area was colonized by this species. Its propagation occurs by rhizomes and by caryopses bearing long silky trichomes that facilitate its wind dispersal. *Ulmus minor* Miller is a deciduous tree, frequent in uncultivated environments and hedges, which can grow up to 30 m tall; it produces, in spring, before budding, a winged fruit (samara) that assures wind dispersal. *Verbena officinalis* L. is a very common, perennial, synanthropic herbaceous species growing in uncultivated land and on the border of roads and paths. Among established species, *Chrysopogon zizanioides* (L.) Roberty, the vetiver plant, is characterized by a large root system that, unlike other grasses, commonly grows upright up to 4 m, making this plant very suitable to stabilize soil and stream banks. Fast-growing tree species are represented by willows (*Salix matsudana* Koidz., *Salix alba* L.) and eucalypt (*Eucalyptus camaldulensis* Dehnh), typical of riverbanks as phreatophytic species, largely used in the phytomanagement of degraded lands.

### 4.3.3 Metal(loid) Concentrations in Plants

The concentration of different metal(loid)s in the leaves of the sampled plant species is shown in Table 4.6. Large variations in metal concentration and distribution among species occurred. Metal(loid) concentrations in leaf of sampled species followed the order Zn > Pb > Cd and As. For most analyzed species, the concentration of As was within the normal concentration range (1.0–1.7 mg kg$^{-1}$) reported for leaf tissues of plants grown in unpolluted areas (according to [21]). Values exceeding this range were found in *Blackstonia perfoliata* (L.) Hudson (4.67 mg kg$^{-1}$), *Verbena officinalis* L. (2.13 mg kg$^{-1}$), and *Ulmus minor* Miller (1.87 mg kg$^{-1}$). However, these values were below the critical range [22]. Cadmium and Zn concentrations in leaves were higher than the normal values in 10 and 8 out of the 12 plant species, respectively. Both *Salix* species showed the highest values of Cd concentration among the sampled plants. Moreover, *Salix alba* L. showed also the highest detected values for Zn concentration. Besides *S. alba* L., *S. matsudana* Koidz. and *Amaranthus retroflexus* L.

**Table 4.6** Concentration (mg kg$^{-1}$ DW, mean ± SE, $n = 5$) of arsenic, cadmium, zinc, and lead in the leaves of 12 species sampled in the study area (landfill island, Venice Lagoon, Italy)

| Plant species | As | Cd | Zn | Pb |
|---|---|---|---|---|
| *Amaranthus retroflexus* L. | 1.16 ± 0.19 bc | 1.94 ± 0.87 c | 841.7 ± 147 a | 9.20 ± 1.2 c |
| *Blackstonia perfoliata* (L.) Hud. | 4.67 ± 0.30 a | 0.78 ± 0.15 c | 200.6 ± 42 c | 56.5 ± 9.4 b |
| *Cornus sanguinea* L. | 1.06 ± 0.04 c | 0.12 ± 0.03 c | 52.5 ± 5.6 c | 13.4 ± 1.5 c |
| *Elaeagnus angustifolia* L. | 0.95 ± 0.03 c | 0.65 ± 0.07 c | 103.7 ± 5.0 c | 12.1 ± 0.6 c |
| *Ulmus minor* Miller | 1.87 ± 0.23 bc | 0.54 ± 0.02 c | 81.5 ± 21 c | 8.27 ± 0.8 c |
| *Phragmites australis* (Cav.) Trin. | 1.29 ± 0.17 bc | 0.11 ± 0.02 c | 65.8 ± 13 c | 21.7 ± 1.4 c |
| *Rubus caesius* L. | 1.31 ± 0.15 bc | 0.63 ± 0.16 c | 184.2 ± 57 c | 8.98 ± 1.4 c |
| *Verbena officinalis* L. | 2.13 ± 0.82 b | 0.40 ± 0.16 c | 186.2 ± 34 c | 20.9 ± 2.8 c |
| *Chrysopogon zizanioides* (L.) | 0.95 ± 0.02 c | 0.82 ± 0.21 c | 100.8 ± 15 c | 263.2 ± 23.2 a |
| *Salix matsudana* Koidz. | 1.09 ± 0.03 bc | 10.93 ± 0.67 a | 698.3 ± 75 ab | 7.32 ± 0.7 c |
| *Salix alba* L. | 1.13 ± 0.05 bc | 10.73 ± 0.89 a | 1079.3 ± 78 a | 6.06 ± 0.2 c |
| *Eucalyptus camaldulensis* Dehnh. | 1.15 ± 0.08 bc | 5.60 ± 1.15 b | 351.6 ± 31 bc | 37.1 ± 5.2 bc |
| Normal range[a] | 1.0–1.7 | 0.05–0.2 | 27–150 | 5–10 |
| Phytotoxic range[b] | 1–20 | 10–20 | 100–500 | 28–200 |

For comparison of means, ANOVA followed by Tukey's test ($p < 0.05$) was performed. Values in columns followed by different letters are significantly different for each heavy metal analyzed

[a] Normal concentration range (mg kg$^{-1}$ DW) of trace elements in plants, according to Kabata-Pendias and Pendias [21]

[b] Phytotoxic concentration range (mg kg$^{-1}$ DW) according to Alloway [22] except for Pb ([23]; [24])

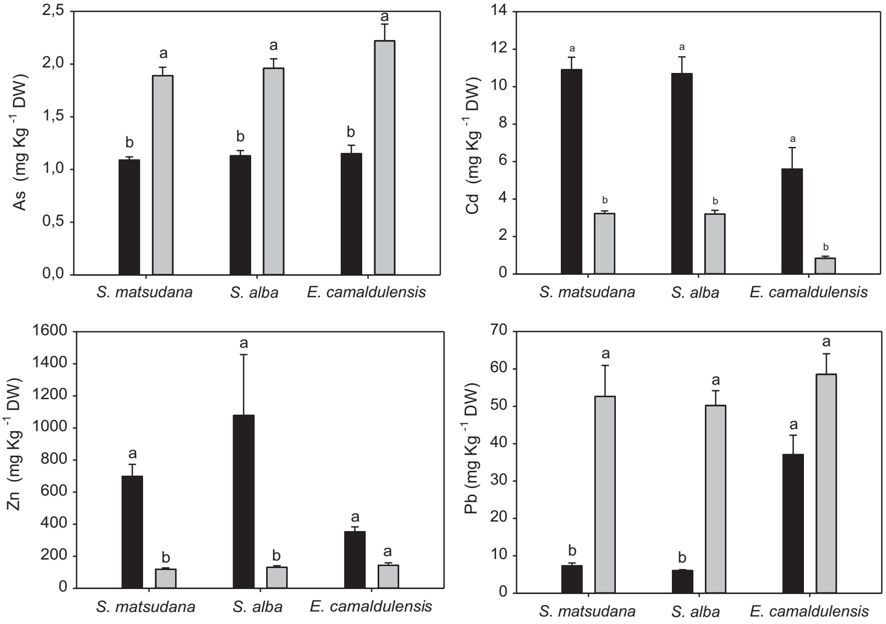


**Fig. 4.1** Concentration (mg $kg^{-1}$ DW, mean ± SE, $n = 5$) of arsenic, cadmium, zinc, and lead in the leaves (black bar) and stem (gray bar) of three woody species sampled in the study area (landfill island, Venice Lagoon, Italy). For comparison of means, ANOVA followed by Tukey's test ($p < 0.05$) was performed. For each metal(loid), values in the bars followed by different letters are significantly different for each plant analyzed

both showed Zn concentration values higher than the critical range reported for plants by Alloway [22]. Lead concentration exceeded the normal value in 7 out of the 12 plant species, reaching the highest value in *Chrysopogon zizanioides* (L.) Roberty leaves. In this plant species, a Pb concentration in the leaves above the critical range was detected [23, 24]. In Fig. 4.1, a comparison between leaf and stem concentration for each heavy metal in the established tree species (*S. alba*, *S. matsudana*, and *E. camaldulensis*) is reported. Generally, a higher As and Pb concentration in stems than in leaves was detected in these plants. Except for *E. camaldulensis*, a remarkable difference between the organs was found for lead concentration. On the contrary, Cd and Zn concentrations were notably higher in leaves than stems.

### 4.3.4 Bioconcentration Factor (BCF)

To better highlight the ability of the plant species recognized in the study area to accumulate metal(loid) in their aboveground organs, the bioconcentration factor (BCF) for leaves (Table 4.7) and stems (Table 4.8) was calculated. As reported in

**Table 4.7** Bioconcentration factor (BCF, mean ± SE, $n = 5$) for different metal(loid)s in leaves of 12 plant species sampled in the study area (landfill island, Venice Lagoon, Italy)

| Plant species | As | Cd | Zn | Pb |
|---|---|---|---|---|
| *Amaranthus retroflexus* L. | 0.06 ± 0.01 bc | 0.23 ± 0.105 c | 1.88 ± 0.33 a | 0.12 ± 0.01 c |
| *Blackstonia perfoliata* (L.) Hud. | 0.23 ± 0.01 a | 0.09 ± 0.018 c | 0.45 ± 0.09 c | 0.75 ± 0.12 b |
| *Cornus sanguinea* L. | 0.05 ± 0.002 bc | 0.02 ± 0.007 c | 0.12 ± 0.01 c | 0.16 ± 0.021 c |
| *Elaeagnus angustifolia* L. | 0.05 ± 0.002 c | 0.08 ± 0.009 c | 0.23 ± 0.01 c | 0.16 ± 0.009 c |
| *Ulmus minor* Miller | 0.09 ± 0.015 bc | 0.06 ± 0.002 c | 0.18 ± 0.04 c | 0.11 ± 0.011 c |
| *Phragmites australis* (Cav.) Trin. | 0.06 ± 0.008 bc | 0.01 ± 0.002 c | 0.15 ± 0.03 c | 0.29 ± 0.02 c |
| *Rubus caesius* L. | 0.07 ± 0.008 bc | 0.08 ± 0.020 c | 0.41 ± 0.12 c | 0.12 ± 0.019 c |
| *Verbena officinalis* L. | 0.11 ± 0.041 b | 0.05 ± 0.019 c | 0.42 ± 0.07 c | 0.28 ± 0.038 c |
| *Chrysopogon zizanioides* (L.) Rob. | 0.05 ± 0.001 c | 0.10 ± 0.026 c | 0.23 ± 0.03 c | 3.48 ± 0.308 a |
| *Salix matsudana* Koidz. | 0.05 ± 0.002 bc | 1.31 ± 0.081 a | 1.56 ± 0.16 ab | 0.10 ± 0.010 c |
| *Salix alba* L. | 0.06 ± 0.002 bc | 1.28 ± 0.107 a | 2.41 ± 0.84 a | 0.08 ± 0.003 c |
| *Eucalyptus camaldulensis* Dehnh. | 0.06 ± 0.004 bc | 0.67 ± 0.137 b | 0.79 ± 0.07 bc | 0.49 ± 0.069 bc |

For comparison of means, ANOVA followed by Tukey's test ($p < 0.05$) was performed. Different letters in the columns indicate significantly differences among species

**Table 4.8** Bioconcentration factor (BCF, mean ± SE, $n = 5$) for different metal(loid)s in the stem of three woody species sampled in the study area (landfill island, Venice Lagoon, Italy)

| Plant species | As | Cd | Zn | Pb |
|---|---|---|---|---|
| *Salix matsudana* Koidz. | 0.09 ± 0.004 a | 0.39 ± 0.017 a | 0.27 ± 0.019 a | 0.70 ± 0.11 a |
| *Salix alba* L. | 0.10 ± 0.004 a | 0.38 ± 0.024 a | 0.29 ± 0.022 a | 0.66 ± 0.05 a |
| *Eucalyptus camaldulensis* Dehnh. | 0.11 ± 0.008 a | 0.10 ± 0.013 b | 0.32 ± 0.034 a | 0.77 ± 0.07 a |

For comparison of means, ANOVA followed by Tukey's test ($p < 0.05$) was performed. Different letters in the columns indicate significantly differences among species

Zacchini et al. [25], the BCF is defined as the ratio between total metal concentration in plant organs and soil. Mean soil metal(loid) concentrations were used. The BCF of As was rather low (0.05–0.1) in all investigated plants, except for *B. perfoliata* where a BCF three times higher than the mean value calculated for all the species was observed. The leaf BCF for Cd and Zn was highly variable among species, ranging from 0.01 to 1.31 for Cd and from 0.12 to 2.41 for Zn. Interestingly, the highest BCF values for both metals were found in the *Salix* species (*S. matsudana* 1.31 for Cd and 1.56 for Zn; *S. alba* 1.28 for Cd and 2.41 for Zn). A notable BCF value (1.88) for Zn was also observed in *A. retroflexus*. The BCF values for Pb ranged between 0.08 and 3.48, with the value of *C. zizanioides* more than sevenfold higher than the mean value calculated for all species.

Stem BCF values (Table 4.8) in the three established woody species were similar for the different metals analyzed, except for Cd where a lower value in *E. camaldulensis* compared with the two *Salix* species was observed.

## 4.4 Discussion

The risk for the ecosystem and human health posed by the concentration of pollutants in the industrial landfills is currently recognized as a serious concern. Worldwide, the reduction of the potential hazard associated to the presence of contaminated sites is perceived as a remarkable environmental challenge. In the last years, the request to face this issue by bio-based technologies able to mitigate and possibly rehabilitate the polluted environments without producing further damages to the ecosystems has been increasing. In this context, the term "phytomanagement" has been utilized to indicate a novel approach based on the exploitation of the different ecosystem services provided by the plant communities [9]. Among them, numerous studies have highlighted the role of plants in metal-polluted areas in promoting the environmental stabilization, pollution control, and mitigation. In fact, it has been reported that a vegetation cover can limit the dispersion of metal-containing soil particles exerted by water and wind erosion and reduce the water transfer along the soil profile toward the groundwater table [26, 27]. Moreover, the presence of a plant community is fundamental to improve the biological activity of the rhizosphere by supporting the growth of microbes and fungi, to enrich soil of organic matter, and to positively affect the biogeochemical cycles [28, 29]. In order to have a successful exploitation of the ecosystem services provided by plants, it is well recognized that a crucial step is the choice of selected plant species that can efficiently colonize and grow in metal-contaminated soils [30, 31]. In this context, natural or assisted selection can represent a valuable tool for identifying metal-accumulating or metal-excluding plants, with the aim to characterize and improve the physiological and biochemical traits useful for a more efficient phytomanagement of degraded industrial sites.

Accordingly, this work was focused on assessing the ability of plants, spontaneous or established, to accumulate arsenic and heavy metals and to give useful indications on their potential use for the phytomanagement of the contaminated site. Previous studies characterizing the contamination of the sediments and waters in the Venice Lagoon have highlighted the role played by the Porto Marghera industrial area as the main source of heavy metals [32, 33]. In the present work, a considerable patched contamination of soil and groundwater by As, Cd, Zn, and, to a lesser extent, Pb was detected in the experimental area (Tables 4.4 and 4.5). These results are consistent with a previous study in this area that reported that As, Zn, and Cd were the main soil and water contaminants with concentrations higher than the Italian law limits, especially in the 1–2 m soil depth layer (https://www.regione.veneto.it/web/ambiente-e-territorio/database-indagini-ambientali-georeferenziate). Patched contamination detected in the area could be ascribed to the different composition of the industrial waste that gave rise to the island over time. Moreover, the different degree of contaminant leaching due to the groundwater table movement and the origin of soil contributed to soil patched contamination.

Several studies have focused on the factors affecting the movement of trace elements along the soil profile and cause groundwater pollution. It is underlined that metal mobility depends on the geochemical properties of soil as well as other environmental factors [34]. Garcia et al. [35] showed that the mobility of As, Cd, Zn, and Pb in a sandy-loam textured soil, similar to that observed on the study area, followed the order Pb < As < Zn and Cd. Data reported in Table 4.4 show a homogeneous Cd distribution in all soil layers because of the high mobility of Cd. On the contrary, lesser mobile elements such as As and Pb accumulated preferentially in the deeper layers sampled. Zinc distribution along the soil profile could be the result of the particular soil composition, i.e., industrial waste, which could have reduced the mobility of this metal despite the movement of seasonal groundwater to the top layer.

The piezometric investigation allowed to detect groundwater contamination by heavy metals in the study area (Table 4.5), highlighting a particular environmental hazard, especially on an island. In fact, the seawater intrusion, a common phenomenon on the island and in coastal waters, represents a serious risk factor for pollutant diffusion to marine ecosystems [36]. Scattered data regarding metal presence in the groundwater samples might be the result of different factors such as the patched distribution of soil contaminants, the leaching processes exerted by rainfall, and the degree of seawater intrusion.

Analysis of metal(loid) concentrations in leaves (Table 4.6) revealed that, as commonly found [30, 37], plants growing on polluted soils showed different adaptive responses depending on the metal element. In fact, plant species found in the survey exhibited different capabilities to exclude or concentrate metals into their organs. As a general trend, spontaneous species showed lower heavy metal concentration ability than established species, except for *B. perfoliata* and *A. retroflexus* for As and Zn, respectively. A notable variability of metal concentrations among plant species occurred, varying from 0.11 to almost 11 mg kg$^{-1}$ for Cd and 52.5 to 1079.3 mg kg$^{-1}$ for Zn. These data are in accordance with those previously reported by Del Rio et al. [38] but are higher than those found by Burgos et al. [39] and Brunetti et al. [40] in the vegetation growing on metal-contaminated sites. The range of As concentration detected on leaves of spontaneous and established plant species varied from 0.9 to 4.67 mg kg$^{-1}$, in agreement with that found by Burgos et al. [39] in spontaneous grasses growing in mine spill-contaminated soil and by Del Rio et al. [38] in the analyses of 99 spontaneous plant species on a polluted site in Mexico. Lead concentration in the analyzed plants ranged from 6.06 to 263.2 mg kg $^{-1}$, consistent with the values reported by Del Rio et al. [38] and Migeon et al. [41]. The high value detected for Pb concentration in *C. zizanioides* confirmed the capability of this plant to extract this metal in different growth conditions such as hydroponics [42], pot [43], and field [44].

Among established tree species, heavy metal concentration in leaves and stems differed markedly (Fig. 4.1). As a common trait, Cd and Zn concentrations in leaves were higher than in stems, whereas As and Pb showed the opposite behavior, as previously reported by Unterbrunner et al. [45] and Tlustos et al. [46] in different woody species. This feature is in accordance with the mobility of metals along plant axis [47, 48] that has been reported as following the order Zn > Cd > Pb and As. Cadmium and Zn concentrations detected in willow leaves are similar to those found by French et al.

[49] for plants growing on industrial waste and higher than those reported by Vamerali et al. [13] in pyrite mine waste near Venice. The high ability of willow to accumulate metals, especially Cd and Zn, in the aboveground parts has been reported by several authors [25, 50–52].

The preferential accumulation of As and Pb in woody plant tissues instead of foliage has to be considered an interesting trait to be exploited for the phytomanagement of metal-contaminated sites. In fact, as discussed by Zacchini et al. [25] and Pietrini et al. [53], the storage of metals in nonrenewable parts of plants (i.e., stems and branches) represents a positive ecological service exerted by the plant, allowing for a more effective removal of metals from soils without recycling in the topsoil by leaf shedding. Moreover, woody biomass obtained by tree plantations can also be used for energy production [12, 54] by appropriated pyrolysis and burning processes, with metal remaining in the flying ashes and specifically filtered to avoid the release into the atmosphere. These aspects should be carefully considered when approaching the decision process involving the plant species to be selected for the phytomanagement of metal-contaminated sites. As a general consideration about the mobility of metal(loid)s in soil and along the plant axis, it should be taken into account that the particular characteristics of the soil, among which its pH, could have affected the metal bioavailability and therefore the metal(loid) accumulation in plants. Anyway, to further characterize the capability of plants growing on the experimental site to accumulate metal(loid)s, the BCF calculation for leaves and stems was performed (Tables 4.7 and 4.8). In all plants analyzed, the BCF value for As was below 1, a recognized threshold for assessing the ability of a plant to accumulate metals [55]. However, the BCF values obtained in this investigation were ten times higher compared with some herbaceous species growing on mine tailing [56], even if these values are notably lower than those of hyperaccumulating ferns [57]. In fast-growing tree plants, stem tissues exhibited a higher BCF value than leaves because of the low mobility of this metal inside plants.

*S. alba* and *S. matsudana* leaves showed BCF values for Cd and Zn higher than 1, confirming the good ability of these *Salicaceae* plants to accumulate these metals. In fact, many authors pointed out the great capability of willows to accumulate Cd and Zn in their organs under different pollution and growing conditions [25, 50, 58]. On the contrary, *Eucalyptus* confirmed the low capacity for metal bioconcentration in the leaf apparatus, as reported by Pietrini et al. [59]. *A. retroflexus*, mostly known for the great ability to accumulate iron [60], according to the results of this survey can be considered also as a valuable Zn accumulator. A remarkable capacity of *Amaranthus* plants to phytoextract and accumulate metals was also put in evidence by Iori et al. [61].

All BCF values for Pb in the analyzed plants were <1 except for *C. zizanioides*, which showed a notable capability to bioaccumulate this metal in the aerial parts. This plant has been investigated for its special affinity and tolerance to toxic heavy metals (especially Zn and Pb) and metalloids and its capability to tolerate several environmentally critical conditions such as temperature, salinity, alkalinity, floods, and the presence of xenobiotics [62]. In this work, *C. zizanioides* exhibited a BCF value several times lower than that reported by Boonyapookana et al. [63] in hydroponics, probably due to the peculiarity of this growing system in exploiting the maximum potential for the plant species to concentrate metals in their organs.

**Table 4.9** Results of principal component analysis (PCA) for the BCF values of metal(loid)s in the leaf tissues of the plant species[a]

| Original variables | Factor 1 | Factor 2 |
|---|---|---|
| As | | 0.733 |
| Cd | −0.914 | |
| Zn | −0.914 | |
| Pb | | −0.686 |
| Eigenvalues | 1.994 | 1.016 |
| Proportion of total variance | 49.85 | 25.41 |
| Cumulative | 49.85 | 75.26 |

[a] Only loadings higher than 60% of the maximum absolute value in each factor are presented

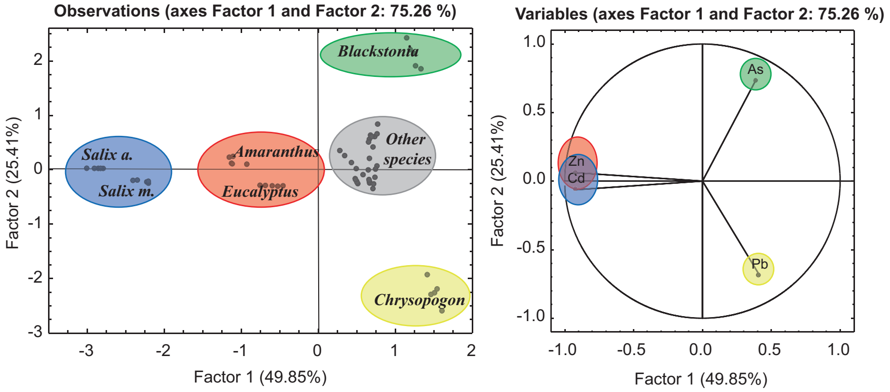


**Fig. 4.2** Scatter plot for species and vectors distributed on the coordinate system of the first two factors resulting from principal component analysis

In order to study the relationship between plant species and metal accumulation in the experimental site and to highlight the different behaviors in metal(loid) leaf bioconcentration, a PCA focused on BCF data calculated for each plant species and metals was conducted (Table 4.9 and Fig. 4.2). PCA produced two significant components that, together, explain 75.26% of the total variance in the data set. The first PCA axis extracted 49.85% of variance for the leaf BCF values of metal(loid)s, with Cd and Zn having the highest contribution to the first axis. The second PCA axis had lower significance (25.41% of variance) and was mostly determined by the contributions of As and Pb. The eigenvalues, variance, cumulative variance, analysis result, eigenvectors, and factor loading values of the three principal components are shown in Table 4.9. As shown in Fig. 4.2a, three plant species were clearly separated in the coordinate systems. *Salix* sp. (*S. alba* and *S. matsudana*), *C. zizanioides*, and *B. perfoliata* were located in three different poles in the scatter plot. Moreover, *A. retroflexus* and *E. camaldulensis* could be considered another group, while the other species showed a distribution close to the origin of the coordinate system but were not separated from each other. The *Salix* sp. was separated under the effect of the first principal component, while *C. zizanioides* and *B. perfoliata* were clearly separated from the other species under the effect of the second principal component as determined by PCA. According to Fig. 4.2b, *Salix* sp. was mostly affected by Cd and Zn which were members of the first principal component, while *C. zizanioides* and *B. perfoliata* were highly affected by Pb and As, respectively, which were members of the second principal component. These results showed that PCA is a useful tool that can be used to highlight species with different capacities to accumulate metal(loid)s and to evaluate their utilization for phytomanagement purposes.

## 4.5 Conclusion

Multiple beneficial functions exerted by a natural vegetation cover in a contaminated industrial soil can be exploited in the phytomanagement. Among them, the limitation of pollutant leaching, the reduction of metal mobility along soil layers, and the positive effect on the biological processes in the rhizosphere should be highlighted, especially in a polluted site located in an island. All spontaneous species, except for *A. retroflexus* toward Zn, exhibited a higher ability to exclude rather than accumulate heavy metals compared with established species. This feature allows proposing such spontaneous species for phytostabilization purposes, increasing the natural value of the polluted area and avoiding the production of a contaminated biomass. On the contrary, the "in situ" recognition of woody plant species that concentrate notable quantities of metals in their aboveground tissues can represent an adding value in terms of remediation of a contaminated site by plants. Beyond these aspects, some peculiar characteristics of woody plants such as *Salix* sp., i.e., adaptability to harsh soil conditions, high biomass productivity, effective nutrient uptake, and high evapotranspiration, should be considered for a successful phytomanagement of an industrial contaminated site.

**Acknowledgments** This work was funded by Eni S.p.A. (contract no. 4900157051 "Experimental activity on phytostabilisation and phytoextraction of heavy metals"). Authors wish to thank Mr. Giuseppe Santarelli and Mr. Giovanni De Simone for their valuable technical assistance.

## References

1. Efroymson RA, Rebecca A, Joseph P et al (2004) A framework for net environmental benefit analysis for remediation or restoration of contaminated sites. Environ Manag 34:315–331
2. Burges A, Epelde L, Blanco F et al (2017) Ecosystem services and plant physiological status during endophyte-assisted phytoremediation of metal contaminated soil. Sci Total Environ 584:329–338
3. Costanza R, d'Arge R, De Groot R et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260
4. Marmiroli M, Pietrini F, Maestri E et al (2011) Growth, physiological and molecular traits in Salicaceae trees investigated for phytoremediation of heavy metals and organics. Tree Physiol 31:1319–1334

5. Dudka S, Miller WP (1999) Accumulation of potentially toxic elements in plants and their transfer to human food chain. J Environ Sci Health B 34:681–708
6. Greger M, Landberg T (1999) Use of willow in phytoextraction. Int J Phytoremediation 1:115–123
7. Cundy AB, Bardos RP, Puschenreiter M et al (2016) Brownfields to green fields: realising wider benefits from practical contaminant phytomanagement strategies. J Environ Manag 184:67–77
8. Epelde L, Becerril JM, Alkorta I, Garbisu C (2014) Adaptive long-term monitoring of soil health in metal phytostabilization: ecological attributes and ecosystem services based on soil microbial parameters. Int J Phytoremediation 16:971–981
9. Domínguez MT, Maranón T, Murillo JM et al (2008) Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: a large-scale phytomanagement case study. Environ Pollut 152:50–59
10. Evangelou MW, Papazoglou EG, Robinson BH, Schulin R (2015) Phytomanagement: phytoremediation and the production of biomass for economic revenue on contaminated land. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 1. Springer International Publishing, Switzerland, pp 115–132
11. Cundy AB, Bardos RP, Church A et al (2013) Developing principles of sustainability and stakeholder engagement for "gentle" remediation approaches: the European context. J Environ Manag 129:283–291
12. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees – a review. Environ Int 29:529–540
13. Vamerali T, Bandiera M, Coletto L et al (2009) Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa, Italy). Environ Pollut 157:887–894
14. Critto A, Agostini P (2009) Using multiple indices to evaluate scenarios for the remediation of contaminated land: the Porto Marghera (Venice, Italy) contaminated site. Environ Sci Pollut Res 16:649–662
15. Zonta R, Botter M, Cassin D et al (2007) Sediment chemical contamination of a shallow water area close to the industrial zone of Porto Marghera (Venice Lagoon, Italy). Mar Pollut Bull 55:529–542
16. Giusti L, Zhang H (2002) Heavy metals and arsenic in sediments, mussels and marine water from Murano (Venice, Italy). Environ Geochem Health 24:47–65
17. Libralato G, Losso C, Arizzi Novelli A et al (2008) Ecotoxicological evaluation of industrial port of Venice (Italy) sediment samples after a decontamination treatment. Environ Pollut 156:644–650
18. Fontaine TA, Moore TD, Burgoa B (2000) Distributions of contaminant concentration and particle size in fluvial sediment. Water Res 34:3473–3477
19. IGV, Italian Guideline Values, Lgs D. Italian Parliament (2006) Testo Unico Ambientale, Norme in materia ambientale, 152/06
20. Giandon P, Garlato A, Ragazzi F (2010) Valori di fondo di metalli e metalloidi nei suoli del veneto. Ars n. 127
21. Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton, FL
22. Alloway BJ (1990) Heavy metals in soil. Blakie and Son Ltd, London
23. Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534
24. Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216
25. Zacchini M, Pietrini F, Scarascia-Mugnozza G et al (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water Air Soil Pollut 197:23–34
26. Foulon J, Zappelini C, Durand A et al (2016) Impact of poplar-based phytomanagement on soil properties and microbial communities in a metal-contaminated site. FEMS Microbiol Ecol 92:fiw163

27. Friesl-Hanl W, Platzer K, Riesing J et al (2017) Non-destructive soil amendment application techniques on heavy metal-contaminated grassland: success and long-term immobilising efficiency. J Environ Manag 186:167–174
28. Bourceret A, Cébron A, Tisserant E et al (2016) The bacterial and fungal diversity of an aged PAH-and heavy metal-contaminated soil is affected by plant cover and edaphic parameters. Microb Ecol 71:711–724
29. Wenzel W (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408
30. Dongmei L, Changqun D (2008) Restoration potential of pioneer plants growing on lead-zinc mine tailing in Lanping, southwest China. J Environ Sci 20:1202–1209
31. Parraga-Aguado I, Querejeta JI, González-Alcaraz MN et al (2014) Usefulness of pioneer vegetation for the phytomanagement of metal(loid)s enriched tailings: grasses vs. shrubs vs. trees. J Environ Manag 133:51–58
32. Bernardello M, Secco T, Pellizzato F, Chinellato M, Sfriso A, Pavoni B (2006) The changing state of contamination in the Lagoon of Venice. Part 2: Heavy metals. Chemosphere 64:1334–1345
33. Mattiuzzo E, Bavero L, Zennaro F, Franco D (2007) Heavy metal behaviour in an experimental free water surface wetland in the Venice Lagoon watershed. Water Air Soil Pollut 183:143–151
34. Zhao X, Dong D, Hua X, Dong S (2009) Investigation of the transport and fate of Pb, Cd, Cr(VI) and As (V) in soil zones derived from moderately contaminated farmland in Northeast, China. J Hazard Mater 170:570–577
35. Garcia I, Diez M, Martin F, Simon M, Dorronsoro C (2009) Mobility of arsenic and heavy metals in a sandy-loam textured and carbonated soil. Pedosphere 19:166–175
36. Förstner U, Wittmann GT (2012) Metal pollution in the aquatic environment. Springer Science & Business Media, New York
37. Viehweger K (2014) How plants cope with heavy metals. Bot Stud 55:35
38. Del Río M, Font R, Almela C et al (2002) Heavy metals and arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the Aznalcóllar mine. J Biotechnol 98:125–137
39. Burgos P, Pérez-de-Mora A, Medejón P et al (2008) Trace elements in wild grasses: a phytoavailability study on remediated field. Environ Geochem Health 30:109–114
40. Brunetti G, Soler-Rovira P, Farrag K, Senesi N (2009) Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region, Southern Italy. Plant Soil 318:285–298
41. Migeon A, Richaud P, Guinet F et al (2009) Metal accumulation by woody species on contaminated sites in the north of France. Water Air Soil Pollut 204:89–101
42. Andra SS, Datta R, Sarkar D et al (2009) Analysis of phytochelatin complexes in the lead tolerant vetiver grass (Vetiveria zizanioides (L)) using liquid chromatography and mass spectrometry. Environ Pollut 157:2173–2183
43. Wilde EW, Brigmon RL, Dunn DL et al (2005) Phytoextraction of lead from firing range soil by Vetiver grass. Chemosphere 61:1451–1457
44. Yang B, Shu WS, Ye ZH et al (2003) Growth and metal accumulation in Vetiver and two Sesbania species on lead/zinc mine tailing. Chemosphere 52:1593–1600
45. Unterbrunner R, Pushenreiter M, Sommer P et al (2007) Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut 148:107–114
46. Tlustos P, Szakova J, Vyslouzilova M et al (2007) Variation in the uptake of arsenic, cadmium, lead and zinc by different species of willows Salix spp. grown in contaminated soils. Centr Eur J Biol 2:254–275
47. Boruvka L, Kozak J, Kristoufkova S (1997) Heavy metal accumulation in plants grown in heavily polluted soils. Folia Microbiol 42:524–526
48. Ko BG, Volgeler I, Bolan NS et al (2007) Mobility of copper, chromium and arsenic from treated timber into grapevines. Sci Total Environ 388:35–42
49. French CJ, Dickinson NM, Putwain PD (2006) Woody biomass phytoremediation of contaminated brownfield land. Environ Pollut 141:387–395

50. Iori V, Pietrini F, Massacci A, Zacchini M (2015) Morpho-physiological responses, heavy metal accumulation and phytoremoval ability in four willow clones exposed to cadmium under hydroponics. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L (eds) Phytoremediation: management of environmental contaminants, vol 1. Springer International Publishing, Switzerland, pp 87–98
51. McBride MB, Martinez CE, Kim B (2016) Zn, Cd, S and trace metal bioaccumulation in willow (Salix spp.) cultivars grown hydroponically. Int J Phytoremediation 18:1178–1186
52. Tőzsér D, Magura T, Simon E (2017) Heavy metal uptake by plant parts of willow species: a meta-analysis. J Hazard Mater 336:101–109
53. Pietrini F, Zacchini M, Pietrosanti L et al (2010) Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. Int J Phytoremediation 12:105–120
54. Bert V, Neu S, Zdanevitch I et al (2017) How to manage plant biomass originated from phytotechnologies? Gathering perceptions from end-users. Int J Phytoremediation 19(10):947–954
55. Baker AJ (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654
56. Chang P, Kim JY, Kim KW (2005) Concentrations of arsenic and heavy metals in vegetation at two abandoned mine tailing in South Korea. Environ Geochem Health 27:109–119
57. Ma LQ, Komar KM, Tu C et al (2001) A fern that hyperaccumulates arsenic-a hardy, versatile, fast growing plant helps to remove arsenic contaminated soils. Nature 409:579
58. Lunackova L, Sottnikova A, Masarovicova E et al (2003) Comparison of cadmium effect on willow and poplar in response to different cultivation conditions. Biol Plant 47:403–411
59. Pietrini F, Iori V, Bianconi D et al (2015) Assessment of physiological and biochemical responses, metal tolerance and accumulation in two eucalypt hybrid clones for phytoremediation of cadmium-contaminated waters. J Environ Manag 162:221–231
60. Chehregani A, Noori M, Yazdi HL (2009) Phytoremediation of heavy-metal-polluted soils: screening for new accumulator plants in Angouran mine (Iran) and evaluation of removal ability. Ecotoxicol Environ Saf 72:1349–1353
61. Iori V, Pietrini F, Cheremisina A et al (2013) Growth responses, metal accumulation and phytoremoval capability in Amaranthus plants exposed to nickel under hydroponics. Water Air Soil Pollut 224:1450–1459
62. Srivastava J, Kayastha S, Jamil S, Srivastava V (2008) Environmental perspectives of Vetiver zizanioides (L.) Nash. Acta Physiol Plant 30:413–417
63. Boonyapookana B, Parkpian P, Techapinyawat S et al (2005) Phytoaccumulation of lead by sunflower (Helianthus annuus), tobacco (Nicotiana tabacum), and vetiver (Vetiveria zizanioides). J Environ Sci Health 40:117–137

# Part III

# Phytoremediation Applications for Contaminated Waters

# Chapter 5
# Role of PGPR in the Phytoremediation of Heavy Metals and Crop Growth Under Municipal Wastewater Irrigation

**Naeem Khan and Asghari Bano**

## 5.1 Introduction

### *5.1.1 Phytoremediation*

Phytoremediation is the process of using green plants for the removal of pollutants from the environment or to reduce their harmful effects. This technique can be used to remove organic as well as inorganic contaminants. Endophytic rhizospheric microorganisms cooperate more meticulously with their host plants and may be more capable to mend the phytoremediation [1]. It is a novel, cost-effective, efficient, environment- and eco-friendly, in situ applicable, and solar-driven remediation strategy [2, 3]. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility with inputs of organic matter [4]. Green plants have an enormous ability to uptake pollutants from the environment and accomplish their detoxification by various mechanisms. Phytoremediation has low installation and maintenance costs compared to other remediation options [5]. Regarding cost, phytoremediation can cost as less as 5% of alternative cleanup methods [6].

---

N. Khan
Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan

A. Bano (✉)
Department of Biosciences, University of Wah, Wah Cantt, Pakistan

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_5

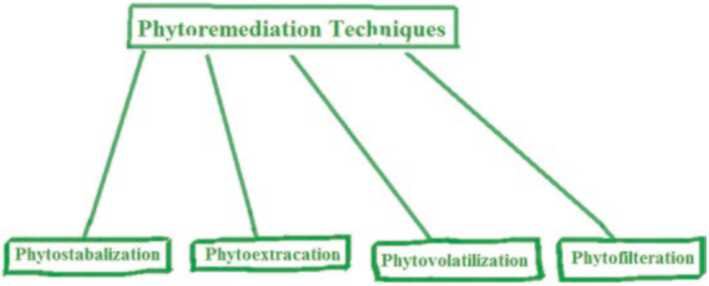


**Fig. 5.1** Phytoremediation techniques for the removal of heavy metals

### *5.1.2 Strategies of Phytoremediation*

Phytoextraction is the most important phytoremediation technique that involves the removal of metals and metalloids from contaminated sites [7]. It is commercially more suitable as compared to other techniques [8]. *Trifolium* spp. are more effective because of their great potential to extract more concentration of a metal [2, 7]. Use of non-hyperaccumulator plants species such as maize and barley requires several cropping seasons to remove heavy metals to acceptable levels through phytoextraction. Although this technique is often used for metals, other elements including As, Se, and organic compounds can also be accumulated [9] (Fig. 5.1).

Phytostabilization refers to the ability of plants to decrease the mobility and bioavailability of a metal either to prevent its leaching to groundwater or its entry into the plants [10]. Additionally, phytostabilization provides structural stability to slopes and loose wastes by plant roots and vegetation cover resulting in the containment of contaminants and therefore limiting their mobility and diffusion in the soil [9]. However, phytostabilization is not a permanent solution as heavy metals remain in the soil and only its movement is restricted.

Phytovolatilization is another approach which converts a heavy metal in volatile form and releases it into the atmosphere through small pores known as stomata [11]. This technique is generally used for the removal of Hg as mercuric ion is transformed into relatively less toxic elemental form.

Phytofiltration is the absorption or concentration of contaminants by roots in hydroponic systems with a continuous effluent flow. Plants with high root surface area and tolerance to contaminants are optimal for this method [9] (Fig. 5.2).

## 5.2 Wastewater

In rural and urban areas of most countries, the application of sewerage and wastewater for irrigation is a regular practice. Small farmers often prefer wastewater where other water sources are also available because wastewater has high nutrient content

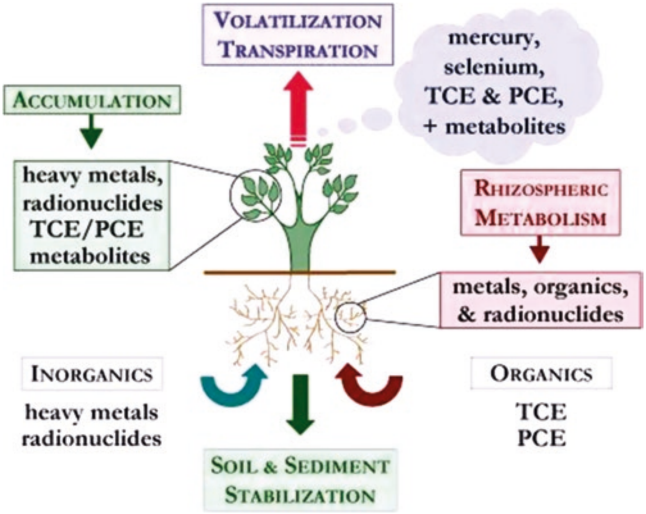


**Fig. 5.2** Phytoremediation accomplished by complex interactions between plants, PGPR, and soil (SREL research: http://archive-srel.uga.edu)

which may reduce or even eliminate the need for other costly chemical fertilizers [12]. Wastewater is extensively used as an inexpensive substitute to conservative irrigation water: supporting livelihoods and generating significant value to the agriculture of urban and peri-urban in spite of the associated health and environmental risks [13]. Farmers often have no alternative, so they depend on unprocessed wastewater. The uses of wastewater in agriculture create key risks to the health of community due to chemical and microbial contaminants. Wastewater use can also produce ecological risks causing soil and groundwater contamination. Irrigation with wastewater can have a number of benefits if managed and implemented properly [14].

### *5.2.1 Types of Wastewater*

There are three different types of wastewater based on source of origination. These include:

*Gray water*: This type of wastewater is generated from bathroom sinks and tubs, laundry machines, etc. Gray water does not contain any urine; therefore, it is very suitable for the purpose of reuse [15].

*Black water*: Black wastewater is usually originated from dishwashers, food sinks, and toilets. Black water contains urine, wipes, toilet paper, and different types of body washes. It has a number of different chemicals and is very risky for health [16].

*Yellow water*: The main component of yellow water is urine collected from different channels. It is yellow in color due to urine [17].

### *5.2.2 Components of Wastewater*

Wastewater consists of a number of different components; these include:

*Suspended solids*: These are solid particles of various sizes suspended in wastewater. These include grits, silt, clay, or other soil particles. These suspended particles are responsible for anaerobic condition if they are disposed untreated [18].

*Pathogens*: Pathogens are organisms that produce diseases. Pathogens are the major component of wastewater that cause disease in farmers. Wastewater contains virus, bacteria, protozoa, and helminths. The number of pathogenic organism in wastewater depends on its origin [19].

*Degradable organic compounds*: These include carbohydrates, fats, and proteins that are present in wastewater. They are measured in terms of BOD and COD. Large quantities of BOD in an environment result in competition between bacteria present in wastewater and other aquatic organisms and deprive them of the oxygen vital for sustaining life. Therefore, wastewater should be treated to reduce BOD before it is released in an environment [18].

*Nutrients*: These are components of food that support life; however, when nutrients are present in higher quantities in wastewater, it supports growth of undesirable organism in an environment. The dominant nutrients of wastewater are nitrogen and phosphorus that are essential for aquatic organisms [19].

*Heavy metals*: Wastewater contains a large number of heavy metals; these include Ni, Cr, Cu, Cd, As, Ag, Hg, and Pb. Heavy metals present in wastewater may enter into the food chain very easily after absorption by living organisms. When they enter into the food chain, they may accumulate in large quantities in the bodies of human beings that may cause serious health risks. Therefore, it is important to treat the wastewater contaminated with heavy metals before its discharge into the environment [20].

*Pollutants*: Wastewater contains a number of different pollutants that may be carcinogenic and cause acute toxicity [21].

### *5.2.3 Reuse of Wastewater for Agriculture*

Although wastewater is a good source of organic compounds and plant nutrients, it also contains unacceptable amounts of soluble salts and heavy metals (Pb, Cd, Cr, Ni, Cu, Fe, and Zn) which enter the soil through wastewater irrigation [22]. These elements have long shelf life and persist long in the soil environment. Soil microbial biomass plays a key role in nutrient cycling.

With the advancement of biotechnology wastewater treatment processes, for example, trickle filter, activated sludge system, oxidation ponds, and aerobic and anaerobic ponds need not as much of land and reliable methods for the treatment of wastewater [23, 24]. Wastewater has a lot of applications, including crop irrigation, aquaculture, irrigation of landscape, industry use, entertainment activity use, various ecological uses, and fake groundwater recharge [25]. It is estimated that total area irrigated with wastewater is about 20 million hectares throughout the world [26]. It has been found that the most common crops of the world mostly lettuce, tomatoes, mangoes, and coconut are watered with sewage water and a large amount of this water is untreated, garrulous through drainpipes into fields of the developing world's vast megacities [27].

Appropriately managed, irrigation of crops with wastewater contributes considerably to supporting livelihoods, food safety, and the value of the surroundings, whereas improperly managed wastewater irrigation may lead to major health risks leading to an expensive load of diarrhea and parasitic diseases and of ecological deprivation (Table 5.1). Wastewater use in agriculture may lead to many excreta-related diseases especially in persons working in the fields irrigated with wastewater and those consuming food irrigated with wastewater, mainly while eaten in raw condition [28]. In low-income countries, the use of wastewater for irrigation cannot be avoided [29].

Sewage and industrial wastewater are commonly used for irrigating agricultural fields in developing countries including India [12]. Continuous use of wastewater for irrigation leads to the enrichment of soil with essential macro- and micronutrients [30]. Micronutrients are beneficial for the growth and metabolism of the plants at lower concentrations, but their higher concentration is toxic. Several micronutrients are heavy metals, and they are known to produce undesirable effects on plants at higher concentrations [11]. An attempt has also been made in the direction of wastewater repossession and its use for irrigation in countries having low water resources of the Mediterranean Basin and the Middle East, for instance, Bahrain, Cyprus, Kuwait, Malta, Israel, Qatar, Oman, Saudi Arabia, and the United Arab Emirates [26].

Untreated wastewater is either used directly or indirectly from polluted streams and rivers because freshwater sources are either out of stock or too luxurious and urban farmers often have no substitute so they used extremely contaminated water. Most of them are urban poor farmers that depend on the income generated from agriculture activities and services rendered for food safety measures [31]. In arid and semiarid regions, use of wastewater in agriculture could be an important consideration for its disposal. Safary and Hajrasoliha [32] showed that 7 years of irrigation with sewage effluent has decreased soil salinity and sodicity and has significantly

**Table 5.1** Increased agricultural productivity (ton/ha/year) made possible through irrigation using domestic wastewater

| Irrigation using | Wheat 8 years[a] | Beans 5 years[a] | Rice 7 years[a] | Potato 4 years[a] | Cotton 3 years[a] |
|---|---|---|---|---|---|
| Raw sewage | 3.34 | 0.9 | 2.97 | 23.11 | 2.56 |
| Primary effluent | 3.45 | 0.87 | 2.94 | 20.78 | 2.3 |
| Effluent from stabilization ponds | 3.45 | 0.78 | 2.98 | 22.31 | 2.41 |
| Water + NPK | 2.7 | 0.72 | 2.03 | 17.16 | 1.7 |

Hespanhol, 2008 (A new paradigm for water resource management)

[a] Number of years used to calculate average productivity

increased organic carbon and nitrogen, available phosphorous, total nitrogen, total phosphorus, and cation-exchange capacity of the topsoil.

## 5.3 Plant Growth-Promoting Rhizobacteria (PGPR)

Bacteria with the intention to inhabit plant roots and promote plant growth are known as PGPR. Soil-plant-microbe interaction has got much importance in recent years. Many types of microorganisms are known to inhabit soil, especially rhizosphere, and play important role in plant development and in remediation of heavy metals. PGPR colonize plant roots, encourage plant growth, and decrease disease or damage due to insects. Presently much research work has done of PGPR, and a large number of them are being commercialized for crops [33]. PGPR improve the nutrient uptake for plants by altering plant hormones level, due to which changes occur in growth and shape of root via increasing root branching, root length, root mass, and the amount of root hairs and thus ultimately enhance the bioremediation of heavy metals [34] (Fig. 5.3).

### *5.3.1 Role of PGPR in Plant Growth and Development*

PGPR can also motivate plant growth ultimately by the eradication of deleterious microorganisms or pathogens present in the rhizosphere [35]. For instance, fluorescent *Pseudomonas* can control root diseases biologically by modulating competitiveness, antibiotic production, siderophores, and hydrogen cyanide production [36]. PGPR can improve the growth and nutrition of plant, pattern of root growth, and plant competitiveness and responses to external pressure. PGPR have also been

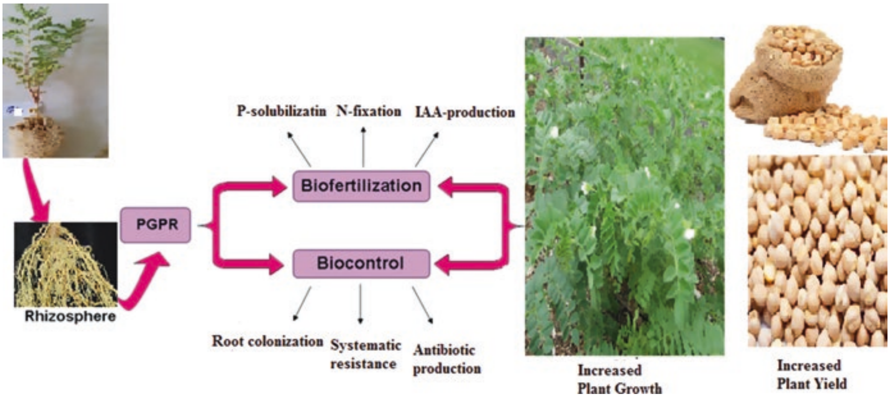


**Fig. 5.3** Plant growth-promoting rhizobacteria (PGPR) and their mechanisms of action

revealed to encourage induced systemic resistance (ISR) to various microorganisms in various crops [37]. PGPR have also the ability to solubilize inorganic and organic phosphates present in soil [35, 38]. The ability of rhizobacteria to promote growth and development of crops may be due to production of phytohormones [39], N fixation [40], and more efficient use of nutrients [39].

### *5.3.2 Role of PGPR in the Remediation of Heavy Metals*

Microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes [41]. The use of rhizobacteria in combination with plants is expected to provide high efficiency for phytoremediation [41, 42]. Therefore, the potential and the exact mechanism of rhizobacteria to enhance phytoremediation of soil heavy metal pollution have recently received some attention [42]. The functioning of associative plant-bacterial symbioses in heavy metal-polluted soil can be affected from the side of both the micropartner (plant-associated bacteria) and the host plant. Thus, bacteria can augment the remediation capacity of plants or reduce the phytotoxicity of the contaminated soil [43]. In addition, plants and bacteria can form specific associations in which the plant provides the bacteria with a specific carbon source that induces the bacteria to reduce the phytotoxicity of the contaminated soil [44]. These biochemical mechanisms increase the remediation activity of bacteria associated with plant roots. Abou-Shanab et al. [41] reported that the addition of *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *Microbacterium arabinogalactanolyticum* to *Alyssum murale* grown in serpentine soil significantly increased the plant uptake of Ni when compared with the uninoculated controls as a result of soil pH reduction. However, heavy metals are known to be toxic to plants and most organisms when present in soils in excessive concentrations.

PGPR improves water uptake, thereby diluting the pollutants particularly Pb that is translocated to shoot thus improving the K:Ca ratio. The benefit of using PGPR is to inhibit the translocation of Ni in shoot and promote the accumulation of Na, K, and Mg. PGPR can be used to reduce the translocation and accumulation of heavy metals—e.g., Ni, Pb, Co, and Mn—alone and in combination [1]. Some micronutrients like zinc, nickel, chromium, and copper are crucial for plant and animal life as well as for microorganisms [45], whereas some metals (e.g., mercury, cadmium, and lead) do not show any clear biotic or physiological role [46]. Thus microbial population either completely inhibit metal ions by inhabiting their different metabolic activities or organisms can develop tolerance mechanisms to higher concentration of metals. Growth-promoting rhizobacteria have developed several mechanisms for their survival under metal-stressed environment. These include mobilizing or transforming metals into inactive form to allow the uptake of heavy metal ions [47]. The activities of the large population of bacteria inhabiting the rhizosphere can also be expected to influence heavy metal uptake by plants. It is reported that under non-sterile soil system, plants showed no iron deficiency symp-

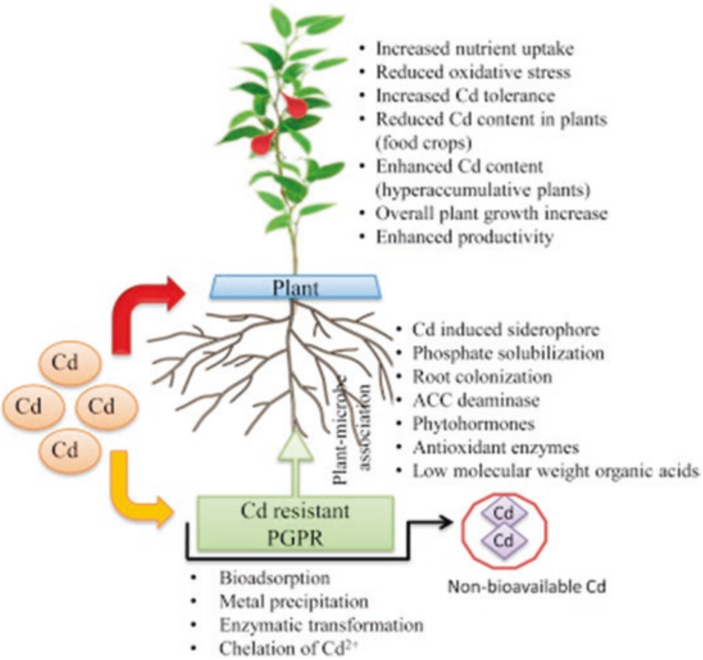


**Fig. 5.4** Plant growth-promoting strategies of PGPR [56]

toms and have fairly high iron level in roots in contrast to plants grown in sterile system. This can attribute to rhizospheric microbial activity, which plays an important role in iron acquisition [48].

PGPR enhance the bioavailability of nutrients and remediation of heavy metals by chelation, acidification, and redox reaction. These PGPR species release various chelating substances that acidify the surrounding environments hence resulting in changes in the redox potential and increasing the remediation of heavy metals [49, 50]. PGPR produce siderophores that enhance the absorption of Fe and reduce the formation of free radicals [50]. They release organic acids and natural chelating agents which reduce the soil pH and sequester soluble ions. Rhizosphere bacteria are also responsible for the production of exopolysaccharides and lipopolysaccharides that are responsible for the removal of metals from contaminated soils through biosorption [51]. Sulfate-reducing bacteria have the capability to change sulfate into hydrogen sulfate which then can react with certain heavy metals (Cd and Zn) and convert them into insoluble form [52]. They have also the ability to detoxify metals through enzymatic and nonenzymatic process. PGPR secreted extracellular enzymes that react with the heavy metals and cleave them, which are then easily assimilated and metabolized [53]. These PGPR species are also very efficient sequester of heavy metals and inorganic pollutant through a process known as biomineralization [54]. Mercury-resistant bacteria have the ability to reduce mercuric ion Hg(II) into vola-

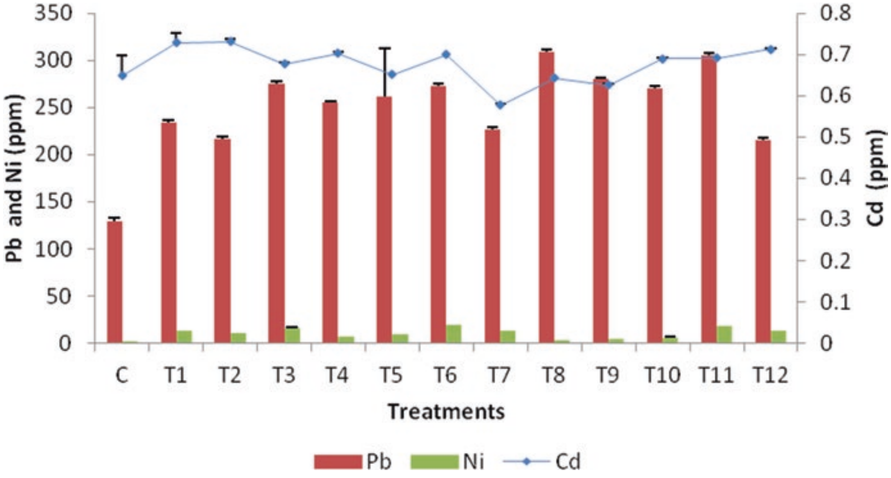


**Fig. 5.5** Effects of silver nanoparticles on phytoremediation potentials and growth of maize plant

tile metallic mercury and Hg(0), which can be easily volatilized from the environment and can be diluted in atmosphere [55] (Fig. 5.4).

Vesicular-arbuscular mycorrhiza fungi also play a key role in the phytoremediation of heavy metals from contaminated sites (Fig. 5.5). It has been reported that these fungi are very effective in the uptake and transport of uranium from soil to roots of associated plants [57]. Liao et al. [49] reported that *Glomus caledonicum* seems to be very promising for the bioremediation of heavy metals. Root colonization with mycorrhizal fungi could also enhance the absorption of zinc [58]. Abu-Elsaoud et al. [59] *carried out experiments with wheat inoculated with F. geosporum* and grown under different concentrations of Zn. They reported that inoculated plants significantly accumulated Zn in their shoot as compared to uninoculated plants.

### 5.3.3 Effects of Silver Nanoparticles on Plant Growth and Remediation of Heavy Metals

Nanoparticles are atomic/molecular aggregates having at least one dimension between 1 and 100 nm [60] that can significantly adjust their physicochemical properties compared to the bulk material [61]. Nanoparticles can be prepared from a full mixture of bulk material and their actions depending on both the chemical composition and on the size and appearance of the particles [62]. Among the newest technological innovations, nanotechnology occupies an important position in renovation of cultivation and food production. The advancement of nanodevices and nanomaterials could unlock new applications in plant biotechnology and cultivation [63]. Currently, the applications of nanotechnology focus in the field of electronics, energy, medication, and biology [64, 65]. Nanoparticles may be produced by physical and chemical methods; however, these methods were too costly. Plant extract and microorganism could be used as cheaper source for the synthesis of nanoparticles [66].

When nanoparticles are applied through foliar applications, they go into stomatal openings or in the course of the bases of trichomes and are then transported to various tissues [67]. However, the accumulation of nanoparticles occurs on the photosynthetic surface which leads to the heating of leaf, causing exchange of gasses due to stomatal obstruction that create changes in various physiological and cellular functions of plants [68]. Nanoparticle accumulation varies among different plants due to reduction potential of ions and the falling capability of plants that depends on the presence of various polyphenols and other heterocyclic compounds present in plants [69]. Application of Ag-nanoparticles had been studied on zucchini plants grown under hydrophonic culture. It had been reported that its application significantly enhance root emergence and growth and do not show any negative impacts except on plant biomass and rate of transpiration [70]. However, the cytotoxic and genotoxic impacts of silver nanoparticles are also studied using root tips of onion and observed the negative impacts of silver nanoparticles have impaired the stages of cell division and caused cell breakdown [71].

Nanoparticles are responsible for physiological and molecular changes in plants. These changes may be positive or negative depending on the type of nanoparticles and plant species [72]. Mehta et al. [73] reported positive effects of silver nanoparticles on nodulation and growth parameters of cowpea in comparison to control plants. They have also been reported that wheat root length and fresh weight were increased in plants treated with silver nanoparticles. Arora et al. [74] reported the beneficial effects of gold nanoparticles on the growth parameters of *Brassica* seedlings. This might be due to their involvement in the production of phytohormone. It has also been reported that nanoparticles are significantly effective in increasing growth and nutrient uptake in radish sprouts and may be helpful in increasing the lignin, pectin, and cellulose contents [75].

The removal of various contaminants from the environment via nanoparticles is known as nanobioremediation. The existing technologies are not very efficient for cleaning up heavy metal contamination. Hence, nanomaterials may be applied effectively for remediation of heavy metals due to its unique physical and chemical properties [76]. Nanotechnology has been proven to be the most effective technology for the treatment of wastewater and increasing the quality of wastewater. Nanotechnology, effectively state many of the water quality issues by means of many diverse types of small size nanoparticles [77]. Nanoparticles played a key role in the development of most effective and efficient water filtration systems. Nanofibers are also important in the treatment of wastewater as they improve the water filtration system due to their small-size pores and high permeability [76, 78]. It has been investigated that silver nanoparticle effects the microbial community in the rhizosphere thus facilitating the remediation of heavy metals [79]. These nanoparticles are also effective for increasing root and shoot growth which ultimately enhance the effectiveness of phytoremediation. Nanoparticles have been successfully being used for the removal of As, Cr, Cd, and Pb from the contaminated sites. Nanotechnology enhances the efficiency of phytoremediation and can also improve the remediation of soil and water contami-

**Table 5.2** Different types of nanomaterials/nanoparticles and their role in the removal of pollutants

| S. No. | Nanomaterials | Pollutant(s) |
|---|---|---|
| 1 | Bimetallic nanoparticles | Chlorinated methane and ethane<br>Polychlorinated biphenyls |
| 2 | Carbonaceous nanomaterials | Heavy metals, herbicides |
| 3 | Nanozeolites | Nitrogen dioxide, toluene |
| 4 | Zerovalent iron nanoparticles | Inorganic ions, organic compounds, inorganic compounds, and heavy metals |
| 5 | $TiO_2$ nanoparticles | Aromatic pollutant, toluene, and phenol |

nated with heavy metals and other organic and inorganic compounds [80]. The application of nanoparticles enhanced the remediation abilities of various plants including *Reseda*, *Scarolia*, *Gundelia*, and *Eleagnum* for Cu, Zn, Ni, and Pb [76]. Jacob et al. [81] reported the accumulation of Ti in wheat and bean plants after treatment with $TiO_2$ nanoparticles. Nanoparticles are also effective for the removal of complex organic compounds which are resistant to microbial and plant degradation; however, biotechnology in combination with nanotechnology could overcome these issues. Iron nanoparticles were found extremely effective for the removal of As, Cd, Hg, and Ni [80]. Khan and Bano [50] found the beneficial effects of silver nanoparticles on the physiology of maize plant and on the remediation of heavy metals when applied alone or in combination with PGPR or sewerage water (Table 5.2).

## 5.4 Conclusion

It is concluded that sewerage water contains different types of microorganism. PGPR strains alone and in combination with sewerage water enhanced the growth and development of plants. Microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes. PGPR improves water uptake, thereby diluting the pollutants particularly Pb that is translocated to shoot thus improving the K:Ca ratio. Nanoparticles could be used effectively for growth promotion and for remediation of heavy metals from contaminated sites.

**Conflict of Interest** The authors declare no conflict of interest.

## References

1. Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254

2. Prasad SM, Singh A (2011) Metabolic responses of Azolla pinnata to cadmium stress: photosynthesis, antioxidative system and phytoremediation. Chem Ecol 27(6):543–555
3. Vithanage M, Dabrowska BB, Mukherjee AB, Sandhi A, Bhattacharya P (2012) Arsenic uptake by plants and possible phytoremediation applications: a brief overview. Environ Chem Lett 10(3):217–224
4. Mench M, Schwitzguébel JP, Schroeder P, Bert V, Gawronski S, Gupta S (2009) Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ Sci Pollut Res 16(7):876
5. Aken BV, Correa PA, Schnoor JL (2009) Phytoremediation of polychlorinated biphenyls: new trends and promises. Environ Sci Technol 44(8):2767–2776
6. Prasad MN (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50(5):686–701
7. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91(7):869–881
8. Ji P, Sun T, Song Y, Ackland ML, Liu Y (2011) Strategies for enhancing the phytoremediation of cadmium-contaminated agricultural soils by Solanum nigrum L. Environ Pollut 159(3):762–768
9. Favas PJ, Pratas J, Varun M, D'Souza R, Paul MS (2014) Accumulation of uranium by aquatic plants in field conditions: prospects for phytoremediation. Sci Total Environ 470:993–1002
10. Erakhrumen AA (2014) Potentials of Rhizophora racemosa for bio-indication and dendroremediation of heavy metal contamination in a mangrove forest, Ondo state, Nigeria. Nig J Agric Food Environ 10(4):1–5
11. Ghosh M, Singh S (2005) A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ 6(4):18
12. Sharma RK, Agrawal M, Marshall F (2007) Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicol Environ Saf 66(2):258–266
13. Pedrero F, Kalavrouziotis I, Alarcón JJ, Koukoulakis P, Asano T (2010) Use of treated municipal wastewater in irrigated agriculture—review of some practices in Spain and Greece. Agric Water Manag 97(9):1233–1241
14. Reiss R, Mackay N, Habig C, Griffin J (2002) An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in the United States. Environ Toxicol Chem 21(11):2483–2492
15. Back DD, Scaringe RP, Ramos C, Samad NA, Gann Sr SD, inventors; Mainstream Engineering Corporation, assignee (1999) Process and system for recycling and reusing gray water. United States patent US 5,868,937, 9 Feb 1999
16. Wu CD, Wei GX (2002) Tea as a functional food for oral health. Nutrition 18(5):443–444
17. Otterpohl R, Braun U, Oldenburg M (2004) Innovative technologies for decentralised water-, wastewater and biowaste management in urban and peri-urban areas. Water Sci Technol 48(11–12):23–32
18. Gupta VK, Kumar R, Nayak A, Saleh TA, Barakat MA (2013) Adsorptive removal of dyes from aqueous solution onto carbon nanotubes: a review. Adv Colloid Interf Sci 193:24–34
19. Gerba CP, Smith JE Jr (2005) Sources of pathogenic microorganisms and their fate during land application of wastes. J Environ Qual 34(1):42
20. Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab J Chem 4(4):361–377
21. Comninellis C (1994) Electrocatalysis in the electrochemical conversion/combustion of organic pollutants for waste water treatment. Electrochim Acta 39(11–12):1857–1862
22. Mapanda F, Mangwayana EN, Nyamangara J, Giller KE (2005) The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agric Ecosyst Environ 107(2):151–165
23. Okoh AI, Odjadjare EE, Igbinosa EO, Osode AN (2007) Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. Afr J Biotechnol 6(25)
24. Elimelech M (2006) The global challenge for adequate and safe water. J Water Supply Res Technol AQUA 55(1):3–10

25. Khan N, Bano A, Rahman MA, Rathinasabapathi B, Babar MA (2018) UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (*Cicer arietinum*) metabolome following long-term drought stress. Plant Cell Environ. https://doi.org/10.1111/pce.13195
26. Jiménez B, Asano T (eds) (2008) Water reuse: an international survey of current practice, issues and needs. IWA, London
27. Pearce P (2004) Trickling filters for upgrading low technology wastewater plants for nitrogen removal. Water Sci Technol 49(11–12):47–52
28. Buechler S, Mekala GD, Keraita B (2006) Wastewater use for urban and peri-urban agriculture. In: van Veenhuizen R (ed) Cities farming for the future: urban agriculture for green and productive cities. RUAF Foundation, The Netherlands, pp 243–273
29. Drechsel P, Keraita B, Amoah P, Abaidoo RC, Raschid-Sally L, Bahri A (2008) Reducing health risks from wastewater use in urban and peri-urban sub-Saharan Africa: applying the 2006 WHO guidelines. Water Sci Technol 57(9):1461–1466
30. Das DC, Kaul RN (1992) Greening wastelands through wastewater. National Wasteland Development Board, Ministry of Environment and Forest, Government of India, New Delhi
31. Smit J, Nasr J, Ratta A (1996) Urban agriculture: food, jobs and sustainable cities, vol 2. The Urban Agriculture Network, Inc., New York, pp 35–37
32. Safary S, Hajrasoliha S (1995) Effects of North Isfahan sewage effluent on the soils of Borkhar region and composition of alfalfa. Paper presented at the 5th Soil Science Congress. Agricultural Vocational School, Karaj, Iran
33. Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Crop Manag 3(1). https://doi.org/10.1094/CM-2004-0301-05-RV
34. Mehboob I, Naveed M, Zahir ZA, Ashraf M (2012) Potential of rhizobia for sustainable production of non-legumes. In: Ashraf M, Öztürk M, Ahmad MSA, Aksoy A (eds) Crop production for agricultural improvement. Springer, Netherlands, pp 659–704
35. Lemanceau P (1992) Beneficial effects of rhizobacteria on plants: example of fluorescent Pseudomonas spp.[plant growth promoting rhizobacteria, PGPR, microbial antagonism, siderophore, bacterial inoculation]. Agronomie. http://agris.fao.org/agris-search/search.do?recordID=FR19930008630
36. Dowling DN, O'Gara F (1994) Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotechnol 12(4):133–141
37. Zhang S, Moyne AL, Reddy MS, Kloepper JW (2002) The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. Biol Control 25(3):288–296
38. Liu WT, Nakamura K, Matsuo T, Mino T (1997) Internal energy-based competition between polyphosphate- and glycogen-accumulating bacteria in biological phosphorus removal reactors—effect of PC feeding ratio. Water Res 31(6):1430–1438
39. Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate-solubilizing Rhizobium leguminosarum biovar. phaseoli. Plant Soil 184(2):311–321
40. Boddey RM, Urquiaga S, Alves BJ, Reis V (2003) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. Plant Soil 252(1):139–149
41. Abou-Shanab RA, Angle JS, Delorme TA, Chaney RL, Van Berkum P, Moawad H et al (2003) Rhizobacterial effects on nickel extraction from soil and uptake by Alyssum murale. New Phytol 158(1):219–224
42. Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by Thlaspi caerulescens. Environ Sci Technol 35(15):3144–3150
43. 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. MSMC541. Int J Phytoremediation 14(3):261–274
44. Rajkumar M, Vara Prasad MN, Freitas H, Ae N (2009) Biotechnological applications of serpentine soil bacteria for phytoremediation of trace metals. Crit Rev Biotechnol 29(2):120–130
45. Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants-a review. Afr J Food Sci 4(5):200–222

46. Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88(11):1707–1719
47. Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51(6):730–750
48. Masalha J, Kosegarten H, Elmaci Ö, Mengel K (2000) The central role of microbial activity for iron acquisition in maize and sunflower. Biol Fertil Soils 30(5):433–439
49. Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50(6):847–853
50. Khan N, Bano A (2016) Modulation of phytoremediation and plant growth by the treatment with PGPR, Ag nanoparticle and untreated municipal wastewater. Int J Phytoremediation 18(12):1258–1269
51. Mishra J, Singh R, Arora NK (2017) Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front Microbiol 8:1706
52. Chibuike G, Obiora S (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Appl Environ Soil Sci. https://doi.org/10.1155/2014/752708
53. Nanda S, Abraham J (2013) Remediation of heavy metal contaminated soil. Afr J Biotechnol 12(21)
54. Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729
55. Khan N, Bano A, Babar MA (2017) The root growth of wheat plants, the water conservation and fertility status of sandy soils influenced by plant growth promoting rhizobacteria. Symbiosis 72(3):195–205
56. Sharma RK, Archana G (2016) Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria. Appl Soil Ecol 107:66–78
57. Rufykiri G, Thiry Y, Wang L, Delvaux B, Declerck S (2002) Uranium uptake and translocation by the arbuscular fungus, Glomus intraradices, under root-organ culture conditions. New Phytol 156(2):275–281
58. Pozo MJ, Cordier C, Dumas-Gaudot E (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to Phytophthora infection in tomato plants. J Exp Bot 53(368):525–534
59. Abu-Elsaoud AM, Nafady NA, Abdel-Azeem AM (2017) Arbuscular mycorrhizal strategy for zinc mycoremediation and diminished translocation to shoots and grains in wheat. PLoS One 12(11):e0188220
60. Ball P (2002) Natural strategies for the molecular engineer. Nanotechnology 13:15–28
61. Nel A, Xia T, Mädler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311:622–627
62. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK et al (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 40(14):4374–4381
63. Scrinis G, Lyons K (2007) The emerging nano-corporate paradigm: nanotechnology and the transformation of nature, food and agri-food systems. Int J Sociol Agric Food 15(2):22–44
64. Hu Y, Xie J, Tong YW, Wang CH (2007) Effect of PEG conformation and particle size on the cellular uptake efficiency of nanoparticles with the HepG2 cells. J Control Release 118(1):7–17
65. Caruthers SD, Wickline SA, Lanza GM (2007) Nanotechnological applications in medicine. CurrOpinBiotechnol 18:26–30
66. Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85(2):162–170
67. Wang WN, Tarafdar JC, Biswas P (2013) Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. J Nanopart Res 15(1):1417
68. Da Silva EC, Da Silva MGA, Meneghetti SMP, Machado G, Alencar MARC, Hickmann JM, Meneghetti MR (2008) Synthesis of colloids based on gold nanoparticles dispersed in castor oil. J Nanopart Res 10(1):201–208
69. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X et al (2007) Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnology 18(10):105104
70. Stampoulis D, Sinha SK, White JC (2009) Assay-dependent phytotoxicity of nanoparticles to plants. Environ Sci Technol 43:9473–9479

71. Kumari M, Mukherjee A, Chandrasekaran N (2009) Genotoxicity of silver nanoparticles in Allium cepa. Sci Total Environ 407:5243–5246
72. Nair R (2016) Effects of nanoparticles on plant growth and development. In: Kole C, Kumar D, Khodakovskaya M (eds) Plant nanotechnology. Springer, Cham
73. Mehta CM, Srivastava R, Arora S, Sharma AK (2016) Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. 3 Biotech 6(2):254
74. Arora S, Sharma P, Kumar S, Nayan R, Khanna PK, Zaidi MGH (2012) Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. Plant Growth Regul 66:303–310. https://doi.org/10.1007/s10725-011-9649-z
75. Zuverza-Mena N, Armendariz R, Peralta-Videa JR, Gardea-Torresdey JL (2016) Effects of silver nanoparticles on radish sprouts: root growth reduction and modifications in the nutritional value. Front Plant Sci 7:90
76. Rizwan M, Singh M, Mitra CK, Morve RK (2014) Ecofriendly application of nanomaterials: nanobioremediation. J Nanoparticles. https://doi.org/10.1155/2014/431787
77. Amin MT, Alazba AA, Manzoor U (2014) A review of removal of pollutants from water/wastewater using different types of nanomaterials. Adv Mater Sci Eng. https://doi.org/10.1155/2014/825910
78. Barhate RS, Ramakrishna S (2007) Nanofibrous filtering media: filtration problems and solutions from tiny materials. J Membr Sci 296(1–2):1–8
79. Fernandes JP, Mucha AP, Francisco T, Gomes CR, Almeida CMR (2017) Silver nanoparticles uptake by salt marsh plants–implications for phytoremediation processes and effects in microbial community dynamics. Mar Pollut Bull 119(1):176–183
80. Yadav KK, Singh JK, Gupta N, Kumar V (2017) A review of nanobioremediation technologies for environmental cleanup: a novel biological approach. J Mater Environ Sci 8:740–757
81. Jacob DL, Borchardt JD, Navaratnam L, Otte ML, Bezbaruah AN (2013) Uptake and translocation of Ti from nanoparticles in crops and wetland plants. Int J Phytoremediation 15(2):142–153

## Chapter 6
# Constructed Wetlands Case Studies for the Treatment of Water Polluted with Fuel and Oil Hydrocarbons

**Alexandros I. Stefanakis**

## 6.1 Introduction

Hydrocarbons are common water contaminants with extensive use, widespread occurrence, and varying chemical and physical properties, that are classified into three main categories: aromatics, aliphatic, and alicyclic. Total petroleum hydrocarbons are derived from petroleum processing, e.g., petrol, diesel, kerosene, and lubricating oils. Lighter hydrocarbons, i.e., with less than 16 carbon atoms, are generally substances with higher solubility and volatility (e.g., benzene). Some substances, such as MTBE and alcohols, are highly soluble, but others (e.g., benzene, toluene, ethylbenzene, and xylenes) have a moderate solubility. Hydrocarbons are the most abundant pollutants in the oil and gas industry. Oil and gas exploration and production generates large amounts of produced water, a liquid by-product that contains high concentration of residual hydrocarbons, among other pollutants [1]. Some hydrocarbon compounds can be toxic; hence, they are usually considered as an environmental hazard [2].

Hydrocarbon contamination is a common environmental problem that affects groundwater and/or surface water quality. The increasing demand for oil and gas, especially, puts more pressure on the natural environment. This environmental issue occurs in industrial areas with extensive industrial facilities, such as chemical and petrochemical industry, oil production and refineries, power generation plants, manufacture industry, plastics and steel production, water cooling plants, etc., where effluents containing hydrocarbons are generated and often released to the

---

A. I. Stefanakis (✉)
Bauer Resources GmbH, Schrobenhausen, Germany

Bauer Nimr LLC, Muscat, Sultanate of Oman

Department of Engineering, German University of Technology in Oman (GUtech),
Halban, Sultanate of Oman
e-mail: alexandros.stefanakis@bauer.de

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_6

environment through accidental spills, improper treatment, and illegal disposal. Hydrocarbons are also contained in urban stormwater runoff, spills from roads, fueling deposits (i.e., tank farms, airports, etc.), and transportation activities, among others [3, 4]. Hydrocarbon contamination can also occur locally near petrol stations. Their extent and migration depends on the interactions with other water pollutants as well.

Naturally occurring microorganisms in water and soils are capable of degrading these substances through various reactions and transformation processes. However, the natural degradation capacity is limited to effectively deal with the high hydrocarbon loads in water resulting from human activities. It is also known that hydrocarbons pose a risk to human health and aquatic ecosystems; hence, hydrocarbon contamination is viewed as major environmental and health hazard that needs to be addressed.

Fuel hydrocarbons, such as BTEX compounds (benzene, toluene, ethylbenzene, and xylenes), MTBE (methyl tert-butyl ether), and phenolic compounds, are commonly found in water [2, 5, 6]. MTBE and BTEX are highly soluble and mobile in water, and both are considered toxic with adverse impacts on humans (e.g., BTEX is a known human carcinogen). Both pollutants are regulated in drinking water (200 μg/L and 5 μg/L, respectively; [7, 8]). Phenols are also present in many waters, e.g., effluents from oil refineries and petrochemical industries, as well as in various wastewaters, e.g., tanneries [9], olive mills [10], and pulp and paper mills [11], while they are also used as pesticides and disinfectants [12]. Due to their toxic effects, phenols are also regulated in water (0.001 μg/L; [13]).

Oil hydrocarbons are also a major concern during oil and gas exploration and processing activities, during which large volumes of produced water are generated. Produced water quality depends on the specific characteristics of the oil fields and wells, the produced hydrocarbon type, and the local geological formations [1]. This water contains high levels of petroleum hydrocarbons and salinity, as well as various organic and inorganic compounds [14, 15]. Hence, it cannot be discharged to the environment without prior treatment, since it can have a negative impact on soil properties, plant productivity, and human health [16]. Typically, most of this wastewater is reinjected into shallow or deep aquifers, which is an energy-consuming practice that also creates several additional environmental concerns [17].

Based on the environmental and human risks associated with hydrocarbon compounds, various technologies have been suggested to remediate water polluted with hydrocarbons, such as membrane separation, thermal treatment, electroagulation, adsorption onto porous media (e.g., activated carbon, zeolites), advanced oxidation processes (e.g., $H_2O_2/O_3$, $H_2O_2$/UV, Fenton process), chemical oxidation, air stripping, and vapor extraction [1, 4, 18–21]. However, most of these techniques require specialized staff; consist of complex mechanical equipment; have high investment, operation, and maintenance costs; require high external energy input; and may be accompanied by operational safety risks; thus, their application is often financially and/or technically infeasible [19, 22]. Therefore, the use of alternative and, specifically, sustainable technologies becomes necessary.

Constructed wetlands (CWs) are a worldwide established natural water treatment technology. CWs achieve high levels of performance with generally reduced construction costs and significantly reduced operation and maintenance costs compared to conventional mechanical technologies [5, 23, 24]. They are known as environmentally friendly treatment systems with multiple environmental, economic, and social advantages. CWs have been widely applied for the treatment of domestic and municipal wastewater [25]. But their good treatment capacity shifted the interest toward their application for various industrial wastewater treatment projects [10, 24, 26–28].

Although this wide topic is currently under research, effluents from the petroleum industry contaminated with various hydrocarbon compounds have been effectively treated with this green technology. In general, there are few pilot and full-scale applications of wetland technology for the remediation of hydrocarbon-contaminated waters. Published literature indicates the use of different constructed wetland designs for the remediation of water contaminated with fuel additives, gasoline production by-products, and other volatile organic compounds [2, 29–32]. Moreover, treatment of water containing oil hydrocarbons (i.e., produced water from oilfields) has also been tested, although available studies in this topic are limited [15, 33–37].

This chapter presents an overview of two case studies with global interest on the research efforts and full-scale application of constructed wetland technology for this type of industrial effluents.

## 6.2 Case Study A: Treatment of Groundwater Contaminated with Fuel Hydrocarbons and Volatile Organic Compounds

### *6.2.1 Location and Background*

The first case study comes from the city of Leuna in NE Germany. The area is characterized by a large industrial mega-site with many chemical companies and refineries siting there for more than 100 years. Due to the great brown coal deposits, the region has been an important economic driver since the industrial revolution. Many of the early chemical industries were first established here, and the area was a preferred location for the chemical industry since the early 1900s. The chemical plant Leuna ("IG Farben" until 1945) was founded during World War I. Many revolutionary chemical inventions and activities took place at the "Leunawerke" industrial complex history, such as ammonia synthesis at an industrial scale (1916), hydrogenation from coal to fuel (since 1927), synthesis of caprolactam for producing nylon (1938), and crude oil manufacturing (1951).

However, during the GDR time, both government and industries showed lack of environmental sensitivity, which resulted in inappropriate hazardous waste management and disposal without any concern for many years. Many plants in Leuna industrial mega-site have been destroyed during World War II (1943–1945). These heavy bombings (approximately 80,000), along with accidental spills and improper handling (leaking underground storage tanks, pipelines, etc.,) over time, caused massive pollution by fuels and fuel additives of several thousand tons [38]. Large-scale processing of ammonia, used as a precursor for nitrogen fertilizers and explosives since the early twentieth century, and petroleum refinement since the 1970s led to massive contamination of groundwater and soil [39]. In fact, all industrial areas in East Germany, including Leuna, suffered severe soil and water contamination; hence, many sites were put out of operation in 1989 and 1990. After the German reunification, brownfield remediation was viewed as a major task. After 1990, the chemical industry site was reconstructed, and today Leuna is one of the largest chemical industry sites in Germany with almost 10,000 employees employed in more than 100 companies. Due to the extensive industrial activities for more than 100 years, groundwater resources in the area are heavily contaminated with high concentrations of various organic chemical compounds [38].

### *6.2.2 Experimental Research Facility and Full-Scale Constructed Wetland*

For the remediation of the site, the "Ecological Major Project Leuna" was established. The first stage of the remediation activities consisted in the construction of a downstream sealing wall (approx. 450 m long) and the flood protection across the chemical factory site in 2005. Since 2001, about 35 measures for groundwater and soil remediation have been realized, and hundreds of thousands tons of contaminated soil have been disposed. In 2003, the cooperation with the Helmholtz-Centre for Environmental Research (UFZ) focused on related remediation research. The core of this collaboration was the development of natural remediation solutions for large-scale contaminated sites. The developed project was based on the technically controlled contaminant transfer from anaerobic to aerobic conditions using constructed wetlands and aerobic trench systems to promote the natural degradation of the main contaminants, i.e., BTEX, MTBE, and ammonium. The experimental facility (Fig. 6.1) was built in 2007 near Leuna refinery in cooperation with the State Agency for Exemption from Contamination Liability in Saxony-Anhalt, the UFZ, and the Central German Asset Management Company as sponsor [40].

The experimental facility focused on the investigation of different natural treatment system designs for the removal of volatile organic compounds. The ultimate goal was to develop an optimum, alternative sustainable biological treatment system to replace the conventional, expensive, and energy-consuming established solutions (pump and treat system along with air stripping and adsorption unit). The treatment efficiency of various pilot-scale constructed wetlands (horizontal and vertical flow) and aerobic trench systems was evaluated for the removal of MTBE and benzene

**Fig. 6.1** The experimental research facility in Leuna, Germany

from the contaminated groundwater. The overall outcome of the project was used to assess the feasibility of applying the preferred design to treat the contaminated groundwater under full-scale conditions.

The experimental facility had central maintenance infrastructure and operated outdoors (Fig. 6.1) next to the industrial area, with the experimental beds exposed to the local climatic conditions. The facility consisted of six pilot-scale horizontal subsurface flow (HSF) CWs, floating plant root mats [2, 41–44], and vertical flow (VF) CWs [45, 46], which were set up during 2007 in order to evaluate the optimized CW design. All examined pilot-scale units had different design and operational characteristics, i.e., planted/unplanted, different plant species, different porous media, and different loading rates. The contaminated groundwater was pumped on-site from the aquifer and applied directly to the different pilot-scale CWs.

More than 70 different organic contaminants were detected in a single groundwater sample in this area, indicating the high levels of contamination. MTBE and benzene were the predominant contaminants (mean concentrations of 3 mg/L and 14 mg/L, respectively). The average concentrations and standard deviations of the main organic and inorganic compounds present in the inflow groundwater during the investigation period are shown in Table 6.1.

The results obtained from the long-term (more than 4 years) operation and monitoring of the various pilot-scale CWs were evaluated to extract the final outcome of the whole project. Results showed that the vertical flow CWs in a two-stage operation provided the optimum effluent quality, i.e., effluent water complying with the target effluent concentrations of 1 μg/L and 200 μg/L for benzene and MTBE, respectively [46, 47]. A module operational plant of 60 $m^3$/day was then constructed consisting of a two-stage vertical flow wetland planted with willows (Fig. 6.2).

The full-scale implementation of the pilot system developed by the UFZ Research Institute is a scaled-up plant constructed by the company Bauer with a flow rate of up to 650 $m^3$/day groundwater, including an infiltration basin of the treated effluent. The system consists of a roughing and a polishing filter and has been presented by Van

**Table 6.1** Statistics of main compounds in the influent groundwater in the experimental facility in Leuna [41, 42, 46]

| Compound | Average concentration (mg/L) ± standard deviation |
|---|---|
| Benzene | 13.9 ± 3 |
| MTBE | 3.0 ± 0.8 |
| $NH_4^+$ | 51 ± 9 |
| $NO_3^-$ | 5.4 ± 2.9 |
| $NO_2^-$ | <0.1 |
| $Cl^-$ | 117 ± 17 |
| $PO_4^{3-}$ | 1.4 ± 0.7 |
| $Fe^{2+}$ | 6.7 ± 2.4 |
| $Ca^{2+}$ | 206 ± 14 |
| $K^+$ | 12.4 ± 0.9 |
| $Na^+$ | 133 ± 8 |
| pH | 7.45 ± 0.4 |
| $O_2$ | 0.1 ± 0.1 |
| TOC | 37 ± 4 |
| COD | 104 ± 9 |
| $BOD_5$ | 56 ± 13 |

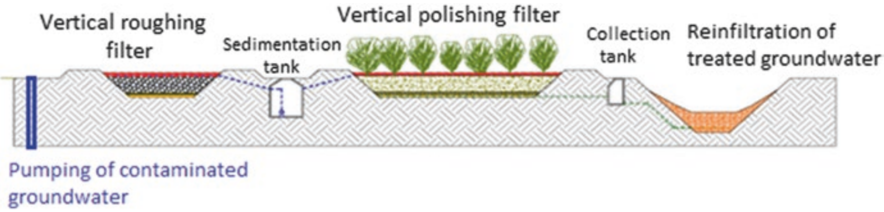


**Fig. 6.2** Typical cross section of the pilot vertical flow constructed wetland treating contaminated groundwater (60 m$^3$/day)

Afferden et al. [46]. The roughing filter, which remained unplanted, included three different media layers (e.g., gravel and expanded clay), with a total depth of 1.55 m, and acts as the first treatment step. Water is distributed across the entire bed surface below the top layer (0.25 m) in order to eliminate the emissions of volatile organic compounds to the atmosphere. The polishing filter had four media layers (including zeolite) with a finer gradation to provide higher attachment surface area for biofilm development and was planted with white willows (*Salix alba*) at an initial density of 5 plants/m$^2$. Willow trees were selected due to their high biomass productivity, high resistance to organic compounds, their ability to adapt to a broad range of climatic conditions, and their common use in wetlands applications [48, 49].

The full-scale plant consists of 17 separate basins of vertical filters combined into 1 central facility and started its operation in 2014 (Fig. 6.3). This groundwater remediation plant replaced an existing technical plant (constructed in 2005) for the treatment of the contaminated groundwater.

**Fig. 6.3** Full-scale vertical flow constructed wetland treating contaminated groundwater in Leuna

The full-scale natural treatment system at the Leuna site achieves similar efficiency with the former high-tech conventional treatment plant. However, this alternative green system has significantly lower operational costs compared to the conventional solution of up to 90%, which is translated to several million euros of resources saving over its operational lifetime. At the same time, the environmental footprint is much lower, since there are no extensive mechanical parts, no chemicals are used for the treatment, and the energy consumption is significantly lower. Hence, this solution is also associated with significantly lower greenhouse gas emissions.

Furthermore, additional research activities in this experimental facility examined the use of wetland systems for the treatment of groundwater contaminated with a variety of hydrocarbons. It is very common that many hydrocarbon compounds (i.e., benzene—BTEX, MTBE, and phenols) are simultaneously present in the contaminated water. MTBE in groundwater is often found along with other gasoline contaminants, usually BTEX [50]. In this case, the water composition becomes more complex, and the appropriate treatment design should be carefully selected, considering the possible interactions between the various contaminants [51]. The fate of MTBE, benzene, and phenolic compounds (phenol and *m*-cresol) simultaneously present in the contaminated groundwater was investigated in the horizontal subsurface flow CW systems of the facility [2]. The two phenolic compounds (influent concentrations of 15 mg/L and 2 mg/L for phenol and *m*-cresol, respectively) were completely removed without any effect on the MTBE and benzene removals (20.2 mg/m$^2$/day and 334.6 mg/m$^2$/day, respectively). This is the first study indicating that CWs can be used for the simultaneous removal of different hydrocarbon compounds. It is also interesting that the planted bed showed higher removal rates, confirming the positive contribution of plants. Further research will investigate the upper efficiency boundaries of constructed wetlands treating a variety of hydrocarbons.

## 6.3 Case Study B: Treatment of Produced Water from Oilfields

### *6.3.1 Location and Background of the Project*

The second case study has to do with one of the largest industrial constructed wetland facilities in the world, designed for the treatment of produced water (i.e., water contaminated with oil hydrocarbons) from an oilfield under desert climatic conditions. This facility is located in the SE corner of the Arabic peninsula in the Middle East, in the southern part of the Sultanate of Oman. The oil production in that area results in large volumes of water with a ratio of oil/water up to 1:10 after the oil extraction process. The previous produced water management practice consisted in the disposal of most of its volume through deep wells into the aquifer. This disposal practice is, however, increasingly viewed as unacceptable and non-environmentally friendly due to both the related high energy consumption and the high recontamination risk of the groundwater resources [15]. Hence, alternative and sustainable treatment and disposal options are highly required in the oil and gas sector. In this frame, the green technology of constructed wetlands possesses all the necessary characteristics to provide a favorable solution. The German company Bauer was awarded in 2008 a design, build, own, operate, and transfer contract to develop this produced water treatment plant. The facility was commissioned in November 2010, and its operation is expected to last for at least 20 years.

### *6.3.2 Facility Description*

The produced water treatment plant is a hybrid system, i.e., it integrates elements of both conventional treatment technologies (gray infrastructure) and natural treatment systems (green infrastructure). Produced water is sent to the intake point of the facility through a pipeline, where upstream separation and recovery of the majority of oil takes place, using passive hydro-cyclone oil separators. The main treatment stage is a constructed wetland system, which receives the effluent from the separators with gravity flow from a long buffer channel. The constructed wetland consists of 36 cells built in a previously arid desert (Fig. 6.4).

**Fig. 6.4** Satellite view of the produced water treatment plant in Oman (photo: Google Earth; image accessed: May 13, 2018)

The treatment capacity of the facility is currently 115,000 m$^3$/day of produced water from the nearby oilfield. The total area of the constructed wetland is 360 ha; the wetland type is a surface flow constructed wetland. In this type, water flows above a soil layer where the plants (reeds) are established and create a dense cluster of reeds. As the water flows through this cluster, it comes in contact with reeds' parts (stems, leaves) and the attached microbial community that degrades the oil hydrocarbons existing in the water [52]. The flow through the wetland cells proceeds with gravity without any use of pumps. Downstream the wetland cells, there is a series of evaporation ponds (EPs) with a total area of 500 ha (Fig. 6.5). These ponds receive and evaporate the treated effluent volume, hence making this facility a zero-discharge system. The evaporation results in residual salt formation that can be processed into industrial grade salt as end product. Another expansion is already ongoing and will be completed in 2019, increasing the total treatment capacity of the facility by 50%, hence reaching a total flow of 175,000 m$^3$/day.

The wetland plant species initially selected for the system was common reeds (*Phragmites australis*). Later, more plant species were introduced, i.e., *Typha domingensis*, *Schoenoplectus littoralis*, *Juncus rigidus*, and *Cyperus* spp., which

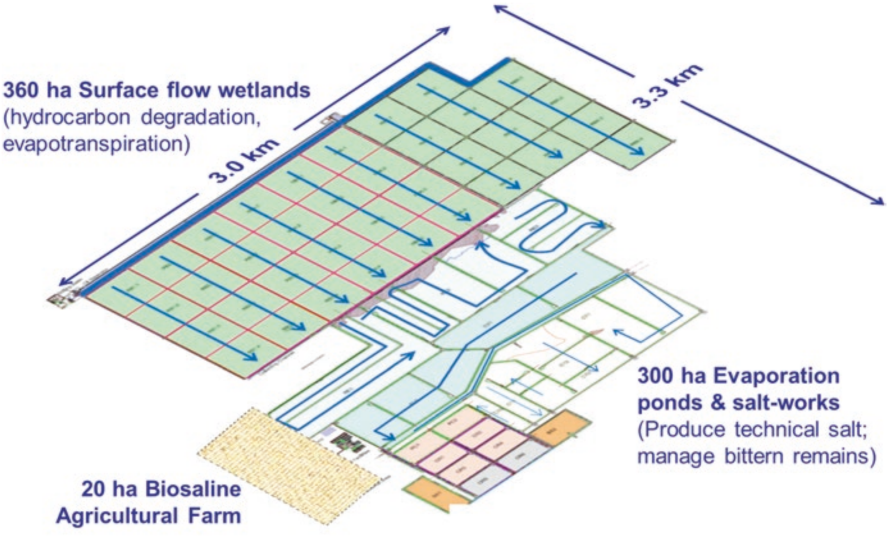


**Fig. 6.5** Schematic overview of the produced water treatment plant in Oman, showing each stage and all current activities in the facility [27]

makes the system a polyculture. However, it was found that after the introduction of more plant species, the plant biomass was enhanced, and the ecosystem became more resilient and healthier [27]. The plant species used are common in SFCWs worldwide and also native in the region. They have been harvested from various locations in the region, for example, in wadis and coastal lagoons, propagated in the established nursery next to the facility, and planted in the SFCW cells. In total, more than two million seedlings have been transplanted (Fig. 6.6).

Moreover, the climate is characterized by typical desert conditions. Highest temperature values are observed in May and June (average daily air temperature of 31.6 °C and 32.8 °C, respectively), while there is practically no precipitation (<2 mm annually) [27].

### 6.3.3 Overall Performance

The quality of the inflow produced water is presented in Table 6.2. The influent produced water has a relatively high salinity (i.e., TDS of approx. 7,000 mg/L; [15, 33, 34]). The main pollutant of interest is the oil in water (OiW), which in some cases can even exceed 500 mg/L. The passive hydro-cyclones and skimmers in the upstream end of the facility remove more than 85% of the hydrocarbons content in produced water. The residual water (after the oil and water separation) has a remaining OiW concentration of 30–40 mg/L and is moving with gravity from the inlet buffer channel to the constructed wetland cells, where it is completely removed. The

**Fig. 6.6** Aerial view of the produced water treatment plant (i.e., constructed wetland cells and evaporation ponds) in the desert of Oman (photo: courtesy of BAUER Resources)

**Table 6.2** Inflow produced water quality at the produced water treatment plant in Oman [15, 27]

| Parameter | Average concentration ± standard deviation |
|---|---|
| Total dissolved solids (mg/L) | 6,810 ± 650 |
| Electricalconductivity (μs/cm) | 13,073 ± 1045 |
| pH | 7.55 ± 0.07 |
| $Cl^-$ (mg/L) | 3,991 ± 493 |
| Suspended solids (mg/L) | 18.9 ± 21.2 |
| Oil in water (mg/L) | 350 ± 150 |
| $BOD_5$ (mg/L) | 15.7 ± 14.7 |
| COD (mg/L) | 122 ± 93 |
| Total nitrogen (mg/L) | 2.46 ± 1.7 |
| $NH_4^+$-N (mg/L) | 1.30 ± 0.9 |
| $NO_2^-$-N (mg/L) | 0.03 ± 0.03 |
| $NO_3^-$-N (mg/L) | 0.08 ± 0.07 |
| Total phosphorus (mg/L) | 0.03 ± 0.03 |
| Boron (mg/L) | 4.5 ± 1.2 |
| $SO_4^{2-}$ (mg/L) | 488 ± 773 |
| $S^{2-}$ (mg/L) | 9.3 ± 15.5 |
| $Ca^{2+}$ (mg/L) | 96.4 ± 31.3 |
| $Mg^{2+}$ (mg/L) | 41 ± 43 |
| $Na^+$ (mg/L) | 2,580 ± 651 |
| $K^+$ (mg/L) | 39.7 ± 10.9 |

effluent water at the wetland outlet has an OiW concentration below the legal discharge limit (<0.5 mg/L). It has also been found that oil hydrocarbons are gradually degraded along the wetland length [15, 27].

Produced water is very poor in nutrients (<3 mg/L for nitrogen and <1 mg/L for phosphorus) and $BOD_5$ (<50 mg/L); thus, the effluent is practically free of $BOD_5$ and nutrients. It is worth mentioning that the COD/BOD ratio of the influent produced water is approx. 8–10, which implies that the majority of the organic matter is likely not readily biodegradable [27]. However, despite this lack of nutrients and the high levels of salinity in the produced water, the wetland system is a healthy ecosystem, and the different plant species survive and propagate quite well in the wetland cells.

The massive wetland plant stands in the wetland cells result in significant evapotranspiration (ET). Approx. 35–40% of the influent water volume is lost through ET, resulting in reduced effluent volume that is discharged to the evaporation ponds. As a result, TDS concentration is increasing along the wetland length and reaches a value of up to 12,000 mg/L in the effluent water [15, 34].

Due to its size and location, this facility is also characterized by an excellent environmental performance. Since its commissioning, five deep well disposal sites in the area have been shut down. This is translated to huge amounts of energy savings. Given also that the wetland is a gravity flow system with practically minimum energy demand, it is estimated that the reduction in energy consumption compared to the previous management practice exceeds 99%. This is also related to the greenhouse gas emissions of the facility and the operation tasks. Considering that the wetland ecosystem acts as a carbon sink as well, these emissions are also significantly reduced; it is estimated that these emissions have been reduced by 99% or more than 1.5 million tons $CO_2$ [34]. It should also be mentioned that this facility alone contributes approx. 4.3% to the country's overall intended nationally determined target to reduce emission by 2%.

The combined system of the SFCW and the EPs is well integrated in the local environment, creating a new ecosystem and habitat for wildlife in the desert. Routine monitoring campaigns and incidental observations indicated that the facility is used by more than 120 migratory bird species as a comfortable stopover as they migrate between Asia and Africa [27].

### *6.3.4 Waste Reuse Activities*

In a continuous effort to make this facility a global reference not just for constructed wetlands technology but also for circular economy practices with minimum waste generation and reuse of waste streams, various activities toward this direction have been undertaken over the last years.

Since 2016, a large-scale agriculture experimental project is running in this facility for the beneficial reuse of the treated effluent [53, 54]. The goal of this project is to investigate the growth of several plant species irrigated with the treated produced

**Fig. 6.7** The agriculture biosaline irrigation research project field of 22 ha at the produced water treatment plant in Oman (photo: courtesy of BAUER Resources)

water under the desert climatic conditions (Fig. 6.7). For this, an irrigation field of 22 ha has been constructed on-site, where different irrigation methods are also under investigation [54]. The species selected are mostly salt tolerant, i.e., they can survive when irrigated with brackish water (up to 12,000 mg/L TDS), and they are adapted to the arid and hot climate of the desert. Another parameter is that the plants under investigation have a commercial value, i.e., they can provide a final product that has a market potential to generate additional revenue (e.g., biofuels, timber or carbon credits, textile, etc.). During the operational period of this project, regular monitoring of the water balance, water quality, soil quality, and plant growth parameters and yield takes place. This research project will provide significant information on the plants able to survive under these environmental conditions (water quality, climate) and also provide a final valuable product, minimizing this way the waste production at the facility and closing the materials cycle. Ultimately, the identified most tolerant plant species with the highest commercial value will be selected for irrigation using the total outflow volume of the constructed wetland system.

Another activity toward the reuse of waste streams in the facility is the implementation of a trial for the production of organic compost. The wetland is dominated by reeds, which continuously produce biomass that is accumulating at the top of the wetland surface. Although this has no effect on the treatment performance, it causes the gradual increase of the water level in the constructed wetland cells. Therefore, an effective solution is required to handle the accumulated biomass and to ensure a sustainable operation of the facility. Since this facility is considered an industrial wastewater treatment plant, the reed biomass is viewed as a contaminated waste material that cannot be transported away from the facility. Hence, on-site compost production seems a reasonable solution for reed biomass management. It also has the advantage that the produced compost can be used as an organic additive to enhance the agricultural production in the adjacent agricultural research project and in the future in the developed agriculture farm irrigated with the treated effluent. The compost can improve the properties of nutrient lacking soils to promote plants growth and health. Currently, the compost trial is ongoing at the facility. The materials used are the reed biomass from the constructed wetland, food waste generated in the kitchen and canteen of the facility accommodation camp, and dried sewage sludge from wastewater treatment plants in nearby accommodation camps of other companies and industrial activities. The successful implementation of this research activity will provide an additional way to recycle waste materials and close the loop of waste generation on-site.

## 6.4 Conclusions

Constructed wetlands technology is already considered today an established treatment option for various wastewaters. New challenges appear in the field of industrial wastewaters. Petrochemical industry and the oil and gas sector are one of the most promising and challenging fields for implementation of such sustainable solutions. Current knowledge and existing examples of large-scale applications presented in this chapter indicate that effective wetland designs can be successfully implemented for the treatment of waters contaminated with a variety of hydrocarbon compounds. Wetland technology can fulfil the need of the industry for sustainable solutions, characterized by a low environmental footprint, low operational costs, minimum energy input, reduced greenhouse gas emissions, and limited maintenance. At the same time, it can bring value to the corporate social responsibility strategy [55], as a tool that both improves the financial position of the industry and contributes to the environmental protection.

## References

1. Jain P, Sharma M, Dureja P, Sarma PM, Lal B (2017) Bioelectrochemical approaches for removal of sulfate, hydrocarbon and salinity from produced water. Chemosphere 166:96–108
2. Stefanakis AI, Seeger E, Dorer C, Sinke A, Thullner M (2016) Performance of pilot-scale horizontal subsurface flow constructed wetlands treating groundwater contaminated with phenols and petroleum derivatives. Ecol Eng 95:514–526
3. Langwaldt JH, Puhakka JA (2000) On-site biological remediation of contaminated groundwater: a review. Environ Pollut 107:187–197
4. Levchuk I, Bhatnagar A, Sillanpää M (2014) Overview of technologies for removal of methyl tert-butyl ether (MTBE) from water. Sci Total Environ 476–477:415–433
5. Stefanakis, A.I., Akratos, C.S,, Tsihrintzis, V.A., 2014. Vertical flow constructed wetlands: eco-engineering systems for wastewater and sludge treatment. 1st ed. Amsterdam, The Netherlands: Elsevier
6. Wu Y, Lerner DN, Banwart SA, Thornton SF, Pickup RW (2006) Persistence of fermentative process to phenolic toxicity in groundwater. J Environ Qual 35:2021–2025

7. USEPA (2009) List of drinking water contaminants and MCLs. https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants
8. USEPA (2015) Integrated Risk Information System (IRIS). http://www.epa.gov/iris
9. Costa CR, Botta CMR, Espindola ELG, Olivi P (2008) Electrochemical treatment of tannery wastewater using DSA® electrodes. J Hazard Mater 153(1–2):616–627
10. Tatoulis T, Stefanakis AI, Frontistis Z, Akratos CS, Tekerlekopoulou AG, Mantzavinos D, Vayenas DV (2016) Treatment of table olive washing water using trickling filters, constructed wetlands and electrooxidation. Environ Sci Pollut Res 24(2):1085–1092. https://doi.org/10.1007/s11356-016-7058-6
11. Abira MA, Van Bruggen JJA, Denny P (2005) Potential of a tropical subsurface constructed wetland to remove phenol from pre-treated pulp and papermill wastewater. Water Sci Technol 51(9):173–176
12. Stottmeister U, Kuschk P, Wiessner A (2010) Full-scale bioremediation and long-term monitoring of a phenolic wastewater disposal lake. Pure Appl Chem 82:161–173
13. WHO (2011) Guidelines for drinking-water quality, 4th edn. WHO, Switzerland
14. Hagström EL, Lyles C, Pattanayek M, De Shields B, Berkman MP (2016) Produced water—emerging challenges, risks, and opportunities. Environ Claims J 28(2):122–139
15. Stefanakis AI, Al-Hadrami A, Prigent S (2017) Treatment of produced water from oilfield in a large Constructed Wetland: 6 years of operation under desert conditions. In: Proceedings, 7th International Symposium for Wetland Pollutant Dynamics and Control (WETPOL), Montana, USA, 21–25 Aug
16. Kim KH, Jahan SA, Kabir E, Brown RJ (2013) A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int 60:71–80
17. Arthur J, Langhus B, Patel C (2005) Technical summary of oil & gas produced water treatment technologies. Tulsa, Oklahoma, pp 1–53
18. Deeb RA, Chu K-H, Shih T, Linder S, Suffet I, Kavanaugh MC (2003) MTBE and other oxygenates: environmental sources, analysis, occurrence, and treatment. Environ Eng Sci 20(5):433–447
19. Stefanakis AI, Thullner M (2016) Fate of phenolic compounds in Constructed Wetlands treating contaminated water. In: Ansari AA et al (eds) Phytoremediation. Springer International Publishing, Switzerland, pp 311–325
20. Fakhru'l-Razi A, Pendashteh A, Abdullah LC, Biak DRA, Madaeni SS, Abidin ZZ (2009) Review of technologies for oil and gas produced water treatment. Journal of Hazardous Materials 170 (2-3):530–551
21. Igunnu ET, Chen GZ3 (2013) Produced water treatment technologies. Int J Low Carbon Tech. 9:157–177
22. Vlasopoulos N, Memon FA, Butler D, Murphy R (2006) Life cycle assessment of wastewater treatment technologies treating petroleum process waters. Sci Total Environ 367(1):58–70
23. Stefanakis AI (2016) Constructed Wetlands: description and benefits of an eco-tech water treatment system. In: McKeown E, Bugyi G (eds) Impact of water pollution on human health and environmental sustainability. Information Science Reference (IGI Global), Hershey, PA, pp 281–303
24. Wu S, Wallace S, Brix H, Kuschk P, Kirui WK, Masi F, Dong R (2015) Treatment of industrial effluents in constructed wetlands: challenges, operational strategies and overall performance. Environ Pollut 201:107–120
25. Stefanakis AI, Tsihrintzis VA (2012) Effects of loading, resting period, temperature, porous media, vegetation and aeration on performance of pilot-scale Vertical Flow Constructed Wetlands. Chem Eng 181–182:416–430
26. Imfeld G, Braeckevelt M, Kuschk P, Richnow HH (2009) Monitoring and assessing processes of organic chemicals removal in constructed wetlands. Chemosphere 74:349–362
27. Stefanakis AI, Prigent S, Breuer R (2018) Integrated produced water management in a desert oilfield using wetland technology and innovative reuse practices. In: Stefanakis AI (ed) Constructed Wetlands for industrial wastewater treatment. Wiley, Chichester

28. Tatoulis T, Akratos CS, Tekerlekopoulou AG, Vayenas DV, Stefanakis AI (2017) A novel horizontal subsurface flow constructed wetland: reducing area requirements and clogging risk. Chemosphere 186:257–268
29. Ballesteros F Jr, Vuong TH, Secondes MF, Tuan PD (2016) Removal efficiencies of constructed wetland and efficacy of plant on treating benzene. Sustain Environ Res 26:93–96
30. Eke PE, Scholz M (2008) Benzene removal with vertical-flow constructed treatment wetlands. J Chem Technol Biotechnol 83:55–63
31. Ranieri E, Gorgoglione A, Montanaro C, Iacovelli A, Gikas P (2014) Removal capacity of BTEX and metals of constructed wetlands under the influence of hydraulic conductivity. Desalin Water Treat. https://doi.org/10.1080/19443994.2014.951963
32. Schultze-Nobre L, Wiessner A, Bartsch C, Paschke H, Stefanakis AI, Aylward LA, Kuschk P (2017) Removal of dimethylphenols and ammonium in laboratory-scale horizontal subsurface flow constructed wetlands. Eng Life Sci 17(12):1224–1233
33. Breuer R, Grissemann E (2011) Produced water treatment using wetlands - reducing the environmental impact of oilfield operations. Soc Petrol Eng J. https://doi.org/10.2118/140124-MS
34. Breuer R, Headley TR, Thaker YI (2012) The first year's operation of the Nimr Water Treatment Plant in Oman-Sustainable produced water management using wetlands. Soc Petrol Eng. https://doi.org/10.2118/156427-MS
35. Ji GD, Sun TH, Ni JR (2007) Surface flow constructed wetland for heavy oil-produced water treatment. Bioresour Technol 98:436–441
36. Knight RL, Robert H, Kadlec H, Ohlendorf M (1999) The use of treatment wetlands for petroleum industry effluents. Environ Sci Technol 33(7):973–980
37. Wallace S, Schmidt M, Larson E (2011) Long term hydrocarbon removal using treatment wetlands. In: SPE Annual Technical Conference and Exhibition, Denver, Colorado, USA, 30 Oct–2 Nov
38. Martienssen M, Fabritius H, Kukla S, Balcke GU, Hasselwander E, Schirmer M (2006) Determination of naturally occurring MTBE biodegradation by analyzing metabolites and biodegradation by-products. J Contam Hydrol 87:37–53
39. UBA (2003) Inventarisierung von Grundwasserschäden und deren Beurteilung in Großprojekten "Ökologische Altlasten" der neuen Bundesländer. Umweltbundesamt. Bearbeiter GICON Großmann Ingenieur Consult GmbH, Auftraggeber
40. Rügner H, Henzler R, Bittens M, Weiß H, Leven C, Bayer P, Finkel M (2007) SAFIRA II – Revitalisierungskonzepte für großskalige Boden- und Grundwasserverunreinigungen. Altlasten Spektrum 1:7–12
41. Chen Z, Kuschk P, Reiche N, Borsdorf H, Kaestner M, Koeser H (2012) Comparative evaluation of pilot scale horizontal subsurface-flow constructed wetlands and plant root mats for treating groundwater contaminated with benzene and MTBE. J Hazard Mater 209–210:510–515
42. Seeger E, Kuschk P, Fazekas H, Grathwohl P, Kaestner M (2011a) Bioremediation of benzene-, MTBE- and ammonia-contaminated groundwater with pilot-scale constructed wetlands. Environ Pollut 159:3769–3776
43. Seeger E, Reiche N, Kuschk P, Borsdorf H, Kaestner M (2011b) Performance evaluation using a three compartment mass balance for the removal of volatile organic compounds in pilot scale Constructed Wetlands. Environ Sci Technol 45:8467–8474
44. Seeger EM, Maier U, Grathwohl P, Kuschk P, Kaestner M (2013) Performance evaluation of different horizontal subsurface flow wetland types by characterization of flow behavior, mass removal and depth-dependent contaminant load. Water Res 47(2):769–780
45. De Biase C, Reger D, Schmidt A, Jechalke S, Reiche N, Martinez-Lavanchy PM, Rosell M, Van Afferden M, Maier U, Oswald SE, Thullner M (2011) Treatment of volatile organic contaminants in a vertical flow filter: relevance of different removal processes. Ecol Eng 37:1292–1303
46. Van Afferden M, Rahman KZ, Mosig P, De Biase C, Thullner M, Oswald SE, Mueller RA (2011) Remediation of groundwater contaminated with MTBE and benzene: the potential of vertical-flow soil filter systems. Water Res 45:5063–5074

47. De Biase C, Maier U, Baeder-Bederski O, Bayer P, Oswald SE, Thullner M (2012) Removal of volatile organic compounds in Vertical Flow Filters: predictions from reactive transport modeling. Ground Water Monit Remed 32(2):106–121
48. Mleczek M, Rutkowski P, Rissmann I, Kaczmarek Z, Golinski P, Szentner K, Strazynska K, Stachowiak A (2010) Biomass productivity and phytoremediation potential of Salix alba and Salix viminalis. Biomass Bioenergy 34:1410–1418
49. Rentz JA, Alvarez PJJ, Schnoor JL (2005) Benzo[a]pyrene cometabolism in the presence of plant root extracts and exudates: implications for phytoremediation. Environ Pollut 136:477–484
50. Wang X, Deshusses MA (2007) Biotreatment of groundwater contaminated with MTBE: interaction of common environmental co-contaminants. Biodegradation 18:37–50
51. Thullner M, Stefanakis AI, Dehestabi S (2018) Constructed Wetlands treating water contaminated with organic hydrocarbons. In: Stefanakis, A.I. (ed.), Constructed Wetlands for industrial wastewater treatment. Wiley-Blackwell, Chichester, UK
52. Abed RMM, Al-Kharusi S, Prigent S, Headley T (2014) Diversity, distribution and hydrocarbon biodegradation capabilities of microbial communities in oil-contaminated cyanobacterial mats from a constructed wetland. PLoS One 9(12):e114570
53. Prigent S, Al-Hadrami A, Headley T, Al-Harrasi W, Stefanakis AI 2016 The reuse of Wetland-treated oilfield produced water for saline irrigation. International Conference of the International Desalination Association (IDA) on water reuse and recycling, Nice, France, 25–27 Sept
54. Stefanakis AI, Al-Hadrami A, Prigent S (2017) Reuse of oilfield produced water treated in a Constructed Wetland for saline irrigation under desert climate. In: 7th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL), Montana, USA, 21–25 Aug
55. Nikolaou I, Stefanakis AI (2018) A novel response of industry to wastewater treatment with constructed wetlands: a managerial view through system dynamic techniques. In: Stefanakis AI (ed) Constructed Wetlands for industrial wastewater treatment. Wiley, Chichester

# Chapter 7
# Tolerance to Metals in Two Species of Fabaceae Grown in Riverbank Sediments Polluted with Chromium, Copper, and Lead

**Gabriel Basílico, Ana Faggi, and Laura de Cabo**

## Abbreviations

| | |
|---|---|
| AGR | Absolute growth rate |
| BCF | Bioconcentration factor |
| $C_f$ | Contamination factor |
| DAP | Days after planting |
| $D_C$ | Degree of contamination |
| RGR | Relative growth rate |
| SOD | Superoxide dismutase enzyme |
| TF | Translocation factor |

## 7.1 Introduction

The Matanza-Riachuelo river (Argentina) is a river of short length (80 km), flow (8 $m^3/s$), and slope (0.35%) that crosses the Pampean plain, a gently rolling landscape built by fluvial dynamics. In the lower basin, the river is almost completely

---

G. Basílico (✉)
Laboratorio de Bioindicadores y Remediación, Universidad de Flores,
Ciudad Autónoma de Buenos Aires, Argentina

A. Faggi
Laboratorio de Bioindicadores y Remediación, Universidad de Flores,
Ciudad Autónoma de Buenos Aires, Argentina

Museo Argentino de Ciencias Naturales – Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina

L. de Cabo
Museo Argentino de Ciencias Naturales – Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_7

rectified and is named Riachuelo. Pollution in the Matanza-Riachuelo river basin has its origin in the discharges of the first salting plants established during the eighteenth century [1]. The intensification and diversification of human activity in the basin, which currently hosts between 3.5 and 4 million inhabitants, resulted in a scenario of chronic pollution of the main river and many of its tributaries. Raw sewage, treated sewage, and untreated effluents from slaughterhouses, tanneries, and other industries comprise the main sources of pollution [2].

This aquatic system is impacted by both organic and inorganic pollutants. In addition to the contribution of the sewage discharges, enrichment in river water organic matter results from industrial effluents, which introduce a variety of organic compounds like aliphatic and aromatic hydrocarbons, pesticides, polychlorinated biphenyls, and persistent organic compounds. Metal entrance to aquatic environments owes not only to industrial effluents but also to stormwater runoff, particularly in urban rivers. Metals such as copper (Cu), chromium (Cr), and lead (Pb) are notable for their toxicity to aquatic and terrestrial biota. In the Matanza-Riachuelo river, these metals are found mainly in the suspended solids and sediments of the bed and the riverbanks [2]. Cu is an essential metal for plant growth that is a cofactor of the enzyme superoxide dismutase (SOD), and it intervenes in several oxide reduction reactions of the electron transport chain [3]. However, very high concentrations of this metal can cause anatomical, morphological, and physiological changes in plants [4]. Trivalent Cr is an essential metal for the metabolism of lipids, carbohydrates, and proteins. Very high concentrations of this metal in the soil cause anatomical, morphological, and physiological changes in plants [4]. Pb is a nonessential metal without a biological role and is phytotoxic after a certain tolerance threshold [3].

Water and sediment contamination are not the only relevant environmental issues in the Matanza-Riachuelo river basin. The riverbanks in the lower basin show severe physical and biological alterations, due to the presence of solid waste, biological invasions, channelization, and other alterations of the fluvial geomorphology. The removal of invasive plant species and the reintroduction of native species are relevant actions among those necessary for the ecological rehabilitation of the banks of this river [5]. During 2015, in a section of the Riachuelo riverbank, a rehabilitation experience was carried out, which aimed to increase local biodiversity and contribute to the phytostabilization of pollutants present in the sediment. The plan consisted in the removal of solid waste and exotic species, the direct planting of native herbaceous and woody species, and the use of bio-roll technique for the plantation of rooted macrophytes. Among the reintroduced native woody species were included *Erythrina crista-galli* L. and *Senna corymbosa* (Lam.) H.S. Irwin & Barneby, both belonging to the Fabaceae family [6].

Pollutants present in the Matanza-Riachuelo riparian sediments could condition the growth of the selected plant species for ecological rehabilitation. However, there are mechanisms by which some plant species can tolerate high levels of pollutants. The most frequent one is the exclusion, where metal entry to the roots is prevented [7]. Metal translocation from the roots to the aerial tissues is the mechanism of accumulating and hyperaccumulating plant species that are used in other phytoextraction strategies. Meanwhile, if the objective of riverbank rehabilitation is the increase in biodiversity and phytostabilization of sediments, it is preferable that species do not translocate metals to the aerial tissues, in order to decrease the risk of metal transfer to the biota [8].

The aims of this work were to evaluate growth and tolerance of *E. crista-galli* and *S. corymbosa* in the Riachuelo riparian sediments and assess potential Cr, Cu, and Pb bioaccumulation in plant tissues.

## 7.2 Materials and Methods

### *7.2.1 Sediment Sampling*

Several samples of approximately 5 kg of surface riparian sediment (0–15 cm) were extracted in a 100-m-long section located in the lower basin of the Matanza-Riachuelo river. The section, located immediately downstream from the Ezequiel Demonty Bridge on the north riverbank (Autonomous City of Buenos Aires), was rehabilitated during 2015 [6]. The sediment samples obtained were mixed, obtaining a single homogenous composite sample that was subsequently sieved through a mesh of 0.5 cm of aperture. Subsequently, subsamples of 1 kg each were obtained.

### *7.2.2* **E. crista-galli** *and* **S. corymbosa** *Bioassay*

Seeds of *E. crista-galli* and *S. corymbosa* were planted in a commercial substrate rich in nutrients and organic matter. The seeds were previously scarified and hydrated, in order to maximize the percentage of germination. After approximately 4 months, individuals of each species were selected, with a similar height of 186 ± 19 and 215 ± 15 mm for *E. crista-galli* and *S. corymbosa*, respectively. The selected individuals were transplanted to pots containing 1 kg of polluted sediment from the banks of the Riachuelo river. Pots have no drainage to prevent metal lixiviation. In each pot was added a volume of 150 mL of deionized water, necessary to obtain the saturation of the substrate. During the bioassay, water losses through evapotranspiration were estimated by pots weighting and compensated by irrigation with a frequency of 2–3 times per week, depending on the ambient temperature. Plants were grown under natural lighting conditions in a greenhouse, during the spring-summer period. At the beginning of the experience and at 90 and 153 days after planting (DAP) in the polluted sediments, three individuals of each species were randomly selected, determining the total height with measuring tape, the basal diameter of the stem with caliber, the total dry weight of the aerial biomass (stem and leaves) and roots, as well as the concentration of metals in aerial biomass and roots. Absolute and relative growth rates (AGR and RGR) were calculated based on dry weight, according to [9].

### 7.2.3 Analytical Determinations

Chemical characterization of the sediment included assessment of pH and electrical conductivity (EC) with a Hanna® multiparameter sensor in the supernatant of a 1:2.5 p/v water suspension previously stirred for 15 min [10]. The percentage of organic matter (% $\mathrm{OM_{LOI}}$, g/100 g) in each sample was determined by ignition at 360 °C in a muffle furnace for 2 h [11], while the percentage of organic carbon (% $\mathrm{OC_{LOI}}$, g/100 g) was estimated by applying a factor of 0.58 to the value of % $\mathrm{OM_{LOI}}$ [12]. The concentrations of Cr, Cu, and Pb were determined in sediment, plant aerial tissues, and roots in individuals of each species. Before the transplantation, the initial concentrations ($C_0$) of metals in aerial tissues and roots of each species were determined, repeating these determinations in extracted samples at 90 and 153 DAP. In all cases, the samples were previously washed several times with tap water and then with deionized water, dried in an oven at 105 °C until constant weight, pulverized, subjected to acid digestion, and analyzed by atomic absorption spectrometry [13]. For both species the total content of metals in aerial tissues and roots was calculated as the product of the biomass and the concentration of each metal in the corresponding tissue. The bioconcentration factor (BCF) was calculated as the quotient between the concentration of metal in roots and in soil. In addition, the translocation factor (TF) was calculated as the quotient between the concentration of metal in aerial part and roots. In both cases, a value greater than 1 indicates bioconcentration or translocation.

Two contamination indices, the contamination factor ($C_\mathrm{f}$) and the degree of contamination ($D_\mathrm{C}$), were calculated according to Eqs. 7.1 and 7.2 [14]:

$$C_\mathrm{f} = \frac{c_\mathrm{m}}{c_\mathrm{ref}} \tag{7.1}$$

$$D_\mathrm{C} = \sum C_f \tag{7.2}$$

where $c_m$ is the average concentration of the metal in the sediment and $c_\mathrm{ref}$ is the reference concentration for that metal. The $C_\mathrm{f}$ values allow classifying the contamination for each metal: <1 low contamination factor, 1–3 moderate contamination factor, 3–6 high contamination factor, and >7 very high contamination factor. The $D_\mathrm{C}$ value allows classifying the degree of contamination, taking into consideration the contribution of each determined metal: <6 low degree of contamination, 6–12 moderate degree of contamination, 12–24 high degree of contamination, and >24 very high degree of contamination.

### 7.2.4 *Statistical Analysis*

In order to evaluate if metal bioconcentration in plant biomass increased with the time of exposure, the existence of statistically significant differences at 90 and 153 DAP was examined by parametric (Student's $t$) or nonparametric (Mann-Whitney $U$) tests according to the data.

### 7.2.5 *In Situ Survey of Vegetation*

During 2016, a periodic survey of the site rehabilitated in 2015 [6] was carried out to establish the general phytosanitary status of reintroduced plants, including *E. crista-galli* and *S. corymbosa* plants.

## 7.3 Results and Discussion

### 7.3.1 *Sediment Characteristics*

The sediment was slightly acidic, with a pH of 6.47 ± 0.08 ($n = 9$). The EC was 2.673 ± 0.553 mS/cm. The % $\mathrm{OM_{LOI}}$ was 7.89 ± 0.37 g/100 g, and the calculated % $\mathrm{OC_{LOI}}$ value was 4.57 ± 0.22 g/100 g. Cr, Cu, and Pb concentrations in the sediment were 155 ± 21 μg/g, 134 ± 27 μg/g, and 144 ± 29 μg/g, respectively. These values were very high compared to the soil considered as a reference (Cr, 19.3 μg/g; Cu, 23.0 μg/g; and Pb, 31.0 μg/g) for the Matanza-Riachuelo river basin by Mendoza et al. [13]. Considering the values of $C_{\mathrm{f}}$ (8.0, 5.8, and 4.6 for Cr, Cu, and Pb, respectively), the chromium contamination was very high, while for copper and lead, it was high. The degree of contamination ($D_{\mathrm{C}} = 18.4$) reflected that sediments can be categorized as highly polluted considering the three metals together.

### 7.3.2 *Growth of* **E. crista-galli** *and* **S. corymbosa** *Plants*

During the bioassay, both species showed exponential growth (Fig. 7.1), with AGR values of 0.049 and 0.062 g/day for *E. crista-galli* and *S. corymbosa*, respectively. The value of the coefficient of determination ($R^2$) was greater than 0.99 in both cases (Fig. 7.1). The RGR value was approximately 0.012 g/g day for both species, with a slightly higher growth in the case of *S. corymbosa*. It should be noted that the adult trees of *E. crista-galli* reach heights greater than 10 m, while those of *S. corymbosa* grows up to 5 m.

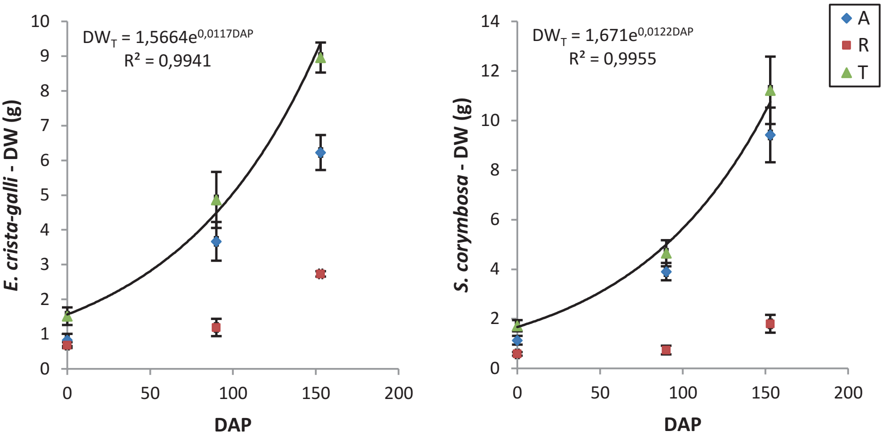


**Fig. 7.1** Aerial (A), roots (R), and total (T) biomass of *E. crista-galli* (left) and *S. corymbosa* (right) throughout the bioassay

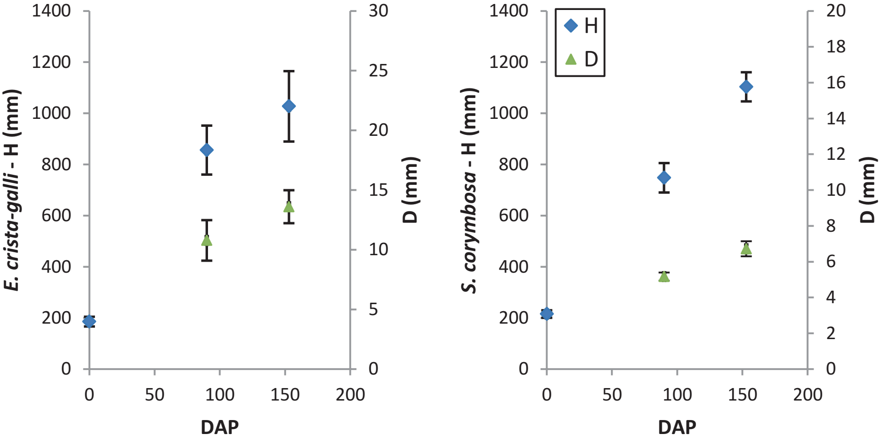


**Fig. 7.2** Height (*H*) and diameter (*D*) of *E. crista-galli* (left) and *S. corymbosa* (right) throughout the bioassay

At the end of the experience, the average height and diameter of *E. crista-galli* were 1057 mm and 14 mm, respectively, whereas for *S. corymbosa*, the average values were 1104 mm and 7 mm, respectively (Fig. 7.2).

### 7.3.3 Metal Bioaccumulation and Translocation

During the bioassay, an increase in Cr and Cu concentrations was observed in the roots of both species, taking into consideration the values found at 153 DAP with respect to the initials ($c_0$) (Table 7.1). An increase of Cu was observed in the aerial

**Table 7.1** Concentrations of Cr, Cu, and Pb in aerial tissues (A) and roots (R) of *E. crista-galli* and *S. corymbosa* throughout the trial. $c_0$, basal concentration; concentration unit, μg/g

| Species | Metal | Tissue | $c_0$ | DAP 90 | DAP 153 | Significance |
|---|---|---|---|---|---|---|
| *E. crista-galli* | Cr | A | <0.5 | (<0.5–0.8) | (<0.5–0.5) | N. S. (*t*-test) |
| | | R | <0.5 | 5.4 ± 1.1 | 3.4 ± 1.3 | N. S. (*t*-test) |
| | Cu | A | 5.7 | 8.4 ± 0.6 | 9.1 ± 1.9 | N. S. (*t*-test) |
| | | R | 5.9 | 25.0 ± 0.7 | 19.8 ± 6.4 | N. S. (*U*-test) |
| | Pb | A | 5.8 | 0.6 ± 0.1 | 3.0 ± 0.4 | N. S. (*U*-test) |
| | | R | 4.0 | 5.3 ± 1.7 | 2.3 ± 1.0 | N. S. (*t*-test) |
| *S. corymbosa* | Cr | A | <0.5 | (<0.5) | (<0.5) | N. S. (*t*-test) |
| | | R | <0.5 | 7.1 ± 1.5 | 8.8 ± 4.1 | N. S. (*t*-test) |
| | Cu | A | 3.4 | 5.9 ± 2.0 | 7.5 ± 0.7 | N. S. (*t*-test) |
| | | R | 9.1 | 81.4 ± 7.9 | 33.4 ± 17.2 | * ($p < 0.05$, *t*-test) |
| | Pb | A | 2.7 | 0.7 ± 0.3 | 3.1 ± 0.6 | ** ($p < 0.01$, *t*-test) |
| | | R | 11.2 | 9.1 ± 5.1 | 6.1 ± 4.7 | N. S. (*U*-test) |

*t*-test student *t* test, *U-test* Mann-Whitney *U*-test

tissues of *E. crista-galli* and of Cu and Pb in those of *S. corymbosa*. The final Pb concentrations in roots were lower than the initial values determined in both species. The same pattern was observed in the aerial tissues of *E. crista-galli*. Cr initial and final concentrations in the aerial tissues of both species were lower than the detection limit (0.5 μg/g). Between 90 and 153 DAP, there was a highly significant increase ($p < 0.01$, *t*-test) of Pb and a significant decrease ($p < 0.05$, *t*-test) of Cu in aerial tissues and roots of *S. corymbosa*, respectively (Table 7.1).

The calculated BCFs were less than 1 in all cases, indicating that the plants cannot be considered as bioaccumulators of any metal, at least under the conditions tested. *E. crista-galli* translocated Pb to the aerial tissues, obtaining a TF of 1.41 at 153 DAP (Table 7.2).

The average total content of Cr, Cu, and Pb in *E. crista-galli* roots increased throughout the experience, as did the Cu content in aerial tissues of both species. The total content of Cu and Pb in the whole plant (roots + aerial part) increased in both species between 90 and 153 DAP, demonstrating the net accumulation of these metals (Fig. 7.3). The values found indicate that, although the examined species are not bioaccumulators of Cu, Cr, or Pb, these metals are absorbed by plants in small quantities over time. The results obtained in this work showed that both species grew well in the riverbank sediments of the Riachuelo river. In addition, it was observed a good growth of the planted specimens in that place during the year 2015 [6]. Several mechanisms have been described for native plants allowing them to grow successfully in highly disturbed environments. In contaminated soils, Brunetti et al. [15] indicate a high metal excluding capacity and low accumulation in aboveground parts, in most of the studied native species they examined. On the contrary, Yoon et al. [16] found several native plants had BCFs or TFs greater than 1. Therefore, we cannot generalize about tolerance mechanisms in native species since it depends not only on the species but also on the metal and the general conditions

**Table 7.2** Factors of bioconcentration (BCF) and translocation (TF) of Cr, Cu, and Pb in *E. crista-galli* and *S. corymbosa*

| Species | Metal | Factor | DAP | |
|---|---|---|---|---|
| | | | 90 | 153 |
| *E. crista-galli* | Cr | BCF | 0.03 ± 0.01 | 0.02 ± 0.01 |
| | | TF | Nc | Nc |
| | Cu | BCF | 0.19 ± 0.01 | 0.15 ± 0.05 |
| | | TF | 0.34 ± 0.03 | 0.50 ± 0.22 |
| | Pb | BCF | 0.04 ± 0.01 | 0.02 ± 0.01 |
| | | TF | 0.12 ± 0.05 | 1.41 ± 0.39 |
| *S. corymbosa* | Cr | BCF | 0.05 ± 0.01 | 0.06 ± 0.03 |
| | | TF | Nc | Nc |
| | Cu | BCF | 0.61 ± 0.06 | 0.25 ± 0.13 |
| | | TF | 0.07 ± 0.02 | 0.28 ± 0.17 |
| | Pb | BCF | 0.06 ± 0.04 | 0.04 ± 0.03 |
| | | TF | 0.08 ± 0.03 | 0.73 ± 0.46 |

*Nc* not calculated (below detection limit)

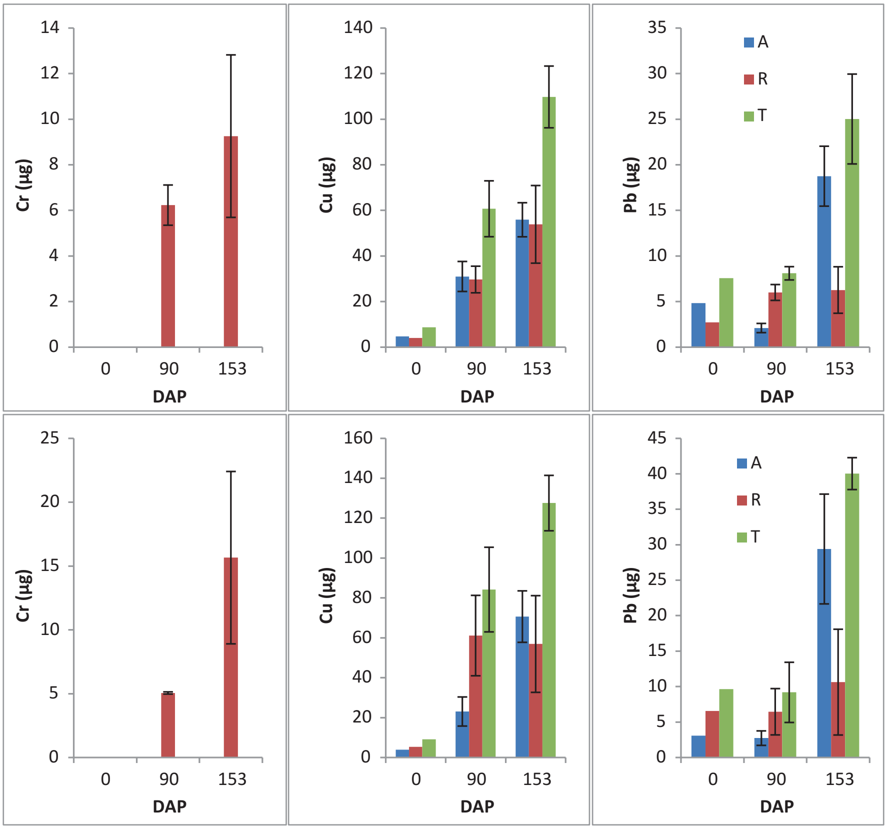


**Fig. 7.3** Net accumulation of Cr, Cu, and Pb in plants of *E. crista-galli* (top) and *S. corymbosa* (bottom)

of the substrate. Although many species of native plants cannot be considered as hyperaccumulators or even as accumulators of metals, their presence in these environments favors the stabilization of the metal in the soil matrix.

The trend observed in net accumulation of metals by *E. crista-galli* makes the species a potential native candidate for phytoremediation purposes, given the larger size of adult trees. Still, being a deciduous tree and given the translocation capacity of Pb toward aerial tissues, the subsequent management of fallen leaves should be assessed in the instance of using this species in the rehabilitation of soils contaminated with this metal.

## 7.4 Conclusions

Our study showed that two native plant species, *E. crista-galli* and *S. corymbosa*, grew well in the Riachuelo contaminated riverbank sediments. None of the species accumulated significant amounts of Cr, Cu, or Pb, and only Pb translocation from the roots to the aerial part was observed in *E. crista-galli* after approximately 5 months of planting, despite the concentration of this metal in the aerial part was low. The species tested showed tolerance not only to the contaminants present in the sediments but also to the environmental conditions of the riverbank through mechanisms that allow their growth in contaminated soils, promoting the stabilization of metals and soil conservation.

**Acknowledgments** The research was supported by grants of the *Universidad de Flores* and *Museo Argentino de Ciencias Naturales "Bernardino Rivadavia."* The authors want to thank the *Gerencia Operativa de Riachuelo y Borde Costero Sur – Agencia de Protección Ambiental (Ciudad Autónoma de Buenos Aires)* that provided assistance during the surveys. The authors are grateful for the revision of the text in English made by Maria Victoria Casares and Luciana Marin.

## References

1. Tudino M, Bonetto C, Conforti V, de Cabo L, Fabrizio de Iorio A, Ferrari L et al (2001) La contaminación del agua. In: Borthagaray AI et al (eds) Diagnóstico Ambiental del Área Metropolitana de Buenos Aires. FADU-UBA, Buenos Aires
2. Bargiela M, deIorio A (2013) La calidad del agua del río Matanza-Riachuelo. Informe Especial: El Saneamiento del Riachuelo 132:12–15
3. Perveen R, Faizan S, Ansari AA (2015) Phytoremediation using leguminous plants: managing cadmium stress with applications of arbuscular mycorrhiza (AM) fungi. In: Ansari AA et al (eds) Phytoremediation, vol 2. Springer International Publishing, Cham, pp 131–142
4. Ambrosini VG, Rosa DJ, Prado JPC, Borghezan M, de Melo GWB, de Sousa Soares RF et al (2015) Reduction of copper phytotoxicity by liming: a study of the root anatomy of young vines (*Vitis labrusca* L.). Plant Physiol Bioch 96:270–280
5. Breuste J, Matanza-Riachuelo FAL c (2014) Una mirada ambiental para recuperar sus riberas. Universidad de Flores, Buenos Aires

6. Basílico G, de Cabo L, Faggi A, Miguel S (2016) Low-tech alternatives for the rehabilitation of aquatic and riparian environments. In: Ansari AA et al (eds) Phytormediation, vol 4. Springer International Publishing, Cham, pp 349–364
7. Becerril JM, Barrutia O, Plazaola JG, Hernández A, Olano JM, Garbisu C (2007) Especies nativas de suelos contaminados por metales: aspectos ecofisiológicos y su uso en fitorremediación. Rev Ecosist 16(2):50–55
8. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals-concepts and applications. Chemosphere 91(7):869–881
9. Hunt R (2003) Growth analysis, individual plants. Encyclopaedia of applied plant sciences. Academic, London, pp 579–588
10. Davies BE (1974) Loss-on ignition as an estimate of soil organic matter. Soil Sci Soc Am Proc 38(1):150–151
11. Schulte EE, Hopkins BG (1996) Estimation of organic matter by weight loss-on-ignition. In: Magdoff FR et al (eds) Soil organic matter: analysis and interpretation. SSSA special publication 46. SSSA, Madison, pp 21–31
12. Eyherabide M, Saínz Rozas H, Barbieri P, Echeverría HE (2014) Comparación de métodos para determinar carbono orgánico en suelo. Cienc Suelo 32(1):13–19
13. Mendoza RE, García IV, de Cabo L, Weigandt CF, de Iorio AF (2015) The interaction of heavy metals and nutrients present in soil and native plants with arbuscular mycorrhizae on the riverside in the Matanza-Riachuelo River Basin (Argentina). Sci Tot Environ 505:555–564
14. Kwon YT, Lee CW (1998) Application of multiple ecological risk indices for the evaluation of heavy metal contamination in a coastal dredging area. Sci Tot Environ 214(1):203–210
15. Brunetti G, Soler-Rovira P, Farrag K, Senesi N (2009) Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region, Southern Italy. Plant Soil 318(1–2):285–298
16. 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 Tot Environ 368(2–3):456–464

# Chapter 8
# Phytoremediation of Industrial Wastewater by Hydrophytes

**Hera Naheed Khan and Muhammad Faisal**

## 8.1 Introduction

Pure uncontaminated water is scarce, and around one billion people around the globe don't have access to it [1]. Two key factors that are responsible for this contamination are industrialization and urbanization [2]. Pollution is a multifaceted phenomenon; a single factor does not contribute to pollution; in fact a range of factors contributes to this evil. Industrialization is one key factor contributing to pollution [3] and industrialization further triggers other factors like release of effluents in water, gases in the air can contribute to ozone layer depletion, access of UV rays to the earth's crust [4] that eventually leads to global warming [5, 6]. Another key factor contributing to environment contamination is overpopulation that ultimately leads to urbanization.

Urbanization is a threat that is destroying our ecosystems, and it's the need to urbanize that increases the pollution both land and air and water [7]. The population of the cities is increasing, and by 2050 it is expected that around two-thirds of the world population will be living in the cities [8]. In the last 200 years, the world has seen a dynamic increase of 48% in the urban population [9]. Megacities are the cities that have a population of around ten million people, and it has been estimated by UN that by 2015, the number of megacities will increase from 2 to almost 22 [10]. But with this mass movement of population to the cities, energy demands have increased, and this calls for exploitation of natural resources that in response generates large amounts of pollution be it land, air, or water [11].

Other than these two factors, there are other anthropogenic activities that play a major role in contaminating our environment, and with rapid population expansion, the quality and quantity of resources are diminishing [12]. Exploitation of resources to fulfill the demands of increasing population generates waste which when exceeds

---

H. N. Khan · M. Faisal (✉)
Department of Microbiology and Molecular Genetics, University of the Punjab,
Lahore, Pakistan

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_8

its recycling limit generates pollution exceeds the limit after which it can’t be recycled or treated generates pollution [13]. Pollution will prolong as long as humans populate the earth, but by controlling population expansion, urbanization, and industrialization, we can control environmental degradation [14].

Availability of clean water is finite; the only thing one can do is to reduce anthropogenic activities that contaminate natural water sources and employ wastewater treatment techniques that are environmentally friendly [15]. Bioremediation is one technique employed all over the world to treat wastewater. This technique is eco-friendly and uses biological agents to control or remove pollutants from water [16]. Biosorption is another phenomenon in which plants are used to absorb metals especially heavy metals from water bodies [17].

## 8.2 Water Pollution

Adulteration of natural resources is pollution, and contamination of surface or groundwater sources is water pollution. Loren Eiseley wrote, “If there is magic in this planet, it is in water,” water is the essence of life. Any factor whether chemical or physical that can adulterate the water bodies leads to water pollution.

The excess of anthropogenic activities has resulted in the production of pollutants that are difficult to control and is responsible for the conversion of natural water habitats into sewage waste depots [18]. According to an estimate, industrial and domestic wastes contribute to a million different types of pollutants to the natural waters thus contaminating them [19]. Some of these pollutants are not harmful and just contribute by adding a bad odor and taste to the fresh water, while most of the contaminants like pesticides, trace elements, plastics, polycyclic compounds, and radioactive materials are a direct threat to humans as well as other organisms [20].

Water pollution directly affects the entire biosphere especially the biota that resides in these water bodies. Water pollution can be caused by either point source pollutants or nonpoint source pollutants [21]. Point source pollutants represent localized pollution-producing bodies like industries or mining areas, whereas nonpoint source pollutants represent unidentified pollution sources [22] like runoff contaminated waters from which pollutants will be carried to other areas (Environment Pollution Centres https://www.environmentalpollutioncenters.org/water/).

Earth is often referred to as the “blue planet” because of the abundance of water on our planet, i.e. ,two-thirds of the planet is covered with waters; only 2.5% is fresh water and out of this most is reserved within glaciers or deepwater sources, and very little is accessible for drinking and other purposes [23].

So, for the survival of our planet, we need to reduce and control this contamination; otherwise with the rate with which we are releasing pollutants in more or less time our freshwater reserves will run out.

## 8.3 Types of Pollutants in Wastewater Especially Industrial Waste

The most dangerous form of pollutants tends to exist in industrial wastewaters. Industrial pollutants vary depending on the type on industry generating it. The effluents in the industrial wastewater are restricted not just to suspended solids, different organic and inorganic materials, certain oils, and dyes that are used frequently in the textile industry [24, 25]. According to an estimate, around one million tons of textile coloring agents are annually being produced [25]. Water contaminated with various pharmaceutical compounds, but most abundantly it tends to be corrupted with metals especially heavy metals. Other than these there is a new term defined as "emerging pollutants" that refers to the existence of such pollutants or chemicals in water whose regulatory status is not yet known, and their impact on either environment or their human health risks have not been defined [26].

The organic pollutants are further classified into the following subtypes [27]:

1. *Oxygen-demanding wastes*: As the name indicates, this type of waste refers to all such waste materials that have higher concentration of biodegradable organic compound; the waste belongs to food and canning industries, leather tanneries, paper industries, breweries, etc. This oxygen-demanding organic waste can be in colloidal, dissolved, or suspended form. As this type of waste has high organic content, hence its degradation requires oxygen that usually occurs via bacterial activity of aerobes. The utilization of oxygen tends to deplete dissolved oxygen thereby affecting the quality of water making it extremely unsuitable for marine organisms; this value of DO when falls below 4.0 mg/ml is a serious indicator of water pollution [28].
2. *Synthetic organic compounds*: This type refers to the organic compounds of synthetic origin that mostly include detergents, pesticides, paints, plastics, pharmaceutical compounds, insecticides, volatile organic compounds (VOCs), etc. The major drawback of these is their inability of biodegradation, and trace amounts of these compounds also tend to make water unhealthy for consumption. Synthetic compounds, i.e., polychlorinated biphenyls (PCBs), have been manufactured and utilized in the industrial sector since the 1930s; these complex chlorobiphenyls are fat soluble and have the ability to move within the body cells and even tissues, thus making such waters extremely unfit for use. Such compounds also tend to persist in the environment as they are degradation resistant [29].
3. *Oil* is a naturally occurring substance that is formed through fossils of plants over a period of millions of years under extremely high-pressure conditions. This is actually a mixture of hydrocarbons that gets degraded by bacterial degradation action. The biodegradation capacity varies from one type of oils to the other with tars showing the slowest biodegradation. Contamination of water bodies with oils occurs through oil spill accidents or wastewater containing oils that enters via refineries, leakage through pipes, etc. As water is lighter than oil so it forms

**Table 8.1** Table of secondary standards (US-EPA)

| Contaminant | Secondary MCL | Noticeable effects above the secondary MCL |
|---|---|---|
| Chloride | 250 mg/L | Salty taste |
| Color | 15 color units | Visible tint |
| Corrosivity | Non-corrosive | Metallic taste; corroded pipes/fixtures staining |
| Fluoride | 2.0 mg/L | Tooth discoloration |
| Foaming agents | 0.5 mg/L | Frothy, cloudy; bitter taste; odor |
| Manganese | 0.05 mg/L | Black to brown color; black staining; bitter metallic taste |
| Odor | 3 ton (threshold odor number) | "Rotten egg," musty, or chemical smell |
| pH | 6.5–8.5 | Low pH: bitter metallic taste; corrosionHigh pH: slippery feel; soda taste; deposits |
| Sulfate | 250 mg/L | Salty taste |
| Total dissolved solids (TDS) | 500 mg/L | Hardness; deposits; colored water; staining; salty taste |

*mg/L* milligrams of substance per liter of water, *MCL* maximum contaminant list

a layer over the surface of water insulating everything underneath with no air contact, this results in reduced dissolved oxygen. It can also affect light transmission by completely blocking the light from entering the water hence affecting photosynthesis of aquatic plants. Certain compounds found in oil are known to be carcinogenic with polycyclic aromatic hydrocarbons (PAH) being one of them.

4. *Nutrients*: Runoff from the fertilizer industry and other sources like sewage and agricultural waste is rich in nitrogen and phosphorous. The excess of these compounds triggers the growth of algae, cyanobacteria, and other water-dwelling weeds thus reducing dissolved oxygen thereby leading to eutrophication of the water body. Eutrophication converts a freshwater body to dead waters that gives off bad odors and becomes unfit for use.

Table 8.1 listed below shows the Maximum Contaminants List issued by EPA that mentions drinking water quality standards to protect people from drinking contaminated water.

## 8.4 Heavy Metal Contaminants

The class of metals with high density and high toxicity even at extremely minute concentrations is referred to as heavy metals [30]. Any metal or metalloid with a density greater than 4 g/cm$^3$ is classified as heavy metals. They are required in very trace amounts by living organisms to carry out their normal developmental or physiological functions [31, 32]; mostly metals act as enzyme catalyst in metabolic reactions, but an increase in their amount may cause variety of carcinogenic effects

**Table 8.2** Heavy metal toxicities in humans

| Sr. no | Metals | Toxicity | Reference |
|---|---|---|---|
| 1 | Arsenic (Ar) | Arsenic-induced carcinogenesis | [33] |
| 2 | Lead (Pb) | Ionic imbalance that impairs the biological metabolism/ production of ROS | [34] |
| 3 | Mercury (Hg) | Produces a neurotoxic compound, responsible for brain damage | [35] |
| 4 | Cadmium (Cd) | Acute and chronic toxicities | [36] |
| 5 | Chromium (Cr) | DNA and protein damage | [37] |
| 6 | Aluminum (Al) | Damaging to neurons, hematopoietic cells, and osseous | [38] |
| 7 | Iron (Fe) | Cellular and DNA damage causing mutations | [39] |
| 8 | Uranium | Reproductive and developmental specifically renal damage | ([40]; [41]) |
| 9 | Nickel (Ni) | Nickel-induced carcinogenesis | [42] |
| 10 | Copper (Cu) | Cu-induced oxidative damage | [43] |

[32]; a list of metal toxicities in humans caused by some heavy metals is mentioned in Table 8.2.

The major problem catered when such metals enter the water bodies is their non-degradable nature and their ability to accumulate [31]. The major heavy metals that tend to be problematic are aluminum, zinc, bismuth, copper, arsenic, nickel, thallium, mercury, uranium, titanium, cadmium, lead, indium, chromium etc.

These metals are introduced in our environment via mining and industrial activities; these metals then react with organic matter and are acted upon by of lithotrophic bacteria to generate their organic toxic forms like monomethyl mercury and dimethyl cadmium that seep through the land and contaminate our underground water reserves [30].

Exposure of human population to even low levels of heavy metal pollutants can result in neurological, behavioral, and developmental disorders [34]. It can also result in insomnia, lack of ability to concentrate, irritability, fatigue, motor and sensory nerve impairments [34], growth disorders like several cancer types due to carcinogenic effect of some metals, impairment of kidney functions, and even autoimmune disorders. Humans get exposed to heavy metals via intake of food [44] and drinking of metal polluted waters [45]. An example of human exposure to contaminants can occur by ingesting of polluted fish or marine foods that are contaminated with methyl mercury; a heavy metal variant. This compound when enters human bodies it can cause damage to vital body organs especially kidneys and lungs [46]. This intermediate of mercury can even cross placental barrier leading to brain damage of the fetus [47]. Similarly accumulation of cadmium inside kidneys and livers of humans can lead to renal failure [48]. Several reports of cadmium occurrences in irrigation as well as sewage waters in India resulted in gallbladder cancers

**Table 8.3** Heavy metal contaminant limits in drinking water issued by US-EPA

| Heavy metals | Maximum concentration in drinking water |
|---|---|
| Lead | 15 ppb |
| Copper | 1.3 ppm |
| Chromium | 100 ppb |
| Arsenic | 10 ppb |
| Mercury | 2 ppb |
| Cadmium | 5 ppb |
| Aluminum | 0.2 ppm |
| Iron | 0.3 ppm |
| Silver | 0.1 ppm |
| Zinc | 5 ppm |

and kidney stones [21, 49]. High chromium and lead concentrations are also known to cause these carcinomas [50]. High exposure of lead can lead to lead poisoning especially in children; this leads to brain and nerve damage causing short-term memory loss, coordination problems, learning disabilities, and reduced intelligence [51]. Arsenic has been known to cause variety of problems including heart disorders, some dermal cancers, and even kidney damage.

As mentioned not all heavy metals have bio-importance and human exposure to even minute concentrations of such metals can have toxic effects (Young 2005; [30]). A list of major concerns posed by heavy metals when they surpass a critical concentration has been mentioned in Table 8.2. Heavy metals intake mostly causes teratogenic effects by disrupting the DNA and causing influential damage to the reproductive and developmental machinery (Table 8.2). The maximum intake limit of the most notorious heavy metal contaminants has been provided by Environmental Protection Authority (EPA) and mentioned in Table 8.3. Table 8.3 clearly shows intake of metals in minute concentrations of even parts per billion can pose serious health threats; hence metal contamination in our drinking water supplies needs to be dealt with an iron hand so that heavy metal toxicities can be prevented.

## 8.5 Phytoremediation

A modern technique that utilizes plants for removing contaminants from the environment is referred to as phytoremediation [18]. It’s a safe, eco-friendly, and economical approach that can be used to remediate large contaminated areas polluted with heavy metals, radioactive materials, and other harmful contaminants; the modern approaches focus on field trials of this technique [21]. This green approach is efficient enough to remediate metals, petroleum, diesel, PAHs, and all types of organic and inorganic pollutants [52]. Scientists also tend to focus on the molecular mechanisms that are responsible for this aspect of plants [53].

Plants with the ability to accumulate 1000 mg/kg of heavy metals like copper, nickel, chromium, lead, or cobalt or more preferably greater than 10,000 mg/kg of such metals are referred to as hyperaccumulators [54, 55]. The efficiency of phytoremediation depends on the limit of plants to accumulate metals and survival of such plants in polluted environments [52]. Sometimes this efficiency can be enhanced by using symbiotic approach, i.e., using combination of plants and microbes to remediate contaminated areas [56]. Microorganisms in this case are mostly plant growth-promoting bacteria that live within the plant roots and along with enhancing plant growth also function in cleaning contaminated waters and soils [57].

For picking up a plant with the potential to decontaminate environment, it (1) should possess increased growth rate and (2) high threshold value to resist metals (3) should show multiple resistance to pests and other plant diseases, (4) should possess a very dense and thick root and shoot system [58] and last but not the least (5) should be least attractive to herbivores, insects, and other animals such that there is least transfer of accumulated metals to upper energy/tropic levels in the food chain [59]; also the plant should not be metal specific so that a single plant type can remediate multiple metals [60, 61]. Another favorable trait might be that the plant should be that area's native species [62].

A commonly occurring hydrophyte *Eleocharis acicularis* has been reported as a hyperaccumulator of copper [63], silver, cadmium, and lead in laboratory and greenhouse experiments [64]; this macrophyte has been associated for the phytoremediation of different heavy metals and metalloids [65]. But still no field study has classified this macrophyte to remediate contaminated waters and soils under natural conditions.

A list of commonly used plants to chelate metals is listed in Table 8.4. Hyperaccumulators are plants that can concentrate up to 1% of metals in its leaves as its dry weight [82]. Hyperaccumulators of nickel are the most commonly

**Table 8.4** Commonly used plants as metal chelators

| Plants | Metals | References |
|---|---|---|
| *Pistia stratiotes* L. (water lettuce) | Cd, Cr, Pb | [66] |
| Duckweed | Cd, Cr, and Pb | [67] |
| *Thlaspi alpestre* | Zn (3%), Pb (1%), Cu | [68] |
| *T. eepeaefolium* | Zn, Pb, Cu | [68] |
| *Silène vulgaris* | Zn, Pb, Cu | [69] |
| *Brassica juncea* | Cu, Zn, Pb, Cd, Ni | [70] |
| *B. rapa* | Cu, Zn | [71] |
| *Minuartia vepva* | Zn, Pb, Cu | [72] |
| *B. napus* | Cu, Zn | [73] |
| *Arabidopsis halleri* | Cd and Zn | [73] |
| *Solanum nigrum* L. | Cd and Th | ([74]; [75]) |
| *Populus deltoides* | As, Hg | [76] |
| *Jatropha* (*Jatropha curcas* L.) | Cd, Cu, Ni, Pb, and Hg | ([77]; [78]) |
| *Astragalus bisulcatus* | Se | [79] |
| *Sebertia acuminate* | Ni | [80] |
| *Haumaniastrum katangense* | Co (0.1%) | [81] |

occurring belonging to almost 277 taxa and 36 various plant families [83]; this is preceded by hyperaccumulators of cobalt, copper, and zinc. Hyperaccumulators for other metals like manganese, lead, cadmium, chromium, and selenium have also been known and mentioned in Table 8.4, but it's the plants belonging to the Brassicaceae family that have been characterized as hyperaccumulators in the temperate zones, whereas [84] plants belonging to the Euphorbiaceae (spurge family) act as hyperaccumulators in the tropical regions [85].

### *8.5.1 Metal Uptake Mechanism in Plants*

Plants have employed a range of strategies to detoxify metals, but the major step is the uptake of metals by plants. The metals are taken up by plant roots, but overaccumulation of metals can have toxic affects causing tissue and cellular damage in plants. In order to avoid this, plants have employed various strategies [86]. One such approach is limiting the movement of toxic metals to plant roots only by mycorrhizal fungi [85]. Plants are also categorized as "accumulators" and "excluders"; accumulators are the plant types that can survive even if they concentrate metals within their bodies, while excluders limit the entry of metals after a specific threshold value [87].

Metal chelation or uptake occurs via the presence of metal-binding proteins and peptides [85]. The translocation and uptake mechanism of ions, metals, and nutrients from the environment occurs via proton pumps, co-transporters and antitransporters, or channels, i.e., proteins that facilitate the transport of ions into the cell [88].

Plants that are hyperaccumulators of heavy metals usually concentrate these metals in concentrations of 100–1000 times more than non-accumulators [87]. Sometimes rhizospheric and mycorrhizal associations in plant also facilitate this increased metal uptake [89]. Different mechanisms are used by plants for detoxifying contaminated areas; these include phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization [90].

1. *Phytoextraction*: This is the absorption of pollutants and their translocation to the aerial parts of the plants typically shoots and sometimes leaves [91].
2. *Rhizofiltration*: This is the removal of contaminants, pollutants, industrial effluents, or heavy metals from contaminated water using plant roots [92]. This can be further categorized into *blastofiltration* (that refers to cleaning of environment, i.e., soil or water using seeds) [93] and *caulofiltration* (this refers to the use of removed and cut shoots of plants for remediation of water or soil) [94]. This filtration of underground water makes water free of contaminants usually metals.
3. *Phytoimmobilization*: This process renders the metals unavailable, thus restricting their mobility within the food chain as well as groundwater [95]. Heavy metal immobilization is done via absorption through the plant roots, detoxification of metals, i.e., conversion to less toxic forms within the rhizosphere, precipitation, or complex formation [96]. This renders the toxic metals unavailable, and their conversion to less toxic forms doesn't affect much.

4. *Phytovolatilization*: After plants uptake toxic metals, pollutants, or industrial effluents through their roots, they sometimes break them or metabolize them like organic forms of contaminants that are usually catabolized using some enzymes like dehalogenases or oxygenases; this phenomenon occurs irrespective of microorganisms. Whereas sometimes heavy metals are transformed to volatile forms that are liberated into the atmosphere, this phenomenon of conversion of metals to vaporous entities and their dissemination to the atmosphere is referred to as phytovolatilization [97]. This process is usually used for metals that have volatile forms especially mercury and selenium. When Se is uptaken by plants, it gets transformed into seleno-amino acids, i.e., selenocysteine and selenomethionine; these are organic forms of selenium [85]. These are bio-methylated and converted to a volatile form, i.e., dimethyl-selenide; this is then dispersed into the atmosphere. Mercury normally exists as a liquid even at room temperature so it can be easily vaporized. But due to its highly reactive nature, it naturally exists as a divalent cation $Hg^{2+}$; therefore this conversion of $Hg^{+2}$ to elemental Hg is catalyzed by bacteria via mercuric reductase, and once in elemental form, it can be easily volatilized [85].
5. *Phytodegradation*: This process refers to the conversion of toxic metals into non-toxic forms using different reduction mechanisms [94]. In this not only heavy metals in fact other toxic compounds and organic pollutants are also converted to their less toxic states by plant enzymes, and the process usually takes place within the plant tissues [98].

An overview of the different methods employed by plants to remediate the environment has been depicted in Fig. 8.1.

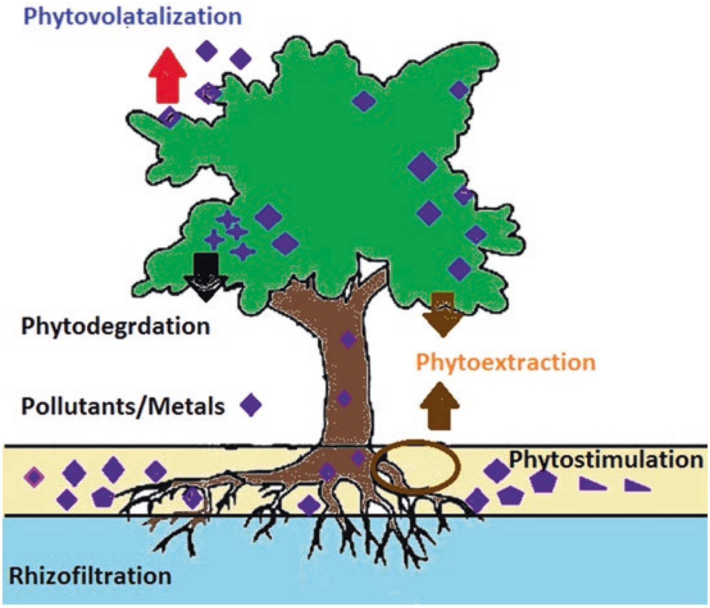


**Fig. 8.1** Commonly used metal uptake and remediation mechanisms in plants

### *8.5.2 Phytoremediation Using Hydrophytes*

Hydrophytes are plants that tend to grow in water; these aquatic plants are the first choice when considering phytoremediation of water [21]. Aquatic plants have been used for this purpose since the 1970s; however over the decades, there is a shift with major focus on induced hyperaccumulator plant species and plant species that work in conjunction with bacteria that reside within the rhizosphere to clean water [99]. Aquatic plants are selected over other terrestrial plants because of their enhanced capability to accumulate almost 1450-fold of heavy metals in water [62]. Another advantage of using aquatic plants is their rapid growth rate and increased biomass production, high pollutant uptake ability, and more pronounced purification because of their direct contact with water [100]. Other than this they are also helpful in regulating oxygen level and food cycle by acting as food source to small fish [101, 102]. These plants can accumulate heavy metals not just in their roots but shoots too, but the roots store relatively higher concentrations than shoots [61].

Hydrophytes are commonly referred to as aquatic macrophytes that include water plants that are large enough to be seen with the naked eye [103]. This class of hydrophytes includes spermatophytes (flowering plants), pteridophytes (ferns), and bryophytes (mosses, hornworts, and liverworts). Although bryophytes are very minuscule yet included within macrophytes, some scientists have included charophytes to this group too. Commonly *Cyanobacteria*, Chlorophyta, Rhodophyta, Xanthophyta, Bryophyta, Pteridophyta, and Spermatophyta represent the seven divisions of macrophytes [104]. These macrophytes are further categorized as submerged plants or emerged plants. Submerged plants are hydrophytes that completely reside within water, while emerged are those that have their roots submerged in water while the remaining plant is above water; then there are free-floating hydrophytes that are like emergent plants but not rooted and rather tend to move here and there above water surface [62, 105].

Reports of emergent and submerged plants are available for their potential role in concentrating heavy metals like water hyacinths, water lettuce, giant duckweed, *Azolla* sp., *Potamogeton pectinatus*, etc. These occur at water depth of around 0.5–3 cm; some examples of submerged plant species are *Potamogeton* sp., mosses, charophytes, *Hydrilla* sp., *Myriophyllum* sp., etc. and emergent species *Typha* sp., *Scirpus* sp., *Phragmites australis*, *Limnocharis flava*, *Spartina* sp., *Cyperus* sp., and *Phragmites* sp. [106]; examples of free-floating macrophytes include *Salvinia* sp., *Eichhornia crassipes*, etc. All these and some other members of these macrophytes clean and concentrate heavy metals, but with varying potentials, the difference in remediation potential can be characterized to the amount of metal present, redox potential, temperature and pH. Also the interaction of metals within the medium can also affect this potential [107].

All these types have acquired their role as remediating agent and employed a unique phenomenon for uptaking and detoxifying contaminated water. Table 8.5 archives most of the commonly known macrophyte accumulators and hyperaccumulators with their maximum metal uptake capacity per weight (g/mg/μg). Some of the macrophytes seem to be capable of accumulating multiple heavy metals like

**Table 8.5** List of macrophytes used for phytoremediation of heavy metals [108]

| Macrophytes | Heavy metal | Accumulation of metals/g |
|---|---|---|
| *Eichhornia crassipes* | Hg<br>Cd<br>Cr<br>Ni<br>Cu | 119 ng Hg<br>1.98 mg Cd<br>2.31 mg Cr<br>1.68 mg Ni<br>314 μg Cu |
| *Elodea densa* | Hg | 177 ng Hg |
| *Elodea canadensis* | Ni | >3500 μg Ni |
| *Egeria densa* | Cd, Cu, Zn | 70.25 mg Cd<br>45.43 mg Cu<br>30.40 mg Zn |
| *Eleocharis acicularis* | Fe, Pb, Zn, Mn, Cr, Cu, Ni | 59,500 μg Fe<br>1120 μg Pb<br>964 μg Zn<br>388 μg Mn<br>265 μg Cr<br>235 μg Cu<br>47 μg Ni |
| *Pistia stratiotes* | Hg<br>Cr, Cd, Ni<br>Zn | 0.57 mg Hg<br>>9 mg Cr<br>2.13 mg Cd<br>10 mg Ni<br>>12 mg Zn |
| *Salvinia natans* | Cr | 7.40 mg Cr |
| *Salvinia auriculata* | Hg<br>Pb | 191 ng Hg<br>494 μg Pb |
| *Lemna gibba* | Ur, As | 897 μg Ur<br>1022 μg As |
| *Lemna minor* | Cu, Ti, Pb | 400 μg Cu<br>221 μg Ti<br>8.62 mg Pb |
| *Ceratophyllum demersum* | As, Cd, Zn | 522 μg As<br>1293 μg Cd<br>57 μg Zn |
| *Potamogeton pusillus* | Cu | 162 μg |
| *Myriophyllum triphyllum* | Cd | 17 μg Cd |
| *Wolffia globosa* | As | >1000 μg As |
| *Typha angustifolia* | Cr, Zn, Cu | 20,210 μg Cr<br>16,325 μg Zn 7022 μg Cu |
| *Typha latifolia* | Zn, Ni, Cu | 340 μg Zn<br>55 μg Ni<br>50 μg Cu |
| *Spirodela polyrhiza* | As | 7.65 nm |
| *Mentha* sp. | Fe | 378 μg |
| *Vallisneria spiralis* | Cr, Cd, Ni | 2.85 mg Cr<br>2.62 mg Cd<br>2.14 mg Ni |

*Eichhornia crassipes* and *Eleocharis acicularis* (Table 8.5) that are capable of accumulating Pb, Cr, Hg, Fe, Ni, Cu, Cd, and Mn in different concentrations.

### 8.5.3 Remediation of Common Heavy Metals Using Free-Floating Macrophytes

The most widely used free-floating macrophytes belong to the genus *Eichhornia*. *E. crassipes* that is commonly known as water hyacinth; a well-known macrophyte with an ability to absorb pollutants from contaminated water [109]. This plant is also known for its ability to grow faster [110], growing over 60 kg per each $m^2$ of water surface, thus an excellent choice for phytoremediation. It was used as an ornamental plant because of its attractive appearance. It belongs to the family Pontederiaceae and is a native of Brazil and Ecuador region [111]. It has long roots that are dispersed in the water; this elaborate root system allows the absorption of metals from the polluted waters. Water hyacinth has been applied to contaminated waters and has been successfully able to remove heavy metals with the highest efficiency for copper and lowest for cadmium with moderate remediation ability for zinc, nickel, and lead [112]. A study conducted on removal of zinc and cadmium from contaminated waters using hyacinths showed that the metals accumulated in the plant biomass (i.e., stems, leaves, roots, and flowers) with maximum metal accumation of 2040 mg/kg for Cd and 9650 mg/kg for Zn observed in the roots [113]. It was reported to decontaminate a liter of polluted water containing 1.5 g/L arsenic has been reported to be decontaminated with just 30 g of dried roots of water hyacinth root in just over a day Emerhi, 2011 reported an 87.52% removal for Cr III in contaminated water sources[114] .

Another free-floating macrophyte commonly known by the name of duckweed has four genera with *Wolffiella* being the smallest plant and the fastest growing too [115]. A group reported that *Lemna* sp. of the duckweeds is the most preferable choice for phytoremediation over other macrophytes; it's known to remove not just metals but phosphorous and nitrogen too from contaminated waters [116]. Species of *Lemna* were found to be more effective than *Azolla* and *Salvinia minima* that are both hyperaccumulators. Several works conducted on *L. minor* indicate its ability to pile up excessive concentrations of different heavy metals including uranium and boron too [62]. Interestingly, Miretzky et al. [60] have reported that dried dead *L. minor* was successful in removing Zn, Cu, and Cd from heavy metal-polluted waters [117].

Another free-floating plant belonging to the family Araceae, namely, *Pistia stratiotes* (L.), famous by the common name of water lettuce was reported in many studies as a phytoremediating plant [118]. Large amounts of heavy metals like iron, magnesium, cadmium, cobalt, manganese, etc. adsorbed to roots of water lettuce. Some reports claim that water lettuce was able to remediate around 80% of Hg in 21 days with most accumulation noted within roots [119].

*Salvinia* sp. are also well known for their metal-absorbing and metal-removing potential in wastewater; it has been tested for removing varying concentrations of a list of heavy metals, namely, Hg, Cr, As, Zn, Cd, Cu, Mn, Ni, and Fe [120].

### *8.5.4 Phytoremediation of Common Heavy Metals Using Emerged and Submerged Macrophytes*

Another class of macrophytes represented by submerged and emerged plants is capable of scavenging heavy metals [121]; *Juncus* sp. and *Typha* sp. are two emergent hydrophytes that can pile up very high concentrations of metals and metalloids both in their roots and shoots with relatively higher concentration in roots [62]. Twelve different species of submerged macrophytes, namely, *Vallisneria*, *Ceratophyllum*, *Myriophyllum*, *Potamogeton* spp., *Elodea*, *Hydrilla*, etc., were tested for their bioremediation potential of the following heavy metals: As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. All plants showed good metal-scavenging capability [107]. *Najas* sp. showed maximum concentration of 1117.65 mg/kg for arsenic, chromium, cobalt, and cadmium [122].

Emergent plants are also well known for their ability to remove heavy metals from wastewater. They tend to accumulate target metals or other pollutants within their organs especially roots and leaves. The metals tend to adsorb and strictly bind to the anionic sites available on the root cell walls [123]. *Phragmites* sp. and *Typha latifolia* are famous hyper-accumulators they can concentrate Cu, Ni, Cd and Pb to about 0.1 % of their dry weight while they are capable of accumalating Fe and Zn to about 1% of their dry weight i.e. roughly about 100 times more [123]. The order of metal removal observed for the two plants separately and when used in combination can be represented in Table 8.6. Table 8.6 clearly shows that the first metal to be removed is Fe, while the last is always Cd for all the three macrophytes, indicating a pattern that the more lethal and toxic a metal is, the longer it takes to get removed from the environment.

**Table 8.6** Preferential metal removal order

| Emergent plants | Metal removal order |
|---|---|
| *P. australis* | Fe > Zn > Cu > Ni > Cr ≅ Pb > Cd |
| *T. latifolia* | Zn > Cu ≅ Fe > Ni > Cr > Pb > Cd |
| *P. australis* and *T. latifolia.* | Fe > Cu > Zn > Ni > Cr > Pb ≅ Cd |

## 8.6 Constructed Wetlands: A Tool for Phytoremediation

These are engineered systems that mimic natural wetlands and are constructed in such a way that they use the naturally available plants and microorganisms in that wetland to treat polluted waters [124]. One major advantage of constructed wetland over natural wetland is the stringent conditions provided. The first wetland using macrophytes to decontaminate wastewater was developed in the 1950s in Germany by Käthe Seidel at the Max Planck Institute in Plön [125]. Since then they have been used and modified over the years and have emerged as a reliable technology. They are categorized into different types depending on the dominating vegetation type (macrophyte used), i.e., free-floating, emergent, or submerged [1]; wetland hydrology, i.e., free surface water or subsurface water; and most importantly the direction of water flow in the wetland systems, i.e., horizontal or vertical.

Seidel developed several experiments for treating different wastewater types, which included livestock wastewater, phenolic wastewater, and dairy wastewaters. He used both horizontal flow and vertical flow systems with subsurface water flow. However, in the Netherlands in 1969, a free-water surface constructed wetland was developed for the first time [126].

Nowadays however both horizontal and vertical constructed wetlands can be combined in a stage manner to produce a hybrid system to achieve higher treatment efficiency. The wetland types have been represented in Fig. 8.2 [127].

### 8.6.1 *Constructed Wetlands with Horizontal Flow*

HF CWs are constructed using rocks and pebbles of around 10–20 mm size that are sealed using impermeable layers, and then wetland plants are added to the above combination [128]. Contaminated water enters the system through a feeding inlet, moves over the rock bed in a horizontal manner, and then finally is discharged via outlet zone. Contaminants and pollutants are removed through a combination of physical, chemical, and biological processes in the filtration zone. These wetlands

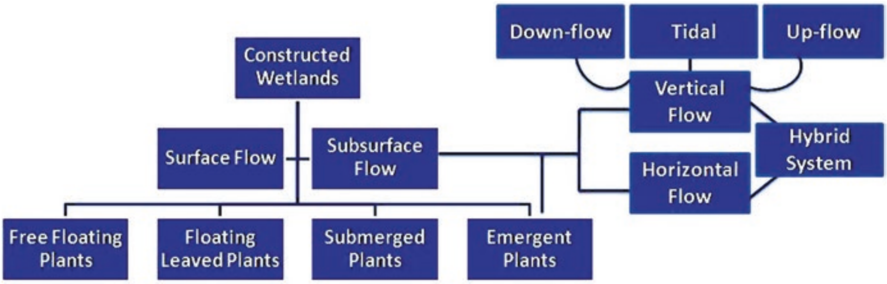


**Fig. 8.2** Diagramatic display of constructed wetlands and their types

provide a mixture of habitats, i.e., aerobic as well as anaerobic, allowing microbial degradation using both aerobic and anaerobic bacteria [129].

Organic compounds get removed via the action of aerobic as well as anaerobic bacteria with anoxic environments dominating in the filtration beds. Nitrogen gets removed via denitrification [130]. Phosphorus removal is very limited, and the very little removal occurs via cation exchange mechanisms; similarly ammonia removal is also extremely limited due to oxygen-deficient condition in the sediment beds [131].

### *8.6.2 Constructed Wetlands with Vertical Flow*

Constructed wetlands with vertical water flow are more complex than horizontal flow, are high maintenance, and were originally introduced by Seidel as a modification to oxygenate anaerobic septic tank effluents. Water is introduced in the form of batches allowed to seep to the sand beds; these systems can't denitrify because they are mostly highly aerobic. These are recommended for treatment of sewage and domestic wastewaters.

### *8.6.3 Heavy Metal Removal Using Constructed Wetlands*

Various studies have been conducted that employ constructed wetlands for treatment of domestic wastewaters, storm runoff waters, agricultural wastewaters, sewage wastewaters, and most importantly industrial wastewaters. Cheng et al., 2002 [132] studied the treatment efficiencies of constructed wetlands for removing heavy metals and observed that removal efficiency was 100% with consistent results for up to 5 months except manganese whose removal efficiency decreased to 42% during the 3rd week. The detection limits of aluminum, cadmium, copper, lead, and zinc were extremely low proving that constructed wetlands using tropical plants is also beneficial.

Metals accumulated in the plant roots both main and lateral roots as well as rhizomes also within the plant leaves and shoots. Major metal accumulation was observed in the lateral roots and then main roots followed by rhizomes and plant leaves; lowest metal concentration was observed in the shoots [133].

## 8.7 Conclusion

Water pollution is increasing with industrial effluents and heavy metals causing increasing health alarms. Nonavailability of clean water due to industrialization and urbanization is a growing concern with estimates of removal of clean water reserves over the coming years. This has prompted scientists to look for available solutions with water remediation being the most plausible of all. Water decontamination using conventional approaches is costly and time taking so scientists moved to the more economical approach of phytoremediation, i.e., using plants especially water-dwelling weeds and hydrophytes to clean water. Macrophytes are the most abundantly used aquatic plants for heavy metal removal because of their innate ability to act as hyperaccumulators for most of the commonly occurring heavy metals. Water hyacinth, water lettuce, and *Lemna* species are the most frequently encountered phytoremediating plants. This approach is cost-effective and also environmentally friendly. The plant's efficiency of phytoremediation is affected by their rate of growth, biomass, and bioaccumulation potential.

Future prospects involve utilization of genetically engineered plants with enhanced metal uptake capacity to ensure absolute removal of metals; these engineered plants shall have rapid growth cycles too to ensure less time and more phytoremediating potential. These can also be used to remove other contaminants from water like hydrocarbons and other toxic and carcinogenic compounds. Another modification to this method will be using rhizospheric plant growth-promoting bacteria that can work in conjunction with the hyperaccumulator plants to give a maximum remediation effect.

## References

1. Kahil MT, Dinar A, Albiac J (2015) Modeling water scarcity and droughts for policy adaptation to climate change in arid and semiarid regions. J Hydrol 522:95–109
2. Kneese AV (2015) Water pollution: economics aspects and research needs. Routledge, London
3. Al-Mulali U, Ozturk I (2015) The effect of energy consumption, urbanization, trade openness, industrial output, and the political stability on the environmental degradation in the MENA (Middle East and North African) region. Energy 84:382–389
4. Martens P (2014) Health and climate change: modelling the impacts of global warming and ozone depletion. Routledge, London
5. Lu Y, Song S, Wang R, Liu Z, Meng J, Sweetman AJ, Jenkins A, Ferrier RC, Li H, Luo W (2015) Impacts of soil and water pollution on food safety and health risks in China. Environ Int 77:5–15
6. Seinfeld JH, Pandis SN (2016) Atmospheric chemistry and physics: from air pollution to climate change. John Wiley & Sons, Hoboken, NJ
7. Stanley MC, Beggs JR, Bassett IE, Burns BR, Dirks KN, Jones DN, Linklater WL, Macinnis-Ng C, Simcock R, Souter-Brown G (2015) Emerging threats in urban ecosystems: a horizon scanning exercise. Front Ecol Environ 13:553–560
8. Elmqvist T, Zipperer W, Güneralp B (2016) Urbanization, habitat loss, biodiversity decline: solution pathways to break the cycle. In: Seta K, Solecki WD, Griffith CA (eds) Routledge handbook of urbanization and global environmental change. Routledge, London, pp 139–151
9. Sun G, Michelsen AM, Sheng Z, Fang AF, Shang Y, Zhang H (2015) Featured collection introduction: water for megacities—challenges and solutions. J Am Water Resour Assoc 51:585–588
10. Cao S, Lv Y, Zheng H, Wang X (2014) Challenges facing China's unbalanced urbanization strategy. Land Use Policy 39:412–415

11. McDonnell MJ, MacGregor-Fors I (2016) The ecological future of cities. Science 352:936–938
12. Tritsch I, Le Tourneau F-M (2016) Population densities and deforestation in the Brazilian Amazon: new insights on the current human settlement patterns. Appl Geogr 76:163–172
13. Sahu K (2016) Heavy metal pollution of air, water and soil—a review. SGAT Bull 2016:16
14. Constant K, Nourry C, Seegmuller T (2014) Population growth in polluting industrialization. Resour Energy Econ 36:229–247
15. Laws EA (2017) Aquatic pollution: an introductory text. John Wiley & Sons, Hoboken, NJ
16. Varjani SJ, Agarwal AK, Gnansounou E, Gurunathan B (2018) Bioremediation: applications for environmental protection and management. Springer, New York, NY
17. Iriel A, Lagorio MG, Cirelli AF (2015) Biosorption of arsenic from groundwater using Vallisneria gigantea plants. Kinetics, equilibrium and photophysical considerations. Chemosphere 138:383–389
18. 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
19. Jenkins SH (2015) Advances in water pollution research: proceedings of the Fourth International Conference Held in Prague 1969. Elsevier, New York City, NY
20. Förstner U, Wittmann GT (2012) Metal pollution in the aquatic environment. Springer, New York, NY
21. Rai PK (2009a) Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Crit Rev Environ Sci Technol 39:697–753
22. Segerson K (2017) Non-point source pollution in an international context. Reference on Natural Resources and Environmental Policy in the Era of Global Change: Volume 1: Game theory. World Scientific 1:115–137
23. Oki T, Kanae S (2006) Global hydrological cycles and world water resources. Science 313:1068–1072
24. Ebrahiem EE, Al-Maghrabi MN, Mobarki AR (2017) Removal of organic pollutants from industrial wastewater by applying photo-Fenton oxidation technology. Arab J Chem 10:S1674–S1679
25. Tichonovas M, Krugly E, Racys V, Hippler R, Kauneliene V, Stasiulaitiene I, Martuzevicius D (2013) Degradation of various textile dyes as wastewater pollutants under dielectric barrier discharge plasma treatment. Chem Eng J 229:9–19
26. Deblonde T, Cossu-Leguille C, Hartemann P (2011) Emerging pollutants in wastewater: a review of the literature. Int J Hyg Environ Health 214:442–448
27. Wegman RC, Melis PH, Josefsson B (1986) Organic pollutants in water. American Chemical Society, Washington, DC
28. Comstock MJ Organic pollutants in water, copyright, advances in chemistry series, foreword, about the editors: sampling, analysis, and toxicity testing. ACS Publications, Washington, DC
29. Potter DW, Pawliszyn J (1994) Rapid determination of polyaromatic hydrocarbons and polychlorinated biphenyls in water using solid-phase microextraction and GC/MS. Environ Sci Technol 28:298–305
30. Duruibe J, Ogwuegbu M, Egwurugwu J (2007) Heavy metal pollution and human biotoxic effects. Int J Phys Sci 2:112–118
31. Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manag 92:407–418
32. Inoue K (2013) Heavy metal toxicity. J Clin Toxicol 3:2161–0495
33. Singh N, Kumar D, Sahu AP (2007) Arsenic in the environment: effects on human health and possible prevention. J Environ Biol 28:359
34. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7:60–72
35. Patrick L (2002) Mercury toxicity and antioxidants: part I: role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity-mercury toxicity. Toxicol Appl Pharmacol 7:456–471

36. Patrick L (2003) Toxic metals and antioxidants: part II the role of antioxidants in arsenic and cadmium toxicity. (Toxic metals part II). Altern Med Rev 8:106–129
37. Stohs S, Bagghi D (2005) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 39:1267–1268
38. Shaw C, Tomljenovic L (2013) Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity. Immunol Res 56:304–316
39. Grazuleviciene R, Nadisauskiene R, Buinauskiene J, Grazulevicius T (2009) Effects of elevated levels of manganese and iron in drinking water on birth outcomes. Pol J Environ Stud 18:819–825
40. Domingo JL (2001) Reproductive and developmental toxicity of natural and depleted uranium: a review. Reprod Toxicol 15:603–609
41. Kurttio P, Harmoinen A, Saha H, Salonen L, Karpas Z, Komulainen H, Auvinen A (2006) Kidney toxicity of ingested uranium from drinking water. Am J Kidney Dis 47:972–982
42. Denkhaus E, Salnikow K (2002) Nickel essentiality, toxicity, and carcinogenicity. Crit Rev Oncol Hematol 42:35–56
43. Gaetke LM, Chow CK (2003) Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189:147–163
44. Mahmood A, Malik RN (2014) Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arab J Chem 7:91–99
45. Wongsasuluk P, Chotpantarat S, Siriwong W, Robson M (2014) Heavy metal contamination and human health risk assessment in drinking water from shallow groundwater wells in an agricultural area in Ubon Ratchathani province, Thailand. Environ Geochem Health 36:169–182
46. Sheehan MC, Burke TA, Navas-Acien A, Breysse PN, McGready J, Fox MA (2014) Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull World Health Organ 92:254–269F
47. Grandjean P, Weihe P, Debes F, Choi AL, Budtz-Jørgensen E (2014) Neurotoxicity from prenatal and postnatal exposure to methylmercury. Neurotoxicol Teratol 43:39–44
48. Matović V, Buha A, Đukić-Ćosić D, Bulat Z (2015) Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food Chem Toxicol 78:130–140
49. García-Esquinas E, Pollan M, Tellez-Plaza M, Francesconi KA, Goessler W, Guallar E, Umans JG, Yeh J, Best LG, Navas-Acien A (2014) Cadmium exposure and cancer mortality in a prospective cohort: the strong heart study. Environ Health Perspect 122:363
50. Tsai T-L, Kuo C-C, Pan W-H, Chung Y-T, Chen C-Y, Wu T-N, Wang S-L (2017) The decline in kidney function with chromium exposure is exacerbated with co-exposure to lead and cadmium. Kidney Int 92(3):710–720
51. Kundu A, Lim JS, Tae IH, Lim J-a, Joo H, Ha M, Bae O-N, Lee BM, Kim HS (2016) Insights into the impact of lead exposure to children through [1H] NMR-based metabolomics. 환경독성보건학회 심포지엄 및 학술대회 10:280–280
52. Sharma S, Singh B, Manchanda V (2015) Phytoremediation: role of terrestrial plants and aquatic macrophytes in the remediation of radionuclides and heavy metal contaminated soil and water. Environ Sci Pollut Res 22:946–962
53. 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
54. van der Ent A, Baker AJ, Reeves RD, Pollard AJ, Schat H (2015) Commentary: toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants. Front Plant Sci 6:554
55. Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174:1–8
56. Annapurna D, Rajkumar M, Prasad M (2016) Potential of Castor bean (Ricinus communis L.) for phytoremediation of metalliferous waste assisted by plant growth-promoting bacte-

ria: possible cogeneration of economic products. Bioremediation and bioeconomy. Elsevier, New York, NY, pp 149–175

57. Weyens N, Thijs S, Popek R, Witters N, Przybysz A, Espenshade J, Gawronska H, Vangronsveld J, Gawronski SW (2015) The role of plant–microbe interactions and their exploitation for phytoremediation of air pollutants. Int J Mol Sci 16:25576–25604
58. Yongpisanphop J, Babel S, Kruatrachue M, Pokethitiyook P (2017) Hydroponic screening of fast-growing tree species for lead phytoremediation potential. Bull Environ Contam Toxicol 99:518–523
59. Callery S, Courtney R (2015) Assessing metal transfer to vegetation and grazers on reclaimed pyritic Zn and Pb tailings. Environ Sci Pollut Res 22:19764–19772
60. Miretzky P, Saralegui A, Cirelli AF (2004) Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). Chemosphere 57:997–1005
61. Mishra VK, Shukla R (2016) Aquatic macrophytes for the removal of heavy metals from coal mining effluent. Phytoremediation. Springer, New York, NY, pp 143–156
62. Rezania S, Taib SM, Din MFM, Dahalan FA, Kamyab H (2016) Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. J Hazard Mater 318:587–599
63. Sakakibara M (2016) Phytoremediation of toxic elements-polluted water and soils by aquatic macrophyte Eleocharis acicularis. AIP conference proceedings. AIP Publishing, Melville, NY, p 020038
64. Yamazaki S, Okazaki K, Kurahashi T, Sakakibara M (2017) Phytoremediation of arsenic-and molybdenum-contaminated alkaline wastewater by Eleocharis acicularis in winter in Japan. IOP conference series: earth and environmental science. IOP Publishing, Bristol, p 012018
65. Nurfitri A, Masayuki S, Koichiro S (2017) Phytoremediation of heavy metal-polluted mine drainage by Eleocharis Acicularis. Environ Sci:13, 131
66. Das S, Goswami S, Talukdar AD (2014) A study on cadmium phytoremediation potential of water lettuce, Pistia stratiotes L. Bull Environ Contam Toxicol 92:169–174
67. Chaudhary E, Sharma P (2014) Duckweed plant: a better future option for phytoremediation. Int J Emerg Sci Eng 2:39–41
68. Dar MI, Khan FA, Rehman F, Masoodi A, Ansari AA, Varshney D, Naushin F, Naikoo MI (2015) Roles of Brassicaceae in phytoremediation of metals and metalloids. Phytoremediation. Springer, New York, NY, pp 201–215
69. Goolsby EW, Mason CM (2015) Toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants. Front Plant Sci 6:33
70. Anjum NA, Umar S, Iqbal M (2014) Assessment of cadmium accumulation, toxicity, and tolerance in Brassicaceae and Fabaceae plants—implications for phytoremediation. Environ Sci Pollut Res 21:10286–10293
71. Angelova V, Ivanova R, Todorov J, Ivanov K (2017) Potential of rapeseed (Brassica napus L.) for phytoremediation of soils contaminated with heavy metals. J Environ Prot Ecol 18:468–478
72. Baker AJ (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654
73. Dhiman SS, Selvaraj C, Li J, Singh R, Zhao X, Kim D, Kim JY, Kang YC, Lee J-K (2016) Phytoremediation of metal-contaminated soils by the hyperaccumulator canola (Brassica napus L.) and the use of its biomass for ethanol production. Fuel 183:107–114
74. Liu H, Yuan M, Tan S, Yang X, Lan Z, Jiang Q, Ye Z, Jing Y (2015) Enhancement of arbuscular mycorrhizal fungus (Glomus versiforme) on the growth and Cd uptake by Cd-hyperaccumulator Solanum nigrum. Appl Soil Ecol 89:44–49
75. Wu Q, Leung JY, Huang X, Yao B, Yuan X, Ma J, Guo S (2015b) Evaluation of the ability of black nightshade Solanum nigrum L. for phytoremediation of thallium-contaminated soil. Environ Sci Pollut Res 22:11478–11487
76. Hussain S, Akram M, Abbas G, Murtaza B, Shahid M, Shah NS, Bibi I, Niazi NK (2017) Arsenic tolerance and phytoremediation potential of Conocarpus erectus L. and Populus deltoides L. Int J Phytoremediation 19(11):985–991

77. Chang F-C, Ko C-H, Tsai M-J, Wang Y-N, Chung C-Y (2014) Phytoremediation of heavy metal contaminated soil by Jatropha curcas. Ecotoxicology 23:1969–1978
78. Marrugo-Negrete J, Durango-Hernández J, Pinedo-Hernández J, Olivero-Verbel J, Díez S (2015) Phytoremediation of mercury-contaminated soils by Jatropha curcas. Chemosphere 127:58–63
79. El-Ramady HR, Abdalla N, Alshaal T, Fári M, Prokisch J, Pilon-Smits EA, Domokos-Szabolcsy É (2015) Selenium phytoremediation by giant reed. Hydrogen production and remediation of carbon and pollutants. Springer, New York, NY, pp 133–198
80. Trivedi S, Ansari AA (2015) Molecular mechanisms in the phytoremediation of heavy metals from coastal waters. Phytoremediation. Springer, New York, NY, pp 219–231
81. Lange B, Ent A, Baker AJM, Echevarria G, Mahy G, Malaisse F, Meerts P, Pourret O, Verbruggen N, Faucon MP (2017) Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytol 213:537–551
82. Reeves RD, Baker AJ, Jaffré T, Erskine PD, Echevarria G, Ent A (2017) A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol 218(2):407–411
83. van der Ent A, Tang Y-T, Sterckeman T, Echevarria G, Morel J-L, Qiu R-L (2017) Nickel hyperaccumulation mechanisms: a review on the current state of knowledge. Plant Soil 423:1–11
84. Merlot S, de la Torre VSG, Hanikenne M (2018) Physiology and molecular biology of trace element hyperaccumulation. Agromining: farming for metals. Springer, New York, NY, pp 93–116
85. Marques AP, Rangel AO, Castro PM (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654
86. Venegas A, Rigol A, Vidal M (2015) Viability of organic wastes and biochars as amendments for the remediation of heavy metal-contaminated soils. Chemosphere 119:190–198
87. Tangahu BV, Sheikh Abdullah SR, 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 2011:939161
88. Thakur S, Singh L, Ab Wahid Z, Siddiqui MF, Atnaw SM, Din MFM (2016) Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. Environ Monit Assess 188:206
89. Ullah A, Heng S, Munis MFH, Fahad S, Yang X (2015) Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ Exp Bot 117:28–40
90. Danh LT, Truong P, Mammucari R, Foster N (2014) A critical review of the arsenic uptake mechanisms and phytoremediation potential of Pteris vittata. Int J Phytoremediation 16:429–453
91. Liu D, Islam E, Ma J, Wang X, Mahmood Q, Jin X, Li T, Yang X, Gupta D (2008) Optimization of chelator-assisted phytoextraction, using EDTA, lead and Sedum alfredii Hance as a model system. Bull Environ Contam Toxicol 81:30–35
92. Yadav AK, Pathak B, Fulekar M (2015) Rhizofiltration of heavy metals (cadmium, lead and zinc) from fly ash leachates using water hyacinth (Eichhornia Crassipes). Int J Environ 4:179–196
93. Gaur N, Flora G, Yadav M, Tiwari A (2014) A review with recent advancements on bioremediation-based abolition of heavy metals. Environ Sci Processes Impacts 16:180–193
94. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881
95. Martínez-Alcalá I, Clemente R, Bernal M (2012) Efficiency of a phytoimmobilisation strategy for heavy metal contaminated soils using white lupin. J Geochem Explor 123:95–100
96. Houben D, Pircar J, Sonnet P (2012) Heavy metal immobilization by cost-effective amendments in a contaminated soil: effects on metal leaching and phytoavailability. J Geochem Explor 123:87–94
97. Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729

98. Bennicelli R, Stępniewska Z, Banach A, Szajnocha K, Ostrowski J (2004) The ability of Azolla caroliniana to remove heavy metals (Hg (II), Cr (III), Cr (VI)) from municipal waste water. Chemosphere 55:141–146
99. Török A, Gulyás Z, Szalai G, Kocsy G, Majdik C (2015) Phytoremediation capacity of aquatic plants is associated with the degree of phytochelatin polymerization. J Hazard Mater 299:371–378
100. Wani R, Ganai B, Shah M, Uqab B (2017) Heavy metal uptake potential of aquatic plants through phytoremediation technique—a review. J Bioremed Biodegr 8:2
101. Mishra VK, Tripathi B, Kim K-H (2009) Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. J Hazard Mater 172:749–754
102. Rai PK (2009b) Microcosm investigation on phytoremediation of Cr using Azolla pinnata. Int J Phytoremediation 12:96–104
103. Pflugmacher S, Kühn S, Lee S-H, Choi J-W, Baik S, Kwon K-S, Contardo-Jara V (2015) Green liver systems® for water purification: using the phytoremediation potential of aquatic macrophytes for the removal of different cyanobacterial toxins from water. Am J Plant Sci 6:1607
104. Kolada A, Pasztaleniec A, Bielczyńska A, Soszka H (2016) Phytoplankton, macrophytes and benthic diatoms in lake classification: consistent, congruent, redundant? Lessons learnt from WFD-compliant monitoring in Poland. Limnol Ecol Manag Inland Waters 59:44–52
105. Pi N, Ng J, Kelly B (2017) Uptake and elimination kinetics of perfluoroalkyl substances in submerged and free-floating aquatic macrophytes: results of mesocosm experiments with Echinodorus horemanii and Eichhornia crassipes. Water Res 117:167–174
106. Newete SW, Byrne MJ (2016) The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. Environ Sci Pollut Res 23:10630–10643
107. Wang Z, Yao L, Liu G, Liu W (2014) Heavy metals in water, sediments and submerged macrophytes in ponds around the Dianchi Lake, China. Ecotoxicol Environ Saf 107:200–206
108. Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, Azolla. Ambio 41:122–137
109. Xiaoyong Z, Yayun T, Jubin Z (2015) Adsorption of Cu(2+) and Pb(2+) from aqueous solution with water hyacinth. Technol Water Treat 2:13
110. Patel S (2012) Threats, management and envisaged utilizations of aquatic weed Eichhornia crassipes: an overview. Rev Environ Sci Biotechnol 11:249–259
111. Chunkao K, Nimpee C, Duangmal K (2012) The King's initiatives using water hyacinth to remove heavy metals and plant nutrients from wastewater through Bueng Makkasan in Bangkok, Thailand. Ecol Eng 39:40–52
112. Gupta P, Roy S, Mahindrakar AB (2012) Treatment of water using water hyacinth, water lettuce and vetiver grass—a review. Resour Environ 2:202–215
113. Singh D, Tiwari A, Gupta R (2012) Phytoremediation of lead from wastewater using aquatic plants. J Agric Technol 8:1–11
114. Emerhi E (2011) Physical and combustion properties of briquettes produced from sawdust of three hardwood species and different organic binders. Adv Appl Sci Res 2:236–246
115. Demim S, Drouiche N, Aouabed A, Benayad T, Couderchet M, Semsari S (2014) Study of heavy metal removal from heavy metal mixture using the CCD method. J Ind Eng Chem 20:512–520
116. Zhang D, Gersberg RM, Ng WJ, Tan SK (2014) Removal of pharmaceuticals and personal care products in aquatic plant-based systems: a review. Environ Pollut 184:620–639
117. Bokhari SH, Ahmad I, Mahmood-Ul-Hassan M, Mohammad A (2016) Phytoremediation potential of Lemna minor L. for heavy metals. Int J Phytoremediation 18:25–32
118. Prajapati SK, Meravi N, Singh S (2012) Phytoremediation of Chromium and Cobalt using Pistia stratiotes: a sustainable approach. Proc Int Acad Ecol Environ Sci 2:136
119. Rodrigues ACD, do Amaral Sobrinho NMB, dos Santos FS, dos Santos AM, Pereira ACC, Lima ESA (2017) Biosorption of toxic metals by water lettuce (Pistia stratiotes) Biomass. Water Air Soil Pollut 228:156

120. Ng YS, Chan DJC (2017) Phytoremediation capabilities of Spirodela polyrhiza, Salvinia molesta and Lemna sp. in synthetic wastewater: a comparative study. Int J Phytoremediation:29053371
121. Ladislas S, El-Mufleh A, Gérente C, Chazarenc F, Andrès Y, Béchet B (2012) Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban stormwater runoff. Water Air Soil Pollut 223:877–888
122. Xing W, Wu H, Hao B, Huang W, Liu G (2013) Bioaccumulation of heavy metals by submerged macrophytes: looking for hyperaccumulators in eutrophic lakes. Environ Sci Technol 47:4695–4703
123. Kumari M, Tripathi B (2015) Efficiency of Phragmites australis and Typha latifolia for heavy metal removal from wastewater. Ecotoxicol Environ Saf 112:80–86
124. Kadlec RH, Knight R, Vymazal J, Brix H, Cooper P, Haberl R (2017) Constructed wetlands for pollution control. IWA Publishing, London
125. Wu H, Zhang J, Ngo HH, Guo W, Hu Z, Liang S, Fan J, Liu H (2015a) A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. Bioresour Technol 175:594–601
126. Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549
127. de la Varga D, Soto M, Arias CA, van Oirschot D, Kilian R, Pascual A, Álvarez JA (2017) Constructed wetlands for industrial wastewater treatment and removal of nutrients. Technologies for the treatment and recovery of nutrients from industrial wastewater. Inform Sci Ref 2018:202–230
128. Abou-Elela SI, Golinielli G, Abou-Taleb EM, Hellal MS (2013) Municipal wastewater treatment in horizontal and vertical flows constructed wetlands. Ecol Eng 61:460–468
129. Pedescoll A, Sidrach-Cardona R, Hijosa-Valsero M, Bécares E (2015) Design parameters affecting metals removal in horizontal constructed wetlands for domestic wastewater treatment. Ecol Eng 80:92–99
130. Zhu H, Yan B, Xu Y, Guan J, Liu S (2014) Removal of nitrogen and COD in horizontal subsurface flow constructed wetlands under different influent C/N ratios. Ecol Eng 63:58–63
131. Vymazal J (2011b) Long-term performance of constructed wetlands with horizontal subsurface flow: ten case studies from the Czech Republic. Ecol Eng 37:54–63
132. 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
133. Vymazal J (2011a) Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674:133–156

# Part IV

# Phytoremediation and Microbial Applications

# Chapter 9
# A Promising Role of Lichens, Their Secondary Metabolites and miRNAs on Treatment of Cancer Disease After Exposure to Carcinogenic Heavy Metals

**Vildan Torun, Elif Değerli, and Demet Cansaran-Duman**

## 9.1 Introduction

### *9.1.1 The Effect of Cancer-Causing Chemicals on Environment*

With the rapid development of industrialization and urbanization in recent years, pollution has reached very serious levels, and this situation threatens public health. One of the most dangerous diseases caused by cancer-causing chemicals is cancer. Cancer-causing chemicals are called substances that cause cancer. Cancer-causing chemicals include solvents, heavy metals, pesticides, radioisotopes, carcinogenic microorganisms, and synthetic derivatives of industrial by-products [1]. Cancer-causing chemicals do not always cause cancer, but many of cancer-causing chemicals induce cancer after prolonged and high exposure. The risk of the development of cancer depends on many factors for people, such as the exposed duration and intensity of exposure, the person's genetic profile and how it is exposed, etc. The International Agency for Research on Cancer (IARC), one of the important sections of the World Health Organization (WHO), has identified cancer-causing potential of different substances. In the past 50 years, the IARC has defined the cancer-causing potential of more than 900 substances. The IARC has classified the substances that have the potential of causing cancer into four different groups. These are Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans; Group 2B, possibly carcinogenic to humans; Group 3, unclassifiable as to carcinogenicity in humans; and Group 4, probably not carcinogenic to humans [2]. As an example to Group 1, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a class I human carcinogen [3] and is shown to cause lymphoma and fibrosarcomas [4]. The

---

V. Torun · E. Değerli · D. Cansaran-Duman (✉)
System Biotechnology Advance Research Unit, Biotechnology Institute, Ankara University, Ankara, Turkey

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_9

carcinogenic effects of TCDD contain oxidative damage of the chromosome, altering signal transduction pathways and the replication cycle [5].

Humans are exposed to cancer-causing chemicals in the environment. Cancer-causing chemicals are deleterious, and there is not enough available information on toxicity effect of these chemicals. In recent years, there is a growing interest around the world to investigate the connection between exposure to toxic and cancer-causing chemicals and incidence of cancer [6]. A few studies have determined the effect of cancer-causing chemicals on the initiation, promotion, and progression of cancer on humans [6]. Exposure to these carcinogens has caused many types of cancer, including skin, lung, and breast cancer, by affecting different cellular mechanism. For example, some carcinogens do not directly affect DNA; they can cause cells to divide faster than normal cycle, which can increase the likelihood of DNA changes [2].

### 9.1.2 The Influence of Heavy Metal to Human Health

Since the age of industrialization, humans have been using heavy metals for industry, agriculture, and medical activities, and heavy metals have polluted the environment either by natural means or by human activities [7]. Environmental pollution due to heavy metal usage negatively affects many people, and the harmful effects of heavy metals on human health cause some disease [6, 8]. Most of heavy metals are cancer-inducing agents. Almost all heavy metals are classified as Group 1 carcinogens by the IARC [6]. Because of lack of regulations and help from the government, large amounts of wastewater containing heavy metal from factories were released into the environment [9]. It is almost impossible to separate heavy metals after they have accumulated into soil and water, such as chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), arsenic (As), and zinc (Zn), all of which lead to risks for human health and the environment [6].

Jarup pointed out that the level of heavy metal toxicity may be different at the lower, long-term exposure, and recurrent long-term exposure. According to these three types of effect levels, the types of diseases also vary. If heavy metal toxicity is at lower levels, it can damage the functioning of the brain, lungs, kidney, liver, and other important organs and blood composition. Long-term exposure to heavy metal toxicity can lead to neurodegenerative diseases in humans such as multiple sclerosis, Parkinson’s, Alzheimer’s disease, and muscular dystrophy. Repeated long-term exposure of some heavy metals may even cause cancer disease [10, 11].

Heavy metals penetrate into the body through air, food, water, or dermal exposure. Heavy metals have to pass through the plasma membrane to enter the cell. The type of membrane transition is different for some metals. For example, if the metal is in a lipophilic form such as methylmercury and arsenic compounds, it easily penetrates the membrane [12]. When protein bound to cadmium-metallothionein, this metal is actively involved by endocytosis into the cell [13]. Other metals, such as lead, can be absorbed by passive diffusion [6, 14]. The genotoxic effect formation of metals consists of two different ways. Firstly, it occurs when macromolecules interact with metals. The other can be made by electrophilic derivatives or binding the metal to DNA [15]. Many studies have shown that many metals directly alter and/or damage DNA [6, 16]. Studies carried out in our laboratory have determined DNA damage that occurs in lichen species after the heavy metal exposure to them. As a result of our study, it is shown that lichens can be used as biomarkers [17–19]. Previous study determined that some heavy metals such as As, manganese (Mn), Pb, and mercury (Hg) promoted cell proliferation by estradiol in estrogen-sensitive cancer cell lines [20, 21]. It is still unknown how overexposure of this trace metal affects human cells in the literature, but some toxicological studies have been conducted.

### *9.1.3 Several Heavy Metals May Cause Cancer Disease*

Heavy metal carcinogenesis is a process driven by their reaction with critical molecules in body cells (Zhang et al. 2007). The role of heavy metals in the development and inhibition of cancer generates many questions about their essential and toxic effects on human health [22, 23]. There is growing evidence that environmental contaminants such as metals play a role in disease genesis especially cancer. Although studies on cancer and metals are limited, this review suggests that there may be a relationship between exposure to certain metal compounds and the risk of cancer [6]. The IARC and National Toxicology Program (NTP) have announced that certain heavy metals such as cadmium (Cd), nickel (Ni), arsenic (As), beryllium (Be), and especially chromium VI ($Cr^{+6}$) have cancer-causing features on animals and human for recent 25 years [24]. It is an undeniable fact that humans are chronically exposed to various heavy metals from airborne particles, soil, water, and food in daily life. In the recent times, the different heavy metals are released to the environment by industrial applications, drug formulation, food additives, mining, refining metal ores, cement manufacturing plants, and gasoline and diesel vehicles [22, 24].

Almost all heavy metals, such as Cu and Zn, serve as various cancer and disease inducers [25–27]. It is a well-known mechanism of heavy metal-induced disruptions that reactive oxygen species (ROS) lead to oxidative stress [28, 29]. According to various reports, tumor suppressor gene expression, damage repair processes, and enzymatic activities concerned in metabolism are damaged by exposing to these compounds via oxidative damage [28, 30]. The risk of heavy metal exposure and the increase of occupational disease and cancer are correlated with the contamination source such as industrial areas [31–34]. Aberrant gene expression and loss of cellular components including DNA, lipids, and proteins derived from accumulation of free radicals from ROS give rise to cell death [35, 36].

Heavy metals as cancer-causing chemicals have been recognized to have a role in induction of malignant human tumor growths. Recently, certain heavy metals showed a close association to cancer. El-Harouny et al. conducted to find out the role of some toxic heavy metals (Mn, Cd, Fe, Cu, Pb, and Zn) in the induction of breast cancer in vivo [22]. One of the other dangerous heavy metals, Mn, is widely used in the industry. A study of Iregren showed that humans exposed to high doses of Mn may suffer neuronal injury, resembling Parkinson's disease [37]. Hirata reported that DNA fragmentation and apoptosis occur in PC12 cells exposed with Mn [38]. To further gain insight into the heavy metal toxicity, Deng et al. findings revealed that Mn exposure of cultured astrocytes from rats induces proliferation of cells, promotes the level of lactate dehydrogenase (LDH) leakage, and inhibits G0/G1 phase arrest and apoptosis [39, 40].

One of the most harmful heavy metals is Cr and it has carcinogenic effect. The carcinogenicity of Cr has been studied since the 1980s. For example, workers in the chromate-producing industry have more risk to have lung cancer according to a case study [41]. Several research groups determined that high concentrations of $Cr^{3+}$ in the body can cause cellular damage [36, 42]. Hexavalent Cr ($Cr^{6+}$) lead to emergence of reactive hydroxyl radicals by the process of reducing $Cr^{6+}$ to $Cr^{3+}$ in human blood vessels; therefore $Cr^{6+}$ is a toxic heavy metal form for human. In addition, it is known that $Cr^{6+}$ serves no essential biological role in humans. Recent research demonstrates that high level of $Cr^{6+}$ in the bloodstream causes blood cell damage and functional degradation of the liver and kidney [43]. Similar phenomena have been observed and reported in previous paper; $Cr^{6+}$ compounds are reduced to the pentavalent form, and they can bind DNA and interrupt the cellular processes [42]. Furthermore, Cr in water or soil damages the human health by absorption with the skin for long term [36, 44].

Cu is currently categorized by the EPA as a Group D carcinogen. A study on some workers who are exposed to mainly Cu has shown increased cancer risks although they were also exposed in the workplace to other chemicals with carcinogenic potential. Therefore, no available data has been presented in the literature to show that the cancer disease that occurs is only the result of the Cu metal effect. As a result of studying with experimental animals exposed to high doses of Cu, delayed growth and development, bone formation, and decreased body weights were observed [45]. Ni is a toxic heavy metal and has been widely detected in the environment, and it can be a harmful to human health. The higher exposure of Ni leads to many cancer types such as nose, larynx, and prostate and especially lung cancer [45]. Some authors have searched that link between lead (Pb) exposure in workers and cancer disease. The amount of Ni on workers' blood was measured after excessive nickel exposure, and the level of Ni on the blood was higher than normal blood lead concentration. Most of these workers have been diagnosed with lung cancer. But, further studies are evaluated to better understand the effect of other factors, such as smoking or exposures to arsenic or other heavy metals on human health [45].

Breast cancer is one of the most common among all cancer types, and the malignancy rate of breast cancer is fairly high [46]. The incidence of breast cancer is approximately over 1,300,000 women, and these breast cancer cases end up 450,000 deaths annually in the world [47]. Diagnosis of breast cancer is generally performed with mammography, biopsy, or serum tumor markers. Mammography is a fairly reliable detection method, and biopsy is used to definitive detection of the cancer [48]. On the other hand, the American Society of Clinical Oncology and other expert panels for screening or diagnosis of breast cancer do not recommend the detection of breast cancer via serum tumor markers because this method is not sufficiently sensitive in early stages of cancer [49]. Mortality of breast cancer can be reduced with early detection by efficient diagnostic tools. Therefore recent studies focus on more sensitive, specific, and noninvasive methods for early detection of breast cancer to increase survival rate of cancer patients [48].

Breast cancer is a heterogeneous disease because it reveals distinct tumor phenotypic features. Therefore breast cancer is commonly classified into subtypes according to some gene expression patterns, including hormone estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2/neu) [50]. In every year, approximately 700,000 hormone receptor-positive breast cancers are diagnosed among women [51]. On the other hand, expression of ER, PR, and HER2/neu is not observed in some types of breast cancer, and this type of breast cancer is defined as triple negative [52]. According to these distinct phenotypic characteristics, breast cancer is treated with different therapeutic approaches. For example, ER-positive (ER+) breast cancer patients are treated with tamoxifen, which is an antagonist to ER [53]. Triple-negative breast cancer is generally treated with chemotherapy because this type of breast cancer does not have specific target [54]. Chemotherapy is commonly applied to breast cancer patients for cancer treatment; however chemotherapy has many side effects such as destroying healthy cells and damaging the immune system of cancer patients. In addition, cancerous cells can gain resistance to chemotherapeutic agents, so chemotherapy becomes less effective on cancer treatment [55]. For this reason, early warning system (used as biomarker) based on biological organisms or novel therapeutic agents should be discovered for effective, less harmful, sensitive, and specific for breast cancer.

Recent studies have demonstrated that indirect or direct effect of certain heavy metals on progression of carcinogenic processes can be realized [6]. Previously, accumulation of heavy metals in breast tissue (unaltered and affected by tumor process) can affect the DNA fragmentation level and tumor cell survival [56]. The carcinogenic effect of heavy metals on human health can be found out because of the mechanisms of DNA structure breach and inhibition of antioxidant protection [15, 57, 58]. As a result of Stoica et al. study, the effect of As on estrogen-regulated genes in human breast cancer cell line MCF-7 was examined [59], and As stimulated cell proliferation and also mimicked the effects of estradiol [6]. The role of metals on cellular response in terms of the p53 status has been indicated in MCF-7 and MDA-MB231 breast cancer cell lines. For example, exposure of Zn to MDA-MB231 cells with mutant p53 results in resistance formation against apoptosis [6, 60].

The total amount of heavy metals accumulated in breast cancer tissue is approximately an average 72.44–10.3 μg/kg. They are accumulated more in the parenchymal component of tumor tissue. The relationship between the heavy metal accumulation in the foci of neoplasia and degree of breast cancer malignancy has been determined in the recent study [57]. Abo El-Atta et al. designed an in vitro study to comprehend the possible mechanism for Cd-induced breast cancer by using normal cultured mammary cells and breast cancer cells. As a result of this study, they stated that $CdCl_2$ is cytotoxic to primary cultured cells [61]. Besides this cytotoxic effect, $CdCl_2$ lead to DNA damage in both mammary cultured cells and breast cancer cells by causing the mutations in their nucleotide sequence. Consequently, Cd could be considered as a chemical carcinogen that may act either as initiator or promoter on mammary cells [22].

Recent studies have determined that miRNAs are linked to cancer, and it has been shown that many miRNAs are dysregulated after exposure to metal carcinogens [50, 62]. miRNAs are evolutionarily conserved, single-stranded, small noncoding RNA molecules, and the length of miRNAs is approximately 18–25 nucleotides [50]. miRNAs have an important function in RNA silencing and act as posttranscriptional gene regulator by targeting specific mRNA [48]. miRNA seed sequence (3–8 nucleotides) specifically matches with 3′ untranslated gene regions of target mRNA, resulting in blocking of translation by mRNA degradation or translational repression [55]. miRNAs participate in many vital cellular processes, including cell proliferation, differentiation, apoptosis, metabolism, and formation of malignancy in tumors, and are involved in many diseases [62].

As, Cd, $Cr^{6+}$, and Ni can be poisonous for human health even at lower concentrations [63]. These heavy metals can also be carcinogens because certain studies have shown that exposure to heavy metals with water, soil, food, or air increases the risk of cancer formation [64, 65]. In addition, some studies on metal carcinogens indicate that being exposed to metal carcinogens can dysregulate many miRNA (Humphries [62]). According to Wang et al., chronic arsenic exposure on human bronchial epithelial cells (HBECs) causes malignant transformation. To understand the effect of arsenic on miRNA expression levels in HBECs, miRNA microarray was performed in this study. As a result of this microarray, six downregulated miRNAs (the five miR-200 family members and miR-205) were observed in HBECs exposed to As compared to control HBECs. In another study, miR-21 was upregulated in human embryo lung fibroblast cells (HELFs) after continuous treatment with As [66].

One of the metal carcinogens is Ni which leads to lung and nasal cancers. According to Ji et al., exposing to Ni in human bronchial epithelial (16HBE) cells brings about the upregulation of DNA methyltransferase 1 (DNMT1). To determine the reason of upregulation of DNMT1 in terms of miRNA, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis was performed, and significant downregulation of miR-152 was observed in nickel-transformed cells [67]. Cr is also one of the metal carcinogens, and it is generally found at three different stages (0, III, and IV) in nature. The form of $Cr^{6+}$ is known as human carcinogen, and it is recognized that $Cr^{6+}$ lead to lung cancer [62, 68]. The effect of $Cr^{6+}$ on lung cancer formation was investigated, and downregulation of expression of miR-143 was found out in $Cr^{6+}$-transformed human bronchial epithelial BEAS-2B cells [69]. In addition, reexpression of miR-143 in $Cr^{6+}$-transformed human bronchial epithelial BEAS-2B cells decreases the malignancy features [70].

Cd is one of the lung cancer-causing carcinogenic agents. Deregulation of miRNAs in Cd causing lung cancer was studied to understand the epigenetic mechanisms behind lung cancer [46]. Differences in the expression level of many miRNAs were observed in Cd-transformed 16HBE cells. According to the microarray results, miR-27b-3p and miR-944 were upregulated and downregulated, respectively, in Cd-transformed 16HBE cells compared to control cell group [71]. Exposure of Cd, As, and Hg leads to change in miRNA expression in mammalian cancer cells [72–75]. In conclusion, all of these studies on heavy metals indicated that gene expression can be activated or suppressed by heavy metals through epigenetic modifications, and these changes can last throughout life [76].

### *9.1.4 Lichens and Secondary Metabolites as New Promising Source of Heavy Metal Exposure*

The main objective of this review is to provide insight into the sources of heavy metals and their harmful effects on the environment and living organisms. Heavy metals have been one of the greatest threats to human and environmental health in recent years because heavy metals can disperse and produce toxic effects to some extent. It is necessary to develop early warning signals or biomarkers that reflect adverse effects on heavy metals. One of the biological samples, lichens are used as biomarkers in studies of heavy metal pollution as an indicator of environmental and human health. Lichen is a form of symbiotic life between fungi and algae or cyanobacteria worldwide [77]. Many studies have shown that lichens are a good biomarker for the response of heavy metal pollution [78, 79]. As a result of these studies, lichen species are particularly promising biomarker for exposure to heavy metals since lichens are easily sampled from nature; they do not have root systems and waxy cuticles, so they provide wet and dry accumulation for mineral nutrients and lichens absorb nutrients directly from the air; they can also pick up and accumulate the elements in their tissues; and thus they reflect atmospheric input of cancer-causing chemicals such as toxic gases and heavy metals in large quantities. Through high levels of heavy metal accumulation capability of lichens, they are used as early warning system of environmental pollution; therefore it is an important clue that reveals the incidence of cancer in human.

In recent years, the biopharmaceutical industry has aimed to generate the natural anticancer agents which contain lichen secondary metabolites. Lichens produce various secondary metabolites that have an important role in some biological activities such as anticancer activity [80]. Recent studies have been revealed that physciosporin, isolated from *Pseudocyphellaria coriacea*, significantly inhibits the migration and invasion in human lung cancer cells [80]. One of the other secondary metabolites is protolichesterinic acid, isolated from Iceland moss, which shows antiproliferative effects on several cancer cell lines as well as breast cancer cell line. In SK-BR-3 cell line, HER2 expression level is downregulated after the exposure of protolichesterinic acid [77]. Usnic acid and norstictic acid also reduce the cell viability on breast cancer cell lines such as MDA-231. Besides reduction of cell viability, norstictic acid inhibits the migration and invasion in MDA-231 cell line [81]. In our previous studies, the effects of usnic and vulpinic acid on the proliferation and apoptosis of different cancer cells were assessed. We demonstrated that both secondary metabolites inhibited the proliferation and induced apoptosis of cancer cells [82, 83]. These studies suggest that lichen secondary metabolites can be a potential therapeutic agent for the treatment of cancer.

## 9.2 Conclusion

The ongoing research works have clearly demonstrated that environmental pollution which mainly resulted from heavy metal can have a detrimental effect on people's health. In this review, we suggested to use lichens to understand the toxicity and carcinogenic mechanisms of heavy metals. This chapter evaluated on induction of oxidative stress, DNA damage, and cell death processes based on heavy metals and a result of circumstance which increases the risk of cancer diseases. Some pharmaceutically important lichens and their secondary metabolites have provided alternative solution for human diseases. The aim of this review is to illustrate and evaluate on the biomarker potential of lichens because they serve as new promising source against heavy metal exposure. Lichens that are vulnerable biological organism need to be investigated in more deep detail to use for solution of heavy metal toxicity.

Many lichen secondary metabolites have antiproliferative effect on different cancer cells, and they are commercially valuable and medicinally important; therefore secondary metabolites from lichens could be potential sources of anticancer drugs. The aim of this review was to provide insights regarding the anticancer properties of lichen secondary metabolites and also to provide information regarding one of the most promising sources for cancer treatment. Thus, we might think lichen secondary metabolites are helpful for prevention of effect of heavy metal-induced cancer disease.

## References

1. Arya A, Arya S, Arya M (2011) Chemical carcinogen and cancer risk: an overview. J Chem Pharm Res 3(5):621–631
2. Mathew BB, Singh H, Biju VG, Krishnamurthy NB (2017) Classification, source, and effect of environmental pollutants and their biodegradation. J Environ Pathol Toxicol Oncol 36(1):55–71
3. Hasegawa R, Futakuchi M, Mizoguchi Y, Yamaguchi T, Shirai T, Ito N et al (1998) Studies of initiation and promotion of carcinogenesis by N-nitroso compounds. Cancer Lett 123(2):185–191

4. Wang TC, Chiou CM, Chang YL (1998) Genetic toxicity of N-methylcarbamate insecticides and their N-nitroso derivatives. Mutagenesis 13(4):405–408
5. Lock EA, Reed CJ, Mcmillan JM, Oatis JE, Schnellmann RG (2007) Lack of formic acid production in rat hepatocytes and human renal proximal tubule cells exposed to chloral hydrate or trichloroacetic acid. Toxicology 230(2–3):234–243
6. Yaghoubi S, Barlow J, Kass PH, Orenstein FS, Cross S, Heath E (2007) Breast cancer and metals: a literature review. Zero Breast Cancer 2007:1–34
7. Juracek KE, Ziegler AC (2006) The legacy of leaded gasoline in bottom sediment of small rural reservoirs. J Environ Qual 35(6):2092–2102
8. Adachi K, Tainosho Y (2004) Characterization of heavy metal particles embedded in tire dust. Environ Int 30(8):1009–1017
9. Su CC (2015) Heavy metal and cancer risk. SM J Public Health Epidemiol 1(4):1019
10. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7(2):60–72
11. Jarup L (2003) Hazards of heavy metal contamination. Br Med Bull 68(1):167–182
12. Lakowicz J, Anderson C (1980) Permeability of lipid bilayers to methyl- mercury chloride: quantification by fluorescence quenching of a carbazole-labeled phospholipid. Chem Biol Interact 30:309–323
13. Antila E, Mussalo-Rauhamaa H, Kantola M, Atroshi F, Westermarck T (1996) Association of cadmium with human breast cancer. Sci Total Environ 186(3):251–256
14. Karmakar N, Jayaraman G (1988) Linear diffusion of lead in the intestinal wall: a theoretical study. IMA J Math Appl Med Biol 5(1):33–43
15. Bont DR, Larebeke NV (2004) Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 19(3):169–185
16. Chakrabarti SK, Bai C, Subramanian KS (2001) DNA-protein cross links induced by nickel compounds in isolated rat lymphocytes, role of reactive oxygen species and specific amino acids. Toxicol Appl Pharmacol 170:153–165
17. Cansaran-Duman D, Altunkaynak E, Aras S (2014) Heavy metal accumulation and genotoxicity indicator capacity of the lichen species, Ramalina pollinaria collected from around an iron-steel factory in Karabük, Turkey. Turk J Bot 38:477–490
18. Cansaran-Duman D, Altunkaynak E, Aslan A, Büyük İ, Aras S (2015) Application of molecular marker to detect DNA damage caused by environmental pollutants in lichen species. Genet Mol Res 14(2):4637–4650
19. Vardar Ç, Başaran E, Cansaran-Duman D, Aras S (2014) Assessment of air pollution genotoxicity in the Province of Kayseri (Central Anatolia) by using Pseudevernia furfuracea (L.) Zopf and AFLP markers. Mutat Res Genet Toxicol Environ Mutagen 759:43–50
20. Wassell J, Rogers SL, Felmingam KL, Bryant RA, Pearson J (2015 Apr) Sex hormones predict the sensory strength and vividness of mental imagery. Biol Psychol 107:61–68
21. Zhang C, Lib P, Wena Y, Fengd G, Liua Y, Zhanga Y et al (2018) The promotion on cell growth of androgen-dependent prostate cancer by antimony via mimicking androgen activity. Toxicol Lett 288:136–142
22. El-Harouny MA, El-Morsi DA, Ahmed BRA, El-Atta HMA (2011) Chronic toxicity of some heavy metals and breast cancer in egyptian females. J Clin Toxicol 1:109
23. Pan J, Plant JA, Voulvoulis N, Oates CJ, Ihlenfeld C (2010) Cadmium levels in Europe: implications for human health. Environ Geochem Health 32:1–12
24. Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF (2007) Cadmium-induced cancers in animals and in humans. Int J Occup Environ Health 13:202–212
25. Fergusson JE (1990) The heavy elements: chemistry, environmental impact and health effects, 1st edn. Pergamon Press, Oxford
26. Hambidge KM, Krebs NF (2007) Zinc deficiency: a special challenge. J Nutr 137(4):1101–1105
27. Stern BR (2010) Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. J Toxicol Environ Health A 73:114–127

28. Bánfalvi G (ed) (2011) Heavy metals, trace elements and their cellular effects, 1st edn. Springer, New York
29. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. EXS 101:133–164
30. Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 1:529–539
31. Grimsrud TK, Andersen A (2012) Unrecognized risks of nickel-related respiratory cancer among Canadian electrolysis workers. Scand J Work Environ Health 38:503–515
32. Grimsrud TK, Berge SR, Martinsen JI, Andersen A (2003) Lung cancer incidence among Norwegian nickel-refinery workers 1953–2000. J Environ Monit 5:190–197
33. Gul N, Shah MT, Khan S, Khattak NU, Muhammad S (2015) Arsenic and heavy metals contamination, risk assessment and their source in drinking water of the Mardan District, Khyber Pakhtunkhwa, Pakistan. J Water Health 13:1073–1084
34. Harvey PJ, Handley HK, Taylor MP (2015) Identification of the sources of metal (lead) contamination in drinking waters in north-eastern Tasmania using lead isotopic compositions. Environ Sci Pollut Res Int 22:12276–12288
35. Bau DT, Wang TS, Chung CH, Wang AS, Wang AS, Jan KY (2002) Oxidative DNA adducts and DNA-protein cross-links are the major DNA lesions induced by arsenite. Environ Health Perspect 110(5):753–756
36. Kim HS, Kin YJ, Seo YR (2015a) An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention. J Cancer Prev 20(4):232–240
37. Iregren A (1990) Psychological test performance in foundry workers exposed to low levels of manganese. Neurotoxicol Teratol 12:673–675
38. Hirata Y (2002) Manganese-induced apoptosis in PC12 cells. Neurotoxicol Teratol 24:639–653
39. Deng Y, Xu D, Xu B, Xu Z, Tian Y, Feng W et al (2011) G0/G1 phase arrest and apoptosis induced by manganese chloride on cultured rat astrocytes and protective effects of riluzole. Biol Trace Elem Res 144(1–3):832–842
40. Hernroth B, Holm I, Gondıkas A, Tassıdıs H (2018) Manganese inhibits viability of prostate cancer cells. Anticancer Res 38:137–145
41. Garg UK, Kaur MP, Garg VK, Sud D (2007) Removal of hexavalent chromium from aqueous solution by agricultural waste biomass. J Hazard Mater 140:60–68
42. Eastmond DA, Macgregor JT, Slesinski RS (2008) Trivalent chromium: assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. Crit Rev Toxicol 38:173–190
43. Hamilton JW, Wetterhahn KE (1986) Chromium (VI)-induced DNA damage in chick embryo liver and blood cells in vivo. Carcinogenesis 7:2085–2088
44. Gammelgaard B, Fullerton A, Avnstorp C, Menné T (1992) Permeation of chromium salts through human skin in vitro. Contact Dermatitis 27:302–310
45. Mahurpawar M (2015) Effects of heavy metals on human health. Int J Res 2015:1–7
46. Raza U, Saatci Ö, Uhlmann S, Ansari SA, Eyüpoğlu E, Yurdusev E et al (2016) The miR-644a/CTBP1/p53 axis suppresses drug resistance by simultaneous inhibition of cell survival and epithelial-mesenchymal transition in breast cancer. Oncotarget 7(31):49859–49877
47. Banin Hirata BK, Maeda Oda JM, Guembarovski RL, Ariza CB, Coral de Oliveira CE, Ehara Watanabe MA (2014) Molecular markers for breast cancer: prediction on tumor behavior. Dis Markers 2014:5131–5158
48. Matamala N, Vargas MT, Campora RG, Minambres R, Arias JI, Menendez P (2015) Tumor microrna expression profiling ıdentifies circulating micrornas for early breast cancer detection. Clin Chem 61(8):1098–1106
49. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S et al (2007) American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 25:5287–5312

50. McDermott AM, Miller N, Wall D, Martyn LM, Ball G, Sweeney KJ et al (2014) Identification and validation of oncologic mirna biomarkers for luminal a-like breast cancer. PLoS One 9(1):e87032
51. Piccart-Gebhart MJ (2011) New developments in hormone receptor–positive disease. Oncologist 16:40–50
52. Foulkes W, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363:1938–1948
53. Kılıç N, Değerli E, Torun V, Altaytaş F, Cansaran-Duman D (2016) Investigation of synergistic effect of tamoxifen and usnic acid on breast cancer cell line. JSM Biol 1(1006):1–4
54. Apuri S (2017) Neoadjuvant and adjuvant therapies for breast cancer. South Med J 110(10):638–642
55. Vimalraj S, Miranda PJ, Ramyakrishna B, Selvamurugan N (2013) Regulation of breast cancer and bone metastasis by microRNAs. Dis Markers 35(5):369–387
56. Romaniuk A, Lyndin M, Moskalenko R, Kuzenko Y, Gladchenko O, Lyndina Y (2015) Pathogenetic mechanisms of heavy metals effect on proapoptotic and proliferative potential of breast cancer. Intervent Med Appl Sci 7(2):63–67
57. Romaniuk A, Lyndin M, Sikora V, Lyndina Y, Romaniuk S, Sikora K (2017) Heavy metals effect on breast cancer progression. J Occup Med Toxicol 12:32
58. Xi H (2008) Does iron have a role in breast cancer? Lancet Oncol 9(8):803–807
59. Stoica A, Pentecost E, Martin MB (2000) Effects of arsenite on estrogen receptor-alpha expression and activity in MCF-7 breast cancer cells. Endocrinology 141(10):3595–3602
60. Ostrakhovitch EA, Cherian MG (2005) 2005. Role of p53 and reactive oxygen species in apoptotic response to copper and zinc in epithelial breast cancer cells. Apoptosis 10(1):111–121
61. Abo El-Atta H M, El-Harouny M A, El-Mansory AM, Badria, FA, El-Bakary AA (2011) Cadmium genotoxicity and breast carcinoma. MD thesis, Mansoura University, pp 164–166
62. Humphries B, Wang Z, Yang C (2016) The role of microRNAs in metal carcinogen-induced cell malignant transformation and tumorigenesis. Food Chem Toxicol 98(Pt A):58–65
63. Duffus JH (2002) Heavy metals—a meaningless term? Pure Appl Chem 74:793–807
64. Hu H (2002) Human health and heavy metals exposure. In: McCally M (ed) Life support: the environment and human health. MIT Press, Cambridge, pp 65–82
65. Langie SAS, Koppen G, Desaulniers D, Al-Mulla F, Al-Temaimi R, Amedei A et al (2015) Causes of genome instability: the effect of low dose chemical exposures in modern society. Carcinogenesis 36:S61–S88
66. Wang Z, Zhao Y, Smith E, Goodall GJ, Drew PA, Brabletz T et al (2011) Reversal and prevention of arsenic-induced human bronchial epithelial cell malignant transformation by microRNA-200b. Toxicol Sci 121:110–122
67. Ji W, Yang L, Yuan J, Yang L, Zhang M, Qi D et al (2013) MicroRNA-152 targets DNA methyltransferase 1 in NiS-transformed cells via a feedback mechanism. Carcinogenesis 34:446–453
68. Agency for Toxic Substances and Disease Registry (ATSDR) (2005) Toxicological profile for Nickel. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA
69. He J, Qian X, Carpenter R, Xu Q, Wang L, Qi Y et al (2013) Repression of mir-143 mediates Cr (VI)-induced tumor angiogenesis via IGF-IR/IRS1/ERK/IL-8 pathway. Toxicol Sci 134:26–38
70. Ngalame NN, Makia NL, Waalkes MP, Tokar EJ (2015) Mitigation of arsenic-induced acquired cancer phenotype in prostate cancer stem cells by miR-143 restoration. Toxicol Appl Pharmacol 15:30159-9
71. Liu Q, Zheng C, Shen H, Zhou Z, Lei Y (2015) MicroRNAs-mRNAs expression profile and their potential role in malignant transformation of human bronchial epithelial cells induced by cadmium. Biomed Res Int:9020–9025
72. Ding YF, Zhu C (2009) The role of microRNAs in copper and cadmium homeostasis. Biochem Biophys Res Commun 386:6–10
73. Marsit CJ, Eddy K, Kelsey KT (2006) MicroRNA responses to cellular stress. Cancer Res 66:10843–10848

74. Wang B, Li Y, Shao C, Tan Y, Cai L (2012) Cadmium and its epigenetic effects. Curr Med Chem 19:2611–2620
75. Zhou ZS, Zeng HQ, Liu ZP, Yang ZM (2012) Genome-wide identification of medicago truncatula microRNAs and their targets reveals their differential regulation by heavy metal. Plant Cell Environ 35:86–99
76. Ho SM, Johnson A, Tarapore P, Janakiram V, Zhang X, Leung YK (2012) Environmental epigenetics and ıts ımplication on disease risk and health outcomes. ILAR J 53(3–4):289–305
77. Bessadóttir M, Skúladóttir EA, Gowan S, Eccles S, Ómarsdóttir S, Ögmundsdóttir H (2014) Effects of anti-proliferative lichen metabolite, protolichesterinic acid on fatty acid synthase, cell signalling and drug response in breast cancer cells. Phytomedicine 21(12):1717–1724
78. Aras S, Kanlıtepe Ç, Cansaran-Duman D, Halıcı MG, Beyaztaş T (2010) Assessment of air pollution genotoxicity by molecular markers in the exposed samples of Pseudevernia furfuracea (L.) Zopf in the province of Kayseri (Central Anatolia). J Environ Monit 12:536–543
79. Cansaran-Duman D (2011) Study on accumulation ability of two lichen species Hypogymnia physodes and Usnea hirta at iron-steel factory site. Turkey J Environ Biol 32:839–844
80. Yang Y, Park SY, Nguyen TT, Yu YH, Nguyen TV, Sun EG et al (2015) Lichen secondary metabolite, physciosporin, inhibits lung cancer cell motility. PLoS One 10(9):e0137889
81. Ebrahim HY, Elsayed HE, Mohyeldin MM, Akl MR, Bhattacharjee J, Egbert S (2016) Norstictic acid inhibits breast cancer cell proliferation, migration, invasion, and in vivo invasive growth through targeting C-Met. Phytother Res 30(4):557–566
82. Dinçsoy AB, Cansaran-Duman D (2017) Changes in apoptosis related gene expression profiles in cancer cell line exposed to usnic acid lichen secondary metabolite. Turk J Biol 41:484–493
83. Kılıç N, Derici K, Büyük İ, Soydam-Aydın S, Aras S, Cansaran-Duman D (2018) Evaluation of in vitro anticancer activity of vulpinic acid and its apoptotic potential using gene expression and protein analysis. Indian J Pharm Edu Res 52(3):46–54

# Chapter 10
# Phytoremediation of Chromium-Polluted Soil Using Plants in Conjunction with Microbes

**Ayesha Siddiqa and Muhammad Faisal**

## 10.1 Introduction

Chromium is a heavy metal that is found naturally in land and aquatic environment, and that is how it is taken in our food. As it is a heavy metal, it is only good to the environment if it occurs only in balanced ratio. However due to anthropogenic actions, it has become an imminent pollutant and has gained international concern regarding ecological, biological, environmental, and health issues. The contamination of heavy metals in land and aquatic environment is of inordinate concern especially in human population nearby industrial areas [1]. However hexavalent chromium has gained more interest in past decades [2]. Chromium was discovered by Louis Nicolas Vauquelin, as an integral element in mineral crocoite (PbCrCO4). Cr is a lustrous, brittle, hard crystalline, gray, tasteless, and odorless heavy metal. It is placed in the periodic table at the 24th position in VIB group. Its atomic number is 24 and atomic mass is 51, comprising the density of 7.15 g/cm$^3$. Its probable crustal and oceanic abundance is $1.02 \times 10^2$ mg/kg and $3 \times 10^{-4}$ mg/L. Chromium is found in +6, +3, and +2 oxidation state, and the elemental selenium is not found on Earth's crust, so it is only found in chromium compounds. In recent studies it is named as the 7th most abundant atom in Earth's crust and positioned as 21st in crustal rocks. One of the major sources of chromium is erosion of mined mineral rocks, distributed due to the volcanic eruption [3]. However the total production of the chromium now is exceeding the 10 million tons per annum, and the major producers of chromite ore are South Africa and India; Kazakhstan and Turkey are the other significant producers. Its Cr VI and Cr III forms are biologically important, but the major concern is Cr VI forms which are potentially toxic to biological systems because of its high solubility, stability, dominance, and permeability across the

---

A. Siddiqa · M. Faisal (✉)
Department of Microbiology and Molecular Genetics, University of the Punjab,
Lahore, Pakistan

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_10

biological membranes [4]. Chromium-polluted environment is equally harmful to the humans, animals, and plants. Its accumulation in soil and water contracts the growth in plants and retards the physical parameters of plant growth [5]. Cr VI+ has known genotoxic, mutagenic, and tumorigenic effects in every living organism. Cr accumulation disrupts the physiology of microbes and reduces the microbial count in agricultural soil. Prolonged exposure to the Cr VI+ may lead to skin, respiratory, and auditory problems and in severe cases may cause ulceration and cancer. Due to its intimidating effects on human health, it is placed among those 17 chemicals that have the greatest hazard to humans by US Environmental Protection Agency. Despite the health issues in all living forms, the usage of chromium in industries has increased drastically. Cr compounds are widely used in laboratory as intermediate reagent, while chromium metal is used in gas, aircraft, petrochemical, and nuclear sector because of its strength [6]. The main industrial consumers of chromium compounds are refractory brick, leather tanning, metallurgical, wood preservation, dye, and pigment industries. A small amount of chromium compounds, i.e., calcium chromate, sodium chromate, etc., are used as depolarizers, metal finishing, corrosion inhibitor, cutting oils, catalyst, and raw material for various other compounds [7]. And these industries are also the anthropogenic sources of chromium in Earth's crust. These industries use a large amount of chromium during processing [1], and this industrial effluent having the major pollutants and contaminants is added in water bodies later on. Besides chromium metal is also being added by some natural resources. Ore minerals are the most concentrated form of chromium existing naturally, and these mineral rocks are the most important natural source of chromium. After limestone and sandstone, shales and sedimentary rocks are reported to have a high concentration of chromium; however the concentration may vary. Secondly the volcanic eruption is being reported to emit large amount of deposited chromium. Chromium is present in the soil in the form of chromite that may be suspended in the air with the soil particles. However the main source of chromium in Earth's crust is the weathering of rocks and parent material; that's why the concentration of chromium in a particular area is dependent on the presence of the type of rocks and parent material. Anthropogenic source of chromium includes the incineration, transportation, and corrosion of industrial and commercial waste materials. Among all the controllable sources of chromium are effluents from tanneries, chrome plating, metal finishing, and textile plant. These all abovementioned sources add a great deal of chromium in the environment.

## 10.2 Toxicity Potential of Chromium

Metals, just like organic pollutants, are not degraded naturally and tend to accumulate in Earth's crust. These accumulated metal contaminants may enter the food chain causing the mutagenic, toxic, and carcinogenic effects on human health. Chromium is a common compound that is used in wide range of industries globally

[8, 9]. It is also an essential compound but is toxic in large quantities. However not all the chromium species are equal in their potential of toxicity [10]; the toxicity of chromium depends upon the oxidation state of chromium [2]. Knowledge of chemical speciation of chromium is important in investigating the ecological hazards associated with the high concentration of chromium in land and water bodies [4, 11–13]. Due to increased inconsistencies among its different chemical species and their biological behavior, chromium speciation is of marked importance [8].

Among all oxidation states, Cr VI is the most toxic form of chromium [14] because of its solubility and oxidative damage to the macromolecules [15, 16], as Cr III is not soluble that is why Cr III is less toxic to living systems. Cr VI rapidly crosses the cellular membranes and reduces into Cr III, which causes the formation of intracellular complexes with macromolecules and DNA, and that is how Cr III brings mutagenic effect and oxidative damage in cellular systems [17]. When Cr VI reduces into Cr III, it forms the reactive intermediates which may lead to tissue injury due to the severe cellular damage [7, 18]. This oxidative stress may cause the lipid peroxidation in kidneys and liver and DNA damage in peripheral blood lymphocytes [8, 19]. In a study it was determined that dose-dependent increase in Cr exposure leads to the increased enzymatic activity in kidneys and particularly in the liver against the oxidative damage [20, 21]. In reported cases it was shown that Cr VI induced the formation of DNA-protein complexes and cross-links that result in the genotoxic effects in living systems [22] and increased activities of the oxidative enzymes [23, 24]. It was also found that the chromium and its compounds might be the cause of single- and double-strand breaks [25, 26] that resulted in the cases of chromatid exchange, formation and accumulation of micronuclei, chromosomal aberrations [27], alteration in DNA replicative and transcriptive processes, and formation of DNA adduct [28–30]. Hexavalent chromium for the first time was described to be associated with cancer (adenocarcinoma) in a worker of chrome pigment factory. Later on chromium was found to increase the risk of cancer in industrial workers by 15-fold. It was reported to cause the neoplastic transformation in vitro (C3H10T1/2 mouse cell line) [31] and induced cancer in vivo [32]. As a result now chromium is known as the lung cancer inducer worldwide. Despite the fact that Cr VI is soluble and its carcinogenicity is governed by its solubility, it is also hypothesized that the particulate Cr VI is the cause of cancer in lung cell [33]. The data obtained from various studies showed that at the site of bifurcation in bronchial cells, the chromium-induced tumor started to develop [29], as particulate Cr VI accumulate there. But so far only a few studies used the primary human lung cells and particulate Cr VI [29], and the rest of them focused on the soluble Cr VI and its genotoxic effects on cellular systems [34]. However there is one thing to be noticed that soluble Cr VI studies only focused in only high concentration of Cr VI in quite acute and continuous exposure that can happen in occupational exposure of chromium compounds in few cases (Table 10.1). But it is opposite to the lower concentration of Cr VI in chronic and irregular exposure, which is common now [29].

**Table 10.1** The summary of Cr-induced cellular and organ damage in plants

| Cr toxicity | Effects on plant cells |
|---|---|
| Genotoxicity | Polyploidization, cell cycle arrest, disruption of mitosis, chromosomal aberrations, DNA breaks, DNA-protein complex formation, RNA amplification, chromosomal breaks, non-redundancy of genes, down- and upregulation of genes |
| Root and stem growth abnormalities | Reduced seed germination, alteration in histology of root and shoot cells, reduced mitotic index in root tip cells, lesser number of secondary and lateral roots, decreased stem growth |
| Leaf growth abnormalities | Reduced photosynthesis, reduced chlorophyll, disorientation of chloroplast in leaf cells, decreased nutrient uptake and water potential, decreased cell division and number of cells in leaves, decreased or increased transpiration |
| Nutrient uptake irregularities | Decreased nutrient uptake, reduced nutrient and water potential, decreased absorption of macronutrients from soil |
| Cr-induced oxidative stress and lipid peroxidation | Inhibition of electron transport process, changes in enzymatic activities, ultrastructural changes in root cells. ROS-induced damage in cellular biochemical process |

These changes occur in dose-dependent manner [4, 35–37, 91]

## 10.3 Phytoremediation of Chromium-Polluted Soil

Phytoremediation is an integrated, novel, eco-friendly, and multidisciplinary technique that is proven to be effective in cleaning the environment from heavy metal contaminants that are of major concern in remediating our degrading environment. It is an ecological approach that is cost-effective, simple, and environment-friendly. The key role in phytoremediation is played by the plants that are used in remediating the metal- or PHA-polluted environment whether in land or aquatic environment [12, 38]. Terrestrial plants and aquatic plants whether free-floating, emerged, or submerged all are used in phytoremediation [39, 40]. The behavior of chromium in soil, its uptake by plants from soil, and its accumulation in plants fluctuate markedly. These processes depend upon the chromium species present in soil, type of plant, and the physicochemical properties of soil.

### *10.3.1 Transfer of Cr from Soil to Plant*

Cr is absorbed by the plants through carriers that are used for the absorption of micronutrients. However the uptake of chromium is dependent on the Cr species and type of plant. The uptake of Cr III does not require any energy that is why it’s a passive mechanism [41]; however the Cr VI is taken up by the plant through energy-mediated and active transportation via the sulfur or phosphorous transporters, as Cr VI has structural similarity with sulfur and phosphorous ([42, 43]). Studies showed that the presence of sulfur in soil inhibited the absorption of Cr VI [13]. Moreover the presence of Cr VI in soil interfered with the absorption of various essential nutrients, i.e., Mg, Ca, Fe, K, Mn, and P, and other compounds, e.g., ammonia and glycine, because of their ionic resemblance [44]. The presence of metabolic inhibitors in soil affects the Cr VI uptake by plants but do not affect the Cr III uptake that shows the active or energy-mediated transportation of Cr VI [45]. As mentioned earlier, Cr VI is absorbed mainly by the increased solubility as compared to Cr III, so the soil-plant transfer index of Cr VI is high.

### *10.3.2 Chromium Accumulation in Plant Roots*

Chromium metal is considered to be the least mobile metal in biological systems. So when Cr is absorbed by the hyperaccumulator plants, it stays in roots, and the very less quantity goes in leaves and shoots, e.g., studies revealed that roots of Cr hyperaccumulators contained 100 times more Cr than shoots ([42, 45], Gomez et al. 2011, Sabir et al. 2015). The concentration of chromium in various parts of plants was measured, and the highest levels of Cr were found in roots and then in stem and leaves, and the lowest level was found in seeds. Prior studies showed that *Iris pseudacorus* accumulated the Cr in the cell wall of root cells, in cytoplasm, and in intercellular spaces of the rhizome [46]. Later on it was discovered that in roots the major proportion of accumulated Cr exist in cell walls (83.2%) and the lesser proportion resides in vacuoles and cytoplasm (57.7%) [47]. This increased accumulation of Cr in roots is possibly because when Cr enters the roots, it forms the insoluble compounds and stays there.

The possible question why the increased levels of metals like Cr are not harmful to most of the plants was answered by many researchers, as Cr is sequestered in the vacuoles of the root cells and this sequestration is a normal mechanism for metal tolerance in plants [48]. Conversely the transportation of Cr in plants from roots to stem and leaves is limited and dependent on the chemical form of Cr, as Cr VI converts into Cr III in roots cells and adheres with the cell wall of root cells.

### *10.3.3 Translocation of Cr Within Plants*

After the Cr is absorbed and accumulated in plant roots, it must be translocated to other parts of the plants. There are several gene families discovered to date that are involved in the translocation and transportation of metals like Pb [49, 50], As [51], and Cd [49]. These gene families are responsible for making the several transporters and facilitators, e.g., heavy metal ATPase (HMA), natural resistance-associated macrophage protein (NRAMP), cation diffusion facilitator (CDF), ATP-binding cassette (ABC), and ZRT- and IRT-like protein (ZIP), and they are responsible for the absorption, sequestration, resistance, and transfer of metals from roots to aerial parts of plant [51]. Despite the discovery and knowledge of these gene families, we are still not fully aware of the transportation method and mechanism of Cr metal in plants.

Cr is transported from roots to shoots by an active transportation method [27] that is used by the essential minerals like sulfur and phosphorus. Brassicaceae family is the hyperaccumulator of sulfur; however these plants upon administration also accumulated high level of Cr which indicated that the Cr was transported and translocated by the sulfur carriers [48]. Moreover the Fe accumulators, i.e., *Brassica rapa* and *Spinacia oleracea*, are also known to accumulate the Cr in roots and its translocation to upper parts of the plants [27]. However the presence of sulfur or Fe in medium reduces the accumulation of Cr that shows the competition between these metals using the same transportation carriers, and it can be due to the precipitation of Fe with Cr [52]. Similarly in other cases, it was reported that the expression of transcription factor NtST1 (a sulfur transporter) in transgenic tobacco *Nicotiana tabacum* was increased as in *Saccharomyces cerevisiae* the expression of MSN1 was increased for the Cr accumulation. We can also use yeast transporter genes in plants to remediate the Cr-polluted soil [42].

In prokaryotes the sulfur or Cr is usually transported through ABC system [53], and this mechanism is similar in many basal plants and algae for the sulfur transportation among organelles. On the contrary, in eukaryotes the multiple sulfate transporters have been identified that have different affinities for different chemical forms, e.g., in *Chlamydomonas reinhardtii*, six membrane sulfate transporters were discovered, and three of them transport Na-sulfate transporters, and the rest of them were for proton-sulfate transportation. Nevertheless these transporters are also used in the uptake and transportation of Cr. Researchers found that under sulfur starvation, the cells start accumulating more sulfur and make sulfur-containing Cr detoxification compounds, i.e., chelators, antioxidant enzymes, or compartmentalization of Cr in vacuoles.

### *10.3.4 Hyperaccumulator Plants of Chromium Metal*

Hyperaccumulators are the plant species that are capable of accumulating a high concentration of metals in both aerial and underground tissues; they have high metal tolerance and good biomass production, and their bioaccumulation and metal translocation factor is greater than 1 (Sabir et al. 2015, [38]). That is why in phytoremediation, the hyperaccumulators are of prime importance, and they have been under research and identification for past decades ([42], Daud et al. 2014). Although the total number of identified hyperaccumulators is almost 500 belonging to 45 different families, Cr hyperaccumulators are less in number. Some identified Cr hyperaccumulators are *Leersia hexandra* (Liu et al. 2011), *Pluchea indica* (Sampanpanish et al. 2006), *Spartina argentinensis* (Gomez et al. 2011), *Amaranthus dubius* (Mellem et al. 2012), *Dyera costulata* (Ghafoori et al. 2011), *Prosopis laevigata* (Buendía-Gonz_alez et al. 2010), *Convolvulus arvensis* (Gardea-Torresdey et al. 2004), and *Pteris vittata* [42].

The mechanism of tolerance in hyperaccumulators is by converting one chemical form of metal into another, i.e., less toxic to other living systems (Fig. 10.1). Hyperaccumulation implies the idea of metal immobilization, translocation in plants parts, and compartmentalization of metals in vacuoles of plant cells. Cr hyperaccumulators have several mechanisms of tolerance, i.e., reductants biotransforming the Cr VI, binding of ligands with increased affinity, and metal compartmentalization within vacuole or cell wall (Fig. 10.1). According to the studies till date, the mechanism of Cr tolerance in plants is solely due to the Cr immobilization or compartmentalization in root cell vacuoles and due to the reduction of Cr VI to Cr III; however there is still room for more research.

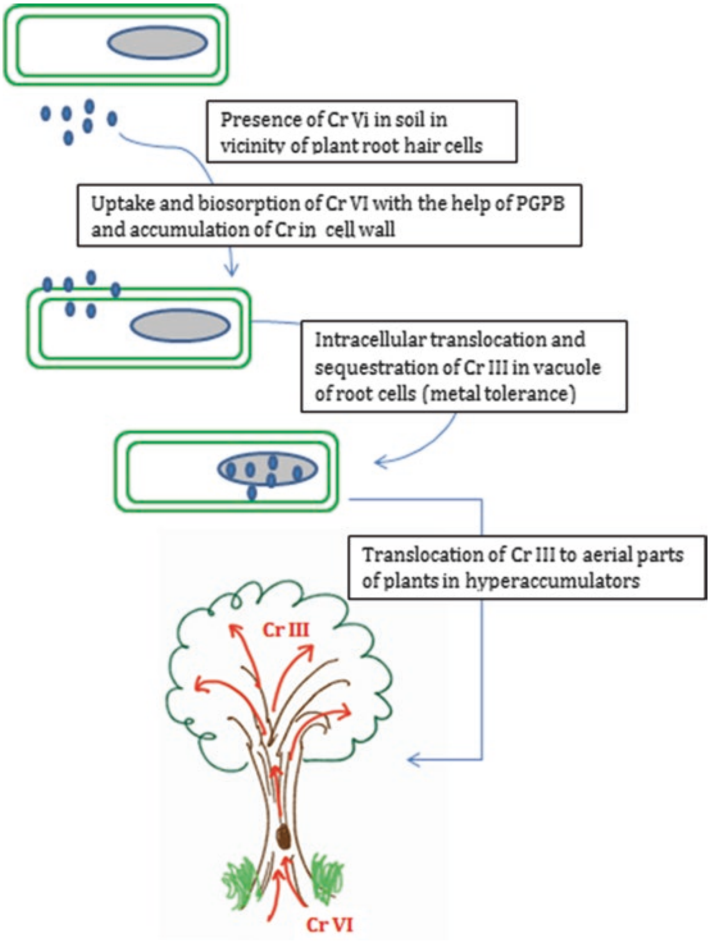


**Fig. 10.1** This figure demonstrates the absorption, sequestration, and translocation of Cr in hyperaccumulator. Hyperaccumulators have special defense and metal tolerance mechanisms that help them to tolerate high concentrations of Cr

### *10.3.5 Factors Affecting the Hyperaccumulation of Cr*

There are numerous factors that affect the efficacy of hyperaccumulation of heavy metals especially chromium, i.e., enhanced absorption by roots, increased translocation of Cr into aerial parts of plants due to increased uptake by xylem, and detoxification of metals by redactors and chelators [54]. Hyperaccumulator plants secrete some organic acids and chelators in the soil so to chelate, absorb, and sequester the metals within plant tissues [55, 56]. These secretions by hyperaccumulator plants are reported to be important in absorbing the metals. Metals are also complexed with secreted organic ligands and later on sequestered by hyperaccumulators [57]. These ligands are mostly amino acids, peptides, organic acids, and several polypeptides.

Several transporter families control the absorbance, hyperaccumulation, hypertolerance, and transportation by xylem hyperloading. These transporter genes are usually overexpressed in the hyperaccumulators in the presence of high metal concentration in their vicinity. Among all the factors affecting phytoremediation or hyperaccumulation, one thing is of key importance that hyperaccumulation is governed by the hypertolerance of metals, and this is accomplished by the internal detoxification, compartmentalization, and complexion of metals [58, 59].

## 10.4 Defense System of Plants Against Cr: Phytochelations

Being able to grow in such a high metal stress, the hyperaccumulators have a few strategies that would help to overcome the stress conditions in their surroundings.

### *10.4.1 Antioxidants*

Activation of antioxidant enzymes is the prior strategy adopted by hyperaccumulators in induced oxidative stress conditions to avoid the deleterious effects of reactive oxygen species (ROS). These enzymes are ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [60]. The type of the antioxidants produced by plant is dependent on the type of the hyperaccumulator and the type of ROS produced in oxidative stress. The mechanism of action of these antioxidants is cumulative as they work in conjugation. In plants the Cr stress can increase or decrease the activity of catalase; till now many cases have been reported where Cr enhanced the catalase activity in the following plants, i.e., *Pistia stratiotes* [61], *Corchorus olitorius* [58], *Gossypium hirsutum* (Daud et al. 2014), *Glycine max* [61], and *Zea mays* [62]. However in *Matricaria chamomilla* [59] and *Triticum aestivum* [63], the Cr stress reduced the catalase activity.

### *10.4.2 Phytochelatins*

Phytochelatins are the polypeptides, rich in cysteine, that are used by plants to chelate the heavy metals present in the soil. These compounds are important in detoxification of metals and homeostasis in plants [54]. Phytochelatins are the necessary compounds that are of vital importance in phytoremediation. During Cr detoxification by plants, Cr VI is reduced to Cr III, and then Cr III is complexed by phytochelatins [13, 64, 65]. These complexes are sent to the vacuoles where they are stored as a mechanism of metal tolerance. Earlier it was thought that like other metals Cr does not induce the production of chelatins; however later it was discovered that Cr stress induced the production of chelatins in roots and shoots, e.g., the production of chelatins in *Vigna radiata*, *Helianthus annuus*, and *Brassica juncea* [13, 55].

## 10.5 Plant-Microbe Interaction in Phytoremediation

Plant growth-promoting bacteria (PGPB) are free-living beneficial bacteria found in soil in close vicinity of roots. These bacteria, if not essentially, definitely take the growth of plants to higher levels. The species of bacteria that can resist the high concentration of metals and interact with hyperaccumulators to promote their growth are of prime importance in phytoremediation. These bacterial species either directly or indirectly assist the hyperaccumulators to remediate the soil while growing well in a polluted environment [56]. Indirect assistance means that PGPB will prevent or decrease the deleterious effects of phytopathogenic organisms and they also act as biocontrol agents against the phytopathogens. This is accomplished by several mechanisms such as competition for the binding sites on roots, production of cell wall-lysing enzymes against fungi, iron depletion in rhizosphere, antibiotic production, and systemic resistance in plants [66]. Direct assistance means that PGPBs produce such substances that promote the growth of plants while also increasing the mineral absorption.

### *10.5.1 Traits of PGPB in Phytoremediation*

The PGPB is capable of conducting the phytoremediation because of their several characteristics, i.e., active production of siderophores, biosurfactants, indole acetic acid, ACC (1-aminocyclopropane-1-carboxylate) deaminases, and organic acids [67]. The functional benefit of siderophores in phytoremediation is to aggravate the chlorophyll biosynthesis by providing the iron to plant [68, 69]. They also alleviate the production of free radicals in rhizosphere, increase the mobility of metals and their absorption by chelation reaction, and thus enhance the phytoremediation [68–71]. Siderophores also protect the plants from phytopathogens by decreasing the iron content in rhizosphere [66]. Biosurfactants work by decreasing the bonding between the metal and soil particles thus increasing the metal availability [72]. Organic acids help by solubilizing and mobilizing the metal [57]. ACC deaminases reduce the inhibitory effects of ethylene produced as a result of metal stress. It also increases the length and width of roots so to increase the effectiveness of phytoremediation [56]. Indole acetic acid upgrades the phytoremediation by increasing the metal absorption and root growth and inducing the physiological changes. These are the traits that make the PGPBs important in phytoremediation [73]. To date many case reports and reviews have been published to describe the mechanism of growth promotion by PGPBs and the significance of microbes in phytoremediation.

### *10.5.2 Reported Cases of PGPB-Assisted Cr Phytoremediation*

Anthropogenic activities due to proliferation and development of human's lifestyle have led to the contamination of land and aquatic environment with toxic chemicals and heavy metals. This contamination is affecting the aquatic life forms and disturbing the habitats of terrestrial life (Table 10.2). In past few decades, new techniques had been tried to detoxify the contamination; however among all phytoremediation is the cheapest, easy, and least dubious method of remediation. Here are some of the reported cases of phytoremediation assisted by the microbes.

### *10.5.3 Cr Phytoremediation with Fungi*

Cr-contaminated soil and water bodies can be remediated in assistance with filamentous fungi and yeast, although this association was discovered and studied in the twentieth century, but no practical use of fungi in remediating the Cr-polluted environment is found till now because of possible environmental hazards. Several fungal strains are isolated from Cr-rich environmental niches and, if they are checked and proven not to be deleterious for human or animal life forms, can be used for method of phytoremediation. The fungal species that have active Cr biosorption mechanisms are *Rhizomucor miehei* [86], *Candida tropicalis*, [87] *Mucor hiemalis* [88], *Aspergillus carbonarius* [89], *Penicillium chrysogenum* [87], and *Saccharomyces cerevisiae* [90].

## 10.6 Conclusion

As a conclusion it is safe to say that phytoremediation of Cr-polluted soil by microorganism is a useful and cheaper method to attain a clean environment and, among all remediation strategies, phytoremediation is also safe for human and animal life. However, in planning the strategy, it is important to choose wisely among

**Table 10.2** The reported cases of phytoremediation of Cr by using hyperaccumulators and their effects on plant growth and development

| Sr. no. | Chromium-resistant PGPB | Chromium-resistant plant | Effect of PGPB on plants | Reference |
|---|---|---|---|---|
| 1 | *Pisum sativum* | *Microbacterium* species | Plant growth promotion, lower toxicity of Cr due to less bioavailability, and absorption of Cr by plant | [74] |
| | | *Rhodococcus erythropolis* | Plant growth promotion even in less than required temperature | [75] |
| 2 | *Zea mays* | *Microbacterium* species | Better plant growth, decreased availability of toxic Cr, and absorption by plants thus provided reduced toxicity of Cr | [76] |
| | | *Agrobacterium tumefaciens* | Increased uptake of Cr VI and improved biomass production | [77] |
| 3 | *Lens esculenta* | *Ochrobactrum intermedium* | Enhanced growth of plant parts like root length, shoot length, number of pods, number of grains, number of plants, weight of grains, etc. | [78] |
| 4 | *Helianthus annuus* (sunflower) | *Brevibacterium* species | Improved plant height, dry and fresh weight, increased seedling growth and auxin content | [79] |
| | | *Ochrobactrum intermedium* | High yield; improved germination; increased length of seedlings, roots, and shoots; improved dry and fresh weight; high-auxin content; and reduced uptake of Cr | [80, 81] |
| 5 | *Cicer arietinum* (chick pea) | *Bacillus* species | Increased plant growth, chlorophyll amount, nodulation, grain protein, and seed yield and improved leghemoglobin. And lesser Cr absorption | [82] |
| | | *Paenibacillus lentimorbus* | Improved plant growth and reduced uptake by plants | Khan et al. 2012 |
| | | *Mesorhizobium* species | Increased nodulation, seed yield, protein matter, and nitrogen in roots and shoots and high dry matter accumulation | [83] |
| 6 | Chili | *Cellulosimicrobium cellulans* | Improved growth in plants and lesser uptake of Cr | [84] |
| 7 | Maize | *Pseudomonas aeruginosa, Pseudomonas fluorescens, Ralstonia metallidurans* | Increased metal mobilization in soil, improved Cr uptake, and increased plant growth | [85] |

hyperaccumulators and microbial species and the type of Cr species in soil. Bacterial species have been used widely among researchers, but fungal strains are not encouraged to be used for bioremediation purposes. These Cr-tolerant microbial species assist the hyperaccumulators by several strategies promoting the physical growth of plants and improving biomass.

## References

1. Gill RA, Ali B, Cui P, Shen E, Farooq MA, Islam F, Ali S, Mao B, Zhou W (2016) Comparative transcriptome profiling of two Brassica napus cultivars under chromium toxicity and its alleviation by reduced glutathione. BMC Genomics 17:885
2. Buters J, Biedermann T (2017) Chromium(VI) contact dermatitis: getting closer to understanding the underlying mechanisms of toxicity and sensitization! J Investig Dermatol 137(2):274–277
3. Pirsaheb M, Hossini H, Jabari Y, Sharafi K, Mohamadi S (2016) Evaluation of heavy metals (chromium, nickel and lead) in topsoil of the residential and industrial area of Kermanshah, Iran. IIOAB J 7(2):410–415
4. Lukina AO, Boutin C, Rowland O, Carpenter DJ (2016) Evaluating trivalent chromium toxicity on wild terrestrial and wetland plants. Chemosphere 162:355–364
5. Ding H, Wang G, Lou L, Lv J (2016) Physiological responses and tolerance of Hibiscus cannabinus L. exposed to chromium. Ecotoxicol Environ Saf 133:509–518
6. Verger L, Dargaud O, Chassé M, Trcera N, Rousse G, Cormier L (2017) Synthesis, properties and uses of chromium-based pigments from the *Manufacture de Sèvres*. J Cult Herit. https://doi.org/10.1016/j.culher.2017.09.012
7. Mahmoud AM, AbdEl-Twab SM (2017) Caffeic acid phenethyl ester protects the brain against hexavalent chromium toxicity by enhancing endogenous antioxidants and modulating the JAK/STAT signaling pathway. Biomed Pharmacother 91:303–311
8. Anjum SA, Ashraf U, Khan I, Tanveer M, Shahid M, Shakoor A, Wang LC (2017) Phytotoxicity of chromium in maize: oxidative damage, osmolyte accumulation. Pedosphere, anti-oxidative defense and chromium uptake 27(2):262–273
9. Islam F, Yasmeen T, Arif MS, Riaz M, Shahzad SM, Imran Q, Ali I (2016) Combined ability of chromium (Cr) tolerant plant growth-promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. Plant Physiol Biochem 108:456–467
10. Ahemad M (2015) Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria. J Genet Eng Biotechnol 13:51–58
11. Ahemad M (2014) Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: paradigms and prospects. Arab J Chem. https://doi.org/10.1016/j.arabjc.2014.11.020
12. Gomes MA, Hauser-Davis RA, Suzuki MS, Vitória AP (2017) Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations. Ecotoxicol Environ Saf 140:55–64
13. Shahid M, Shamshad S, Rafiq M, Khalid S, Bibi I, Khan N, Dumat C, Rashid MI (2017) Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. Chemosphere 178:513–533
14. Qambrani NA, Hwang JH, EunOh S (2016) Comparison of chromium III and VI toxicities in water using sulfur-oxidizing bacterial bioassays. Chemosphere 160:342–348
15. Pradhan D, BehariSukla L, Sawyer M, Rahman P (2017) Recent bioreduction of hexavalent chromium in wastewater treatment: a review. J Ind Eng Chem 55:1–20
16. Pandey S, Singh NK, Bansal AK, Arutchelvan V, Sarkar S (2015) Alleviation of toxic hexavalent chromium using indigenous aerobic bacteria isolated from contaminated tannery industry sites. Prep Biochem Biotechnol 46:517–523
17. Prasad R, Kumar M, Trivedi SP (2017) Antigenotoxic effect of turmeric powder extract curcumin against chromium trioxide induced genotoxicity in fish Channa punctatus. J Entomol Zool Stud 5(1):89–94
18. Bondy SC (2016) Inflammation, aging, and oxidative stress. In: Bondy SC, Cambell A (eds) Metal toxicity, inflammation and oxidative stress, 1st edn. Springer, Cham
19. Suh M, Thompson CM, Kirman CR, Carakostas MC, Haws LC, Harris MA, Proctor DM (2014) High concentrations of hexavalent chromium in drinking water alter iron homeostasis in F344 rats and B6C3F1 mice. Food Chem Toxicol 65:381–388

20. Kart A, Koc E, Dalginli KY et al (2016) The therapeutic role of glutathione in oxidative stress and oxidative DNA damage caused by hexavalent chromium. Biol Trace Elem Res 174:387. https://doi.org/10.1007/s12011-016-0733-0
21. Tian X, Patel K, Ridpath JR, Chen Y, Zhou Y-H, Neo D et al (2016) Homologous recombination and translesion DNA synthesis play critical roles on tolerating DNA damage caused by trace levels of hexavalent chromium. PLoS One 11(12):e0167503. https://doi.org/10.1371/journal.pone.016750
22. Wakeman TP, Aimin Yang A, Dalal NS, Boohaker RJ, Zeng Q, Ding Q, Xu B (2017) DNA mismatch repair protein Mlh1 is required for tetravalent chromium intermediate-induced DNA damage. Oncotarget 8(48):83975–83985
23. Hartikainen ES, Hatakka A, Kahkonen MA (2013) Impact of cadmium, chromium, cobalt, lithium and manganese to the growth of fungi and production of enzymes. Expert Opin Environ Biol 2(3). https://doi.org/10.4172/2325-9655.1000109
24. Rafi S, Shoaib A, Awan ZA, Rizvi NB, Nafisa, Shafiq M (2017) Chromium tolerance, oxidative stress response, morphological characteristics, and FTIR studies of phytopathogenic fungus *Sclerotium rolfsii*. Folia Microbiol 62(3):207–219
25. Luczak MW, Green SE, Zhitkovich A (2016) Different ATM signaling in response to chromium(VI) metabolism via ascorbate and nonascorbate reduction: implications for *in vitro* models and toxicogenomics. Environ Health Perspect 124(1):61–66. https://doi.org/10.1289/ehp.1409434
26. Park S, Li C, Zhao H, Darzynkiewicz Z, Xu D (2016) Gene 33/Mig6 inhibits hexavalent chromium-induced DNA damage and cell transformation in human lung epithelial cells. Oncotarget 7(8):8916–8930. https://doi.org/10.18632/oncotarget.6866
27. Cervantes C, Campos-Garc J, Devars S, Corona FG, Tavera HL, Torres-Guzma JC, Moreno-Sänchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25:335–347
28. Rao MY, Narechania MB (2016) The genotoxic effects of anti-cancer drug gossypol on human lymphocytes and its mitigation by melatonin. Drug Chem Toxicol 39(4). https://doi.org/10.3109/01480545.2015.1039646
29. Wise JP, Wise SS, Little JE (2002) The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cells. Mutat Res 517:221–229
30. Wise SS, Holmes AL, Liou L, Adam RM, Wise JP (2016) Hexavalent chromium induces chromosome instability in human urothelial cells. Toxicol Appl Pharmacol 296:54–60
31. Patierno SR, Banh D, Landolph JR (1988) Transformation of C3H/10T1/2 mouse embryo cells by insoluble lead chromate but not soluble calcium chromate: relationship to mutagenesis and internalization of lead chromate particles. Cancer Res 47:3815–3823
32. Ishikawa Y, Nakagawa K, Satoh Y, Kitagawa T, Sugano H, Hirano T, Tsuchiya E (1994) Characteristics of chromate workers' cancers, chromium lung deposition and pre-cancerous bronchial lesions: an autopsy study. Br J Cancer 70:160–166
33. Browning CL, Speer RM, Wise JP (2017) Molecular mechanisms of chromium-induced carcinogenesis. In: Mudipalli A, Zelikoff J (eds) Essential and non-essential metals. Humana, Cham
34. Wise SS, Wise C, Xie H, Guillette LJ, Zhu C, Wise JP Jr, Wise JP Sr (2016) Hexavalent chromium is cytotoxic and genotoxic to American alligator cells. Aquat Toxicol 171:30–36
35. Kamran M, Eqani S, Katsoyiannis A, Xu R, Bibi S, Benizri E, Chaudhary H (2017) Phytoextraction of chromium (Cr) and influence of Pseudomonas putida on Eruca sativa growth. J Geochem Explor 182:269–274
36. López-Luna J, Silva-Silva M, Martinez-Vargas S, Mijangos-Ricardez O, González-Chávez M, Solís-Domínguez F, Cuevas-Díaz M (2016) Magnetite nanoparticle (NP) uptake by wheat plants and its effect on cadmium and chromium toxicological behavior. Sci Total Environ 565:941–950
37. Sharma P, Kumar A, Bhardwaj R (2016) Plant steroidal hormone epibrassinolide regulate—heavy metal stress tolerance in Oryza sativa L. by modulating antioxidant defense expression. Environ Exp Bot 122:1–9

38. Farid M, Ali S, Akram NA, Rizwan M, Abbas F, Bukhari S, Saeed R (2017) Phytomanagement of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. Environ Sci Pollut Res 24(20):16845
39. Gu CS, Liu LQ, Deng YM, Zhang YX, Wang ZQ, Yuan HY, Huang SZ (2017) *De novo* characterization of the *Iris lactea* var. *chinensis* transcriptome and an analysis of genes under cadmium or lead exposure. Ecotoxicol Environ Saf 144:507–513
40. Wani RA, Ganai BA, Shah MA, Uqab B (2017) Heavy metal uptake potential of aquatic plants through phytoremediation technique – a review. J Bioremed Biodegr 8:4. https://doi.org/10.4172/2155-6199.1000404
41. Souza FB, Lima Brandao H, Hackbarth FV, AAU S, Boaventura RA, SMGU S, Vilar VJ (2016) Marine macro-alga Sargassum cymosum as electron donor for hexavalent chromium reduction to trivalent state in aqueous solutions. Chem Eng J 283:903–910
42. Oliveira LM, Gress J, De J, Rathinasabapathi B, Marchi G, Chen Y, Ma LQ (2016) Sulfate and chromate increased each other's uptake and translocation in As-hyperaccumulator Pteris vittata. Chemosphere 147:36–43
43. Oliveira LM, Ma LQ, Santos JA, Guilherme LR, Lessl JT (2014) Effects of arsenate, chromate, and sulfate on arsenic and chromium uptake and translocation by arsenic hyperaccumulator Pteris vittata L. Environ Pollut 184:187–192
44. Ma Q, Cao X, Ma J, Tan X, Xie Y, Xiao H, Wu L (2017) Hexavalent chromium stress enhances the uptake of nitrate but reduces the uptake of ammonium and glycine in pak choi (Brassica chinensis L.). Ecotoxicol Environ Safe 139:384–393
45. Sobariu DL, Fertu DIT, Diaconu M, Pavel LV, Hlihor RM, Drăgoi EN, Curteanu S, Lenz M, Corvini PFX, Gavrilescu M (2017) Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. New Biotechnol 39:125–134
46. Tiwari KK, Dwivedi S, Singh N, Rai U, Tripathi R (2009) Chromium (VI) induced phytotoxicity and oxidative stress in pea (Pisum sativum L.): biochemical changes and translocation of essential nutrients. J Environ Biol 30(3):389–394
47. Liu J, Duan CQ, Zhang X, Zhu Y, Hu C (2009) Subcellular distribution of chromium in accumulating plant Leersia hexandra Swartz. Plant Soil 322:187–195
48. Mahmud JA, Hasanuzzaman M, Nahar K, Rahman A, Hossain MS, Fujita M (2017) Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in Brassica juncea L. Ecotoxicol Environ Saf 144:216–226
49. Dalyan E, Yüzbaşıoğlu E, Keskin BC, Yıldızhan Y, Memon A, Ünal M, Yüksel B (2017) The identification of genes associated with Pb and Cd response mechanism in Brassica juncea L. by using *Arabidopsis* expression array. Environ Exp Bot 139:105–115
50. Tang CF, Song JF, Hu X, Hu X, Zhao Y, Li B, Ou D, Peng L (2017) Exogenous spermidine enhanced Pb tolerance in Salix matsudana by promoting Pb accumulation in roots and spermidine, nitric oxide, and antioxidant system levels in leaves. Ecol Eng 107:41–48
51. Xiao KQ, Li LG, Ma LP, Zhang SY, Bao P, Zhang T, Zhu YG (2016) Metagenomic analysis revealed highly diverse microbial arsenic metabolism genes in paddy soils with low-arsenic contents. Environ Pollut 211:1–8
52. Nakashita AM (2016) Combinatorial use of sulfur-responsive regions of sulfate transporters provides a highly sensitive plant-based system for detecting selenate and chromate in the environment. Soil Sci Plant Nutr 62(4):386–391
53. Murphy T, Kirkham C, Johnson A, Brauer A, Koszelak-Rosenblum M, Malkowski M (2016) Sulfate-binding protein, CysP, is a candidate vaccine antigen of Moraxella catarrhalis. Vaccine 34:3855–3861
54. Huda AKM, Haque MA, Zaman R, Swaraz AM, Kabir AH (2017) Silicon ameliorates chromium toxicity through phytochelatin-mediated vacuolar sequestration in the roots of Oryza sativa (L). Int J Phytoremediation 19(3):246–253
55. Diwan H, Khan I, Ahmad A, Iqbal M (2010) Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and Vigna radiata in response to chromium treatments. Plant Growth Regul 61:97–107
56. Kumari S, Varma A, Tuteja N, Choudhary DK (2016) Bacterial ACC-deaminase: an eco-friendly strategy to cope abiotic stresses for sustainable agriculture. In: Choudhary D, Varma

A, Tuteja N (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore

57. Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174:14–25
58. Islam MK, Khanam S, Lee SY, Alam I, Huhl MR (2014) The interaction of arsenic (As) and chromium (Cr) influences growth and antioxidant status in tossa jute (‘Corchorus olitorius’). Plant Omics 7:499
59. Kovacik J, Babula P, Hedbavny J, Klejdus B (2014) Hexavalent chromium damages chamomile plants by alteration of antioxidants and its uptake is prevented by calcium. J Hazard Mater 273:110–117
60. Farid M, Ali S, Rizwan M, Ali Q, Abbas F, Bukhari SA, Saeed R, Wu L (2017) Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicol Environ Saf 145:90. https://doi.org/10.1016/j.ecoenv.2017.07.016
61. Ganesh KS, Baskaran L, Rajasekaran S, Sumathi K, Chidambaram A, Sundaramoorthy P (2008) Chromium stress induced alterations in biochemical and enzyme metabolism in aquatic and terrestrial plants. Colloids Surf B Biointerfaces 63(2):159–163
62. Hua ZJ, Keli Y, ZhongGui Z, WuSheng J, DongHua L (2009) Antioxidant response system and chlorophyll fluorescence in chromium (VI)-treated Zea mays L. seedlings. Acta Biol Cracov Ser Bot 51:23–33
63. Adrees M, Ali S, Iqbal M, Bharwana SA, Siddiqi Z, Farid M, Ali Q, Saeed R, Rizwan M (2015) Mannitol alleviates chromium toxicity in wheat plants in relation to growth, yield, stimulation of anti-oxidative enzymes, oxidative stress and Cr uptake in sand and soil media. Ecotoxicol Environ Saf 122:1–8
64. Shahid M, Dumat C, Khalid S, Niazi NK, Antunes PMC (2017) Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system. Rev Environ Contam Toxicol 241:73–137
65. Wu Z, McGrouther K, Chen D, Wu W, Wang H (2013) Subcellular distribution of metals within Brassica chinensis L. in response to elevated lead and chromium stress. J Agric Food Chem 61:4715–4722
66. Khasheii B, Anvari S, Jamalli A (2016) Frequency evaluation of genes encoding siderophores and the effects of different concentrations of Fe ions on growth rate of uropathogenic Escherichia coli. Iran J Microbiol 8(6):359–365
67. Kong Z, Glick BR (2017) The role of plant growth-promoting bacteria in metal phytoremediation. Adv Microbl Physiol 71:97–132
68. Chen Y, Yang W, Chao Y, Wang S, Tang YT, Qiu RL (2017) Metal-tolerant *Enterobacter* sp. strain EG16 enhanced phytoremediation using Hibiscus cannabinus via siderophore-mediated plant growth promotion under metal contamination. Plant Soil 413:203–216
69. Chen X, Liu X, Zhang X, Cao L, Hu XX (2017) Phytoremediation effect of Scirpus triqueter inoculated plant-growth-promoting bacteria (PGPB) on different fractions of pyrene and Ni in co-contaminated soils. J Hazard Mater 325:319–326
70. Liu X, Fu JW, Silva ED, Shi XX, Cao Y, Bapathi BR, Chen Y, Ma LQ (2017) Microbial siderophores and root exudates enhanced goethite dissolution and Fe/As uptake by As-hyperaccumulator Pteris vittata. Environ Pollut 223:230–237
71. Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. J Hazard Mater 320:36–44
72. Wang B, Wang Q, Liu W, Liu X, Hou J, Teng Y, Luo Y, Christie P (2017) Biosurfactant-producing microorganism *Pseudomonas* sp. SB assists the phytoremediation of DDT-contaminated soil by two grass species. Chemosphere 182:137–142
73. Huang G, Guo G, Yao S, Zhang N, Hu H (2016) Organic acids, amino acids compositions in the root exudates and Cu-accumulation in castor (Ricinus communis L.) under Cu stress. Int J Phytoremediation 18(1):33–40

74. Soni SK, Singh R, Singh M, Awasthi A, Wasnik K, Kalra A (2014) Pretreatment of Cr(VI)-amended soil with chromate-reducing rhizobacteria decreases plant toxicity and increases the yield of Pisum sativum. Environ Contam Toxicol 66:616–627
75. Trivedi P, Pandey A, Sa T (2007) Chromate reducing and plant growth promoting activities of psychrotrophic *Rhodococcus erythropolis* MtCC 7905. Basic Microbiol 47:513–517
76. Soni SK, Singh R, Awasthi A, Kalra A (2014) A Cr(VI)-reducing *Microbacterium* sp. strain SUCR140 enhances growth and yield of *Zea mays* in Cr(VI) amended soil through reduced chromium toxicity and improves colonization of arbuscular mycorrhizal fungi. Int Environ Sci Pollut Res Int 21(3):1971–1979
77. Rosariastuti R, Prijambada ID, Ngadiman N, Prawidyarini GS, Putri AR (2013) Isolation and identification of plant growth promoting and chromium uptake enhancing bacteria from soil contaminated by leather tanning industrial waste. J Basic Appl Sci 9:243–251
78. Faisal M (2013) Inoculation of plant growth promoting bacteria *Ochrobactrum intermedium, Brevibacterium* sp. and Bacillus cereus induce plant growth parameters. J Appl Biotechnol 1:45–53
79. Faisal M, Hasnain S (2006) Plant growth promotion by *Brevibacterium* under chromium stress. Res J Bot 1(1):24–29
80. Faisal M, Hasnain S (2006) Growth stimulatory effect of *Ochrobactrum intermedium* and Bacillus cereus on Vigna radiata. Lett Appl Microbiol 43:461–466
81. Faisal M, Hasnain S (2005) Bacterial Cr(VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. Biotechnol Lett 27(13):943–947
82. 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
83. Wani PA, Khan MS, Zaidi A (2008) Chromium-reducing and plant growth-promoting Mesorhizobium improves chickpea growth in chromium-amended soil. Biotechnol Lett 30:159–163
84. Chatterjee S, Sau GB, Mukherjee SK (2009) Plant growth promotion by a hexavalent chromium reducing bacterial strain, *Cellulosimicrobium cellulans* KUCr3. World J Microbiol Biotechnol 10:1829–1836
85. Braud A, Jezequel K, Bazot S, Lebeau T (2009) Enhanced phytoextraction of an agricultural Cr- and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 74:280–286
86. Wales DS, Sagar BF (1990) Recovery of metal ions by microfungal filters. J Chem Technol Biotechnol 49:345–355
87. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250
88. Pillichshammer M, Pumpel T, Poder R, Eller K, Klima J, Schinner F (1995) Biosorption of chromium to fungi. Biometals 8:117–121
89. Asheh S, Duvnjak Z (1995) Adsorption of copper and chromium by *Aspergillus carbonarius*. Biotechnol Prog 11:638–642
90. Rapoport AI, Muter OA (1995) Biosorption of hexavalent chromium by yeast. Process Biochem 30:145–149
91. Loubna E, Hafidi M, Silvestre J, Kallerhoff J, Merlina G, Pinelli E (2015) Efficiency of co-composting process to remove genotoxicity from sewage sludge contaminated with hexavalent chromium. Ecol Eng 82:355–360
92. Gómez SR, Naranjoa EM, Buenoa IV, Feldmanb SR (2011) Accumulation and tolerance characteristics of chromium in a cordgrass Cr-hyperaccumulator, Spartina argentinensis. J Hazard Mater 185(2–3):862–869
93. Buendía-González L, Orozco-Villafuerte J, Cruz-Sosa F, Barrera-Díaz CE, Vernon-Carte EJ (2010) Prosopis laevigata a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant. Bioresour Technol 101(15):5862–5867
94. Ghafoori M, Majid N, Islam M, Luhat S (2011) Bioaccumulation of heavy metals by Dyera costulata cultivated in sewage sludge contaminated soil. Afr J Biotechnology 10(52):10674–10682

95. Mellem JJ (2009) Translocation and accumulation of Cr, Hg, As, Pb, Cu and Ni by Amaranthus dubius (Amaranthaceae) from contaminated sites. J Environ Sci Health A Tox Hazard Subst Environ Eng 44(6):568–575
96. Gardea-Torresdeyab JL, Peralta-Videaa JR, Montesb M, Rosaa G, Corral-Diaz B (2004) Bioaccumulation of cadmium, chromium and copper by Convolvulus arvensis L.: impact on plant growth and uptake of nutritional elements. Bioresour Technol 92(3):229–235
97. Daud M K, Mei L, Azizullah A, Dawood M, Ali I, Mahmood Q, Ullah W (2016) Leaf-based physiological, metabolic, and ultrastructural changes in cultivated cotton cultivars under cadmium stress mediated by glutathione. Environ Sci Pollut Res 23(15):15551–15564
98. Jie Liu J, Duan C, Zhang X, Zhu Y, LuX (2011) Potential of Leersia hexandra Swartz for phytoextraction of Cr from soil. J Hazard Mater 188(1–3):85–91
99. Sampanpanish P, Pongsapich W, Khaodhiar S, Khan E (2006) Chromium Removal from Soil by Phytoremediation with Weed Plant Species in Thailand. Water Air Soil Pollut 6(1-2):191–206
100. Khan N, Mishra A, Chauhan PS, Sharma YK, Nautiyal CS (2012) Paenibacillus lentimorbus enhances growth of chickpea (Cicer arietinum L.) in chromium-amended soil. Antonie van Leeuwenhoek 101(2):453–459
101. Sabir M, Waraich EA, Hakeem KR, Ozturk M, Ahmed HR, Shahid M (2015) Phytoremediation: Mechanism and adaptation. In: Khalid RH, Sabir M, Ozturk M, Murmet M (eds) Soil remediation and plants. Elsevier Academic Press, pp 85–105

# Part V

# Phytoremediation Applications of Organic Contaminants

# Chapter 11
# Biological Aspects of Selenium and Silicon Nanoparticles in the Terrestrial Environments

**Hassan El-Ramady, Tarek Alshaal, Nevien Elhawat, Eman El-Nahrawy, Alaa El-Dein Omara, Sahar El-Nahrawy, Tamer Elsakhawy, Azza Ghazi, Neama Abdalla, and Miklós Fári**

## 11.1 Introduction

Nanotechnology is an emerging technology with great wealth of applications involving the synthesis and application of nanomaterials (1–100 nm). Recently, there is a great emphasis on nanoparticle synthesis methods including chemical,

---

H. El-Ramady
Faculty of Agriculture, Soil and Water Department, Kafrelsheikh University,
Kafr El-Sheikh, Egypt

T. Alshaal
Faculty of Agriculture, Soil and Water Department, Kafrelsheikh University,
Kafr El-Sheikh, Egypt

Agricultural Botanic, Plant Physiology and Biotechnology Department, University of
Debrecen, Debrecen, Hungary

N. Elhawat (✉)
Agricultural Botanic, Plant Physiology and Biotechnology Department, University of
Debrecen, Debrecen, Hungary

Faculty of Home Economic, Department of Biological and Environmental Sciences, Al-Azhar
University, Cairo, Egypt

E. El-Nahrawy · A. E.-D. Omara · S. El-Nahrawy · T. Elsakhawy · A. Ghazi
Agricultural Microbiology Department, Soil, Water and Environment Research Institute
(SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC),
Kafr El-Sheikh, Egypt

N. Abdalla
Genetic Engineering Division, Plant Biotechnology Department, National Research Center,
Giza, Egypt

M. Fári
Agricultural Botanic, Plant Physiology and Biotechnology Department, University of
Debrecen, Debrecen, Hungary
e-mail: fari@agr.unideb.hu

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_11

physical, and biological methods [1–3]. These physical and chemical methods involve high energy requirements, low material conversions, use of hazardous chemicals and toxic solvents, as well as generation of hazardous by-products especially when applied in some fields [4]. On the other hand, there is an increasing use of biological approaches for synthesis of nanomaterials which involve high-yield, low-cost, nontoxic, biocompatible, and eco-friendly [2, 5, 6]. Biosynthesis of nanomaterials refers to the phenomenon that employs biological process either by microorganisms or plant extracts which can provide inorganic metabolites either intracellular or extracellular [7, 8]. The biosynthesis of Se and Si were primarily reported using microbes such as bacteria and fungi with a few from plant origin. Among plants, the biosynthesis of nano-Se was reported using *Vitis vinifera* (raisin) fruit [9], *Trigonella foenum-graecum* (fenugreek) from seed extract [10], and *Capsicum annuum* from polygonal leaves extract [11], but the biosynthesis of nano-Si was reported using *Poaceae* [12], *Equisetaceae* [13], nettle stinging hairs [14], and diatoms (frustules) [15].

Concerning the biogenic synthesis of Se nanoparticles by different genus of bacteria, many species have been reported including *Bacillus cereus* [16], *Bacillus subtilis* [17], *Sulfurospirillum barnesii*, *Bacillus selenitireducens*, *Selenihalanaerobacter shriftii*, and *Shewanella* sp. [18]. For the biosynthesis of Si nanoparticles, *Actinobacteria* sp. can produce some enzymes that hydrolyze the metal precursor ($K_2SiF_6$) to form Si/$SiO_2$ nanocomposites [19]. On the other hand, few fungi can synthesize Se nanoparticles such as *Aspergillus terreus* [20] and *Alternaria alternate* [21], whereas *Fusarium oxysporum* can produce Si nanoparticles of average size 7–8 nm [22]. There are common benefits regarding nano-Se and nano-Si for higher plants including protection plants against diseases and abiotic stress. There are a few studies regarding the biological role under stress conditions of both Se and Si in higher plants such as Khattab et al. [23], Tang et al. [24], and Shekari et al. [25].

Regarding the biological roles of Si in agriculture, these roles include the alleviated effects of abiotic stresses such as salt stress [26, 27], drought stress [28, 29], metal toxicity [30, 31], radiation damage [32], nutrient imbalance [33], high temperature [34, 35], and plant leaf senescence [36] as well as biotic stress [37]. The same biological effects could be recognized for Se. On the other hand, a few studies have been published concerning the biological role of Si nanoparticles under stress conditions including salt stress [38], drought [39], metal toxicity [40], radiation damage [33], and nutrient imbalance [32]. Si nanoparticles could be mainly applied in agricultural field through Si fertilizers and nutrient supplements. Many studies have been reported regarding the beneficial effects of Si nanoparticles on plants (e.g., [38, 41–43]). Therefore, it could present a detailed outlook in this chapter about the beneficial roles of Se and Si nanoparticles and their biological role for higher plants in the terrestrial environments. Also, biogenic synthesis of their elements by plant and microorganisms (bacteria and fungi) will be also highlighted.

## 11.2 Selenium and Silicon: More Than Beneficial Elements for Higher Plants

### *11.2.1 Selenium in the Terrestrial Environments*

Since 1817, when the Swedish scientist Berzelius discovered this element and named it selenium (*selene* means Moon), it has puzzled the researchers with its ever-changing appearance, akin to moon, from being toxic to carcinogenic and then carcinostatic [44]. At high doses, Se can be toxic, but it is also an essential element for human health [45–50]. Se is a metalloid from chalcogen (ore forming) family in the periodic table (group16). It can exist in a range of oxidation states from (+6) to selenide (−2) [51]. Elemental Se ($Se^0$) has been known to exist in various allotropic forms, as red amorphous form, black vitreous form, three (α, β, γ) of red crystalline monoclinic forms, and gray/black crystalline hexagonal (also referred to as trigonal) form which is also the most stable form, and some more allotropes are being discovered. The red amorphous form is believed to be mainly consisting of a mixture of small rings and deformed hexagonal-form-like chains of selenium atoms [52, 53]. Industrially, Se is produced as a by-product of copper refining; it is used in glass, steel, electronics, ceramics, and pigment manufacturing [54]. The photoconductivity of Se found its use in a photometer by Siemens, 1875; a photophone by Graham Bell, 1880; an optophone by Fourniere d'Albe, 1912; and the talking films in 1921 [55].

The global distribution of Se in the terrestrial environments depends on both of regional and broad-scale processes. Therefore, the local Se distribution is governed by small- and microscale processes that related to mobility and bioavailability, i.e., the uptake, speciation, solubility, and partitioning of Se. So, plants can assist the region of Se by redistribution through volatilization to the atmosphere, as well as decrease mobility by accumulating into plant tissues [56]. A lot of reports about Se in different environments including soils, waters, sediments, air, crops, and food systems can be found in different studies [57–61]. Therefore, the bioavailability of Se element in the terrestrial environments is related to many factors that make it available to an organism [62]. These factors include soil physiochemical properties, i.e., soil pH, Eh, total organic carbon, and chelates, which can effect on the uptake and accumulation of Se and other nutrient elements by plants [60, 61]. Also, some organic acids exuded by roots may play important roles in determining bioavailability of Se and other mineral nutrient elements in the soil [63–65].

In addition, it is reported that it is a priority to know the Se bioavailability, and the absorbed and used amount by the organism due to the absorbed and transformed Se into a biological available form is usually a fraction [66]. Generally, it should involve a complete evaluation of bioavailability measurements of total Se content, amount actually absorbed, absorbable fraction, and percent utilized by the organism [59–61]. Directly or indirectly, the natural and biological cycle of Se element depends upon the soil–plant system [56]. Also, the soil pH can have a marked influence on the Se content of the plants. For example, in alkaline soils, chemical oxidation produces selenate which is available for plants [67]. The biogeochemical cycle of Se is very important, where some objects like soils, microorganism, crop, grass, animal, or human being. This biogeochemistry cycle will be started after its entry into agroecosystems, i.e., input, translation, transportation, accumulation, and output [58, 68, 69]. The understanding of Se biogeochemistry in different natural environments and its factors controlling Se speciation in natural environments should be addressed for more details [70]. In addition, Se could be lost into the atmosphere through biomethylation process through plants and soil microorganisms. Therefore, Se mobilization across soil–plant–atmosphere interfaces is thus of crucial importance for human Se status [45, 56].

### *11.2.2 Selenium Uptake Metabolism*

The uptake and metabolism of Se are very important processes for the Se nutrition of humans, animals, and plants. Therefore, in recent years, many studies have been published concerning the uptake and metabolism of selenium by plants [56, 71, 72]. The uptake of Se by plants is mainly affected by soil factors, of which the most significant are pH, Eh, water regime, clay content, soil organic matter, cation exchange capacity, nutrient balance, and concentration of other trace elements as well as the plant-specific ability [73]. On the other hand, climatic conditions also are affected partly and indirectly on the rate of Se uptake, which is due to the water flow phenomenon. Also, the greater ambient temperature influences a higher uptake of Se by plants. Several factors depend on the uptake of Se by plants, but in this case, when Se is present in soluble forms, it is readily absorbed by plants, although differences between plant species are very pronounced [45, 48, 56, 58, 71]. Plants mainly can uptake Se from soil solution via sulfate transporters in the form of selenate ($SeO_4^{2-}$) by plant roots as an active uptake. There is a strong competition between S and Se uptake because both anions use the sulfate transporter in root plasma membrane. In this pathway, selenate is reduced to selenite ($SeO_3^{2-}$), and then a reduction to selenide form ($Se^{2-}$) can happen. On the other hand, Se organic forms such as selenomethionine (SeMet) or selenocysteine (SeCys) are also taken up actively by plant roots. So, the similarity in Se and S uptake by plants may help in the uptake and metabolism of Se via pathways of S transporters [46, 71].

Regarding Se metabolism in higher plants, different plant capacities to extract and accumulate this element is evidently related to different metabolic strategies [74]. The availability of Se in soil is also controlled by several soil factors, among which pH is believed to be the most pronounced, as reviewed by Kabata-Pendias [73]. There is a positive linear correlation in most cases between Se content of soils and Se in plant tissues [73]. Otherwise, the complex effect of variable factors on Se uptake by plants can significantly alter the relation between Se in plants and soils. Several compounds of Se have been identified in different plant types and recorded by several authors ([46, 69, 75, 76]). Several plants have the ability to convert Se into volatile compounds and then released into the atmosphere, where this could be defined as mechanism tolerance of Se. Thus, reducing their Se load is an important metabolic activity of a variety of different plant types. The rate of Se volatilization varies widely among plant species [77]. Rates of volatilization and plant species are related to some factors, which are depending on concentration and chemical form of Se and S in the soil, through different seasons of the year. Kapolna and Fodor [78] showed that the main Se species is selenate when fed to plant with $SeO_4^{2-}$, whereas in plants fed with $SeO_3^{2-}$, SeMet and selenomethionine Se-oxide hydrate (SeOMet) dominate in Indian mustard (*Brassica juncea*). Also, the major Se compound in Se-enriched garlic (*Allium sativum*), onion (*Allium cepa*), leek (*Allium ampeloprasum*), and broccoli (*Brassica oleracea*) is SeMet and SeCys, as reported in many studies ([58, 79, 80]).

### 11.2.3 Silicon in the Terrestrial Environments

Silicon (Si) is the second most abundant element after oxygen in the earth's crust which comprises 28.8% by dry weight, and all different plant types contain Si in their tissues as well [32, 33]. Si has an atomic weight of 28.09 and has atomic number 14 on the periodic table of elements [81]. On the other hand, Si as a combined form with oxygen is ubiquitous in nature. Therefore, Si constitutes about 16 g $kg^{-1}$ of soils, with a mean of 330 g $kg^{-1}$, depending on the sample technique, depth and arithmetic procedure associated with determination [82, 83] and its presence in the form of silicic acid [$Si(OH)_4$] (or its ionized form, $Si(OH)_3O_2$, which predominates at $pH_9$) facilitates its uptake by plants. Since there is still no evidence showing that Si is involved in the metabolism of plants, Si has not been recognized as an essential element according to the criteria of essentiality established by Arnon and Stout [84]. However, beneficial effects of Si have been observed in a wide variety of plant species. Nevertheless Si is not (or hardly) considered as beneficial: there is a lot of skepticism in regular medicine because Si has been considered to be inert in humans. In 1973 the Joint FAO/WHO Expert Committee on Food Additives says: "data on orally administered silica and silicates appear to substantiate the biological inertness of these compounds." Si has not yet been listed among the essential elements for higher plants partly because its roles in plant biology are poorly understood and direct evidence is still lacking that it is a part of plant constitutes or enzymes [85–87]. However, there is an increasing body of evidence showing that Si is beneficial for the growth of many plants, especially under various abiotic (e.g., aluminum, salt, and heavy metal toxicity) and biotic stresses (e.g., plant diseases and pests) [32, 33, 85, 88–90].

Terrestrial plants contain Si in appreciable concentrations, ranging from a fraction of 1% of the dry matter to several percent, and in some plants up to 10% or even higher [86]. Higher plants containing Si can be classified according to the amount of Si they contain [91]. The *Cyperaceae* and wetland species of *Gramineae* typically contain 10–15% (dry shoot weight), dry land *Gramineae* generally has 1–3% Si, and, finally, most dicotyledonous species have 0.5% or less. There are fairly distinct differences in the distribution and characteristics of Si deposition between $C_3$ and $C_4$ plants, with $C_3$-type grasses producing more oval phytoliths and $C_4$ grasses tending to produce the same type of silica bodies [92]. In agriculture, Si mainly has several significant roles including plant pathology, nanotechnology, biotechnology, and plant stress as well as plant physiology. Several publications have been issued concerning many topics of silicon including the uptake, transport, and then accumulation of silicon in higher plants (e.g., [26, 32, 33, 87, 93]).

The accumulation of Si in plants may attribute to be an effective uptake system mediated not only by passive diffusion but also by specific transporters like Lsi transporters as a high elucidator for rice, barley, and maize or moderate for pumpkin and soybean or Si transporter genes (GmNIP2-1 and GmNIP2-2) for soybean [87]. It is worth to mention that Si is the sole element that has no detrimental effects on plants even if it is taken up and accumulated excessively in plant tissues due to the fact that silicic acid possesses the unique physicochemical properties of dissociation at physiological pH ranges and polymerization [26, 89]. At the end of the growing season, after the decay of organic plant matter, silica phytoliths accumulate as a meta-stable component of the underlying soil. Phytoliths mean the plant stone and have many properties like a rigid, microscopic structure made of silica, found in some plant tissues and persisting after the plant decaying. Furthermore, phytoliths are ubiquitous in soils resulted in the rain forests [94–96] or from grasslands [95, 97] or from temperate deciduous and evergreen forests [98] or from wetland areas [99–101].

The contribution of phytoliths to the biogeochemical cycling of Si was first reported by Bartoli [98] followed by many authors (e.g., [101–103]). They proved that phytoliths are an importance of the Si internal cycle (recycling of Si within soils to plants) helping in the nutrient availability for ecosystem productivity and for the internal bio-cycling of materials. It is well known that Si in silicate form is the main component of the Earth's crust, where Si oxides represent about 90% of all minerals [104]. Concerning the biogeochemistry of Si, the dissolving of orthosilicic acid ($H_4SiO_4$) could happen from the reaction between dissolved soil $CO_2$ and silicate weathering released from minerals of silicates. These dissolved silicates (i.e., orthosilicic acid) may be transported through soils and exported into rivers and eventually the ocean (Fig. 11.1). It is estimated that the consumption of global $CO_2$ through chemical weathering process is about 0.26 Gt C year$^{-1}$ [105], where the $CO_2$ concentration could impact on both the silicate weathering rate and the biosphere development [106]. Therefore, the global Si-cycle is complex interactions of chemical, biological, and geological processes, acting on a wide variety of spatial and temporal scales (Fig. 11.1). The transport of Si along the terrestrial ecosystems is mainly controlled by a complex set of terrestrial and aquatic processes, and the Si elemental cycle is receiving increasing attention due to its global environmental impacts [26, 104, 107].

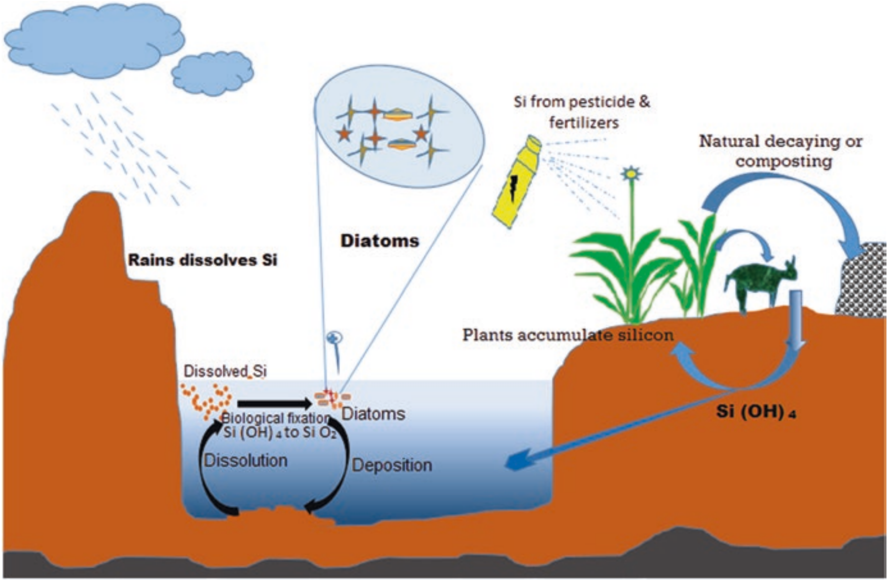


**Fig. 11.1** The silicon cycle in the agroecosystems. This cycle includes some sources of silicon in the nature like diatom (*Bacillariophyceae*), pesticides, and fertilizers. The natural decay or composting of silicon as well as the interactions of silicon in aquatic environments and the role on rainfall in dissolving this element could be also noticed. It could be considered terrestrial ecosystems as reactors in which silicon released through silicate weathering with vertical and horizontal translocation and temporary and permanent immobilization

### *11.2.4 Silicon Uptake and Metabolism*

Silicon is a beneficial element for plant growth. Based on such important roles of Si in plants, Epstein and Bloom [108] have proposed Si as "quasi-essential" for the growth of higher plants. These beneficial effects are mostly expressed through Si deposition in the leaves, stems, and hulls, although other mechanisms have also been proposed [109, 110]. Therefore, Si contents vary greatly in plant tissues, depending much upon plant species, which is attributed mainly to the differences in the mechanisms for Si uptake and transport [86, 89, 111, 112]. Uptake of Si in the root tip region (0–10 mm) comprising both the apical meristem and the elongation zone is also much lower than that in the basal regions (>10 mm from the root tips). These observations indicate that the site of Si uptake is located in the mature regions of the roots rather than the root tips [113]. On the other hand, Si is taken up in the form of an uncharged molecule of silicic acid [114]. Three different modes of Si uptake have been proposed for plants having different degrees of Si accumulation, i.e., active, passive, and rejective uptake [115]. Plants with an active mode of uptake take up Si faster than water, resulting in a depletion of Si in the solution, whereas plants with a passive mode of uptake take up Si at a rate that is similar to the uptake rate of water; thus, no significant changes in the concentration of Si in the uptake solution are observed. By contrast, plants with a rejective mode of uptake tend to exclude Si, which is demonstrated by the increasing concentration of Si in the solution. However, the mechanisms involved in the different uptake modes are not understood ([93, 116]).

The characteristic function of Si is to help plants to overcome multiple biotic and abiotic stresses [26, 32, 33]. For example, Si plays an important role in increasing the resistance of plants to pests and pathogens such as blast on rice [117] and powdery mildew on cucumber [118, 119]. In Japan, Si has been recognized as an "agronomically essential element" for rice. It is a typical Si-accumulating plant, because high accumulation of Si is required for optimal growth and sustainable production of rice [107, 120]. Therefore, the Si effect is characterized by a larger effect associated with a greater Si accumulation in the shoots. However, Si accumulation in the shoots varies considerably among plant species, ranging from 0.1% to 10% Si in the dry weight [121]. Also, Si is effective in preventing lodging in rice by increasing the thickness of the culm wall and the size of the vascular bundles [122], thereby enhancing the strength of the stems. Also, Si alleviates the effects of other abiotic stresses including salt stress [25, 26, 39], metal toxicity [30, 31], drought stress [28, 39], radiation damage [32], nutrient imbalance [33], high temperature [34, 35], plant leaf senescence [36], and freezing [85, 87, 121, 123] as well as biotic stress [37].

## 11.3 Selenium and Silicon Nanoparticles: Their Biological Role for Plants

### *11.3.1 Selenium Nanoparticles and Their Biological Role for Plants*

Nano-Se could be defined as nano-elemental selenium or nano-Se manufactured for use in nutritional supplements and developed for applications in Se fertilization [124]. It is bright red, highly stable, and of nano-size in the redox state of zero ($Se^0$). Nano-Se has a higher efficiency in upregulating selenoenzymes and exhibits less toxicity than selenite [64]. As well known, $Se^0$ is rare, occurring mostly in sedimentary rocks [125]. From three allotropes of $Se^0$, the gray and the black ones are biologically inert, which may be due to their insolubility [126]. A lot of reports are also appearing on enhanced efficacy of nanoscale Se in reproduction, digestion, growth, and immune modulation [58, 69, 127]. Moreover, the toxicity impact of Se has been reduced with synthesized nano-Se proposed by [128], and new physiological and biological properties such as its role in microbial inhibition and fatty liver prevention have been explored that were not elucidated earlier. Also, nano-Se can be administered to reduce oxidative stress and to increase the productivity of stress-ridden fish and livestock [69, 129, 130].

For years, biological effects of organic forms of Se and some salts have been studied, but recently, elemental Se nanoparticles have gained some attention as a possible source of this beneficial component [131–133]. The biological roles of Se nanoparticles depend on size, playing an important role in range of 5–200 nm [134]. Also, the transmission electron micrograph showed that the separated Se nanoparticles were spherical in range of 80–220 nm in size [135]. These nanoparticles also have the antioxidant properties of hollow spherical nanoparticles of Se [124]. It is found also that Se nanoparticles have an excellent bioavailability, a high biological activity, and low toxicity [136]. Therefore, Se nanoparticles are gaining importance in electronics and optics due to their enhanced photoconducting, semiconducting, catalytic, and photoelectrical properties [137, 138]. Se nanoparticles exhibit low cytotoxicity compared to Se compounds and possess excellent anticancer and therapeutic activities making them apt to medicinal applications [69, 139–141]. Se is required as a cofactor for glutathione peroxidases and thioredoxin reductase in animals, which is supplied in their meal as an essential element. However, studies have shown that Se nanoparticles are utilized more efficiently than inorganic and organic Se [58, 142, 143].

Many studies involved the different biological roles of Se nanoparticles on plants and their biosynthesis [58, 64, 65, 144, 145]. So, these Se nanoparticles have potential applications in both biological and nanotechnological fields such as more applications for Se nanoparticles, which represented the development of safer Se vitamins and food additives [60, 61, 130, 146], new chemo-preventatives [132, 133], novel antibiotic coatings [147], anticancer treatments [139, 148], and in vivo fluorescent dyes for bioimaging applications ([149]). Se nanoparticles can also be used in electronic devices including solar cells [150], photosensors [151], and chemical sensors [17]. Also, it is reported that inorganic nanoparticles can be used by synthetic polymer in different forms such as encapsulated or stabilized, i.e., dendrimers, hyperbranched polymers, and linear polymers [152].

There are great opportunities for the intervention of nanotechnologies in the area of fertilizers and plant nutrition [58] especially using nano-Se in fertilization field, which plays important role for increasing crop yield and agricultural productivity as well as food security [153]. Recently, several patents and products containing nanomaterials for crop nutrition and protection are increasing (e.g., [154, 155]). Few studies have been published concerning the comparison between Se nanoparticles and other inorganic Se forms in higher plants [144, 148, 156–159]. These studies involved different biological and physiological effects on different plants including rice [157], tobacco [144, 156], tomato [158], and giant reed [148]. The stimulating effect of Se nanoparticles had already been detected during the rooting stage [156]. She reported that Se nanoparticles stimulated the root regeneration in higher concentrations giving more extensive and dense roots when Se nanoparticles were applied with rate of 50–100 mg $L^{-1}$ and fresh weight increased significantly as well. Also, Se nanoparticles were taken up by tobacco callus cultures and resulted in good rooted tobacco plantlets. The biological effects of Se nanoparticles were different from the selenate ion ($SeO_3^{2-}$) in plant tissue culture. It is found that concentration range of Se nanoparticles is 50–100 mg $kg^{-1}$, and 40% stimulated the organogenesis and the growth of root system significantly, while selenite did not show these effects at any concentration, moreover, inhibited both callus growth and root regeneration totally in 50–100 mg $kg^{-1}$ concentrations.

### *11.3.2 Silicon Nanoparticles and Their Biological Role for Plants*

Nowadays, the polymer nanocomposites have been gained the attention of most researchers. The unique properties of these materials have been allocated to the attention of most scientists [160]. There is a great case for the nanocomposite properties which can be received making compatibility between the nanoparticles and the polymer [161]. Si nanoparticles have a great deal of biomedical research due to their stability, low toxicity, and ability to be functionalized with a range of molecules and polymers. Silica particles with very small particle size and high surface area have a wide use in polymer industry and surface coating. The level of these particles includes three hydroxyl groups of chemicals, hydrogen, and hydroxyl groups attached to the siloxane groups (functional groups in organosilicon chemistry with the Si–O–Si linkage). Therefore, the particles are hydrophilic, although the siloxane groups are hydrophobic alone,possibly there are hydrophobic silica particles using hydrophobic materials such as hexamethyl disilane, i.e., organo-silicon compound with the formula $Si_2(CH_3)_6$ [162]. Many investigations have been done on the use of silica particles of different polymers (e.g., [132, 133, 163]). Due to surface properties of the nanoparticles, it is found that silica nanoparticles could improve the strength, modulus, hardness, creep resistance, and adhesion of materials [164–166].

A lot of attention has been given by researchers for the application of nano-Si in agriculture. Si nanoparticles have greater surface area and higher reactivity comparing with the bulk form. Si nanoparticles could help in increasing the uptake of nutrients by plants due to higher solubility and surface reactivity as well as its application for mitigating different stress conditions. Therefore, the application of Si nanoparticles have provided new solutions to problems in plants and food science in agriculture systems in order to enhance the quality of plant products [32, 33, 167]. Si nanoparticles play great roles in plants under stressful conditions, whereas under normal conditions, its role is often minimal or even nonexistent. Therefore, the role of Si nanoparticles in plant health and the productivity of soils are receiving increased attention in the agricultural sciences [168]. For example, Si in the form of $H_4SiO_4$ or dissolved silicate is readily uptaken by plants and is deposited as a solid amorphous Si [73, 169].

Few studies have been published concerning the biological role of Si nanoparticles under stress conditions including salt stress (e.g., [38, 39]), metal toxicity [40, 170, 171], drought [39], radiation damage [33], nutrient imbalance [32], high temperature, and freezing. For radiation damage, Tossi et al. [172] reported that

UV-B causes reduction in maize growth by inducing membrane damage. Additionally, some apparent signs such as plant dwarfism, notable changes in shoots, and marked reduction in length and width of leaf in wheat seedlings were also noticed, which might be linked with modification in cell division or cell elongation [173]. Besides this, decline in growth of wheat seedlings exposed to UV-B could be correlated with high levels of reactive oxygen species (ROS) that cause negative impact on macromolecules such as lipids (lipid peroxidation), and it was also accompanied by a fluctuation in NO level [32, 174, 175]. Furthermore, Si nanoparticles appear to be more efficient in mitigating UV-B stress than Si, which is related to its greater availability to wheat seedlings than Si as indicated by data of Si accumulation [176, 177]. However, Si and Si nanoparticles managed these alterations as it is known that Si plays as a supportive role in minimizing internal injuries of plant structures caused by different stresses [178–180]. Tripathi et al. [33] showed that UV-B radiation negatively influenced growth of wheat seedlings through interference in photosynthesis due to decreased levels of antioxidants (superoxide dismutase and peroxidase) and enhanced level of oxidative stress. However, pre-addition of either Si or Si nanoparticles protect wheat seedlings against UV-B stress by protecting photosynthesis and regulating level of oxidative stress. Therefore, Si nanoparticles might protect wheat seedlings through NO-mediated triggering of antioxidant defense system [173, 181, 182].

For metal toxicity, Tripathi et al. [183] have also reported that Si nanoparticles may mitigate negative impact of Cr(VI) on PS II photochemistry in pea seedlings. Furthermore, Genty et al. [184] suggested that enhanced values of Si nanoparticles under stressful conditions may be due to downregulation of PS II to avoid excess reduction in order to equivalent the reduced requirement for electrons through NADPH utilization. It is reported that Si nanoparticles-mediated amelioration of Cr(VI) phytotoxicity may be assigned to more than one mechanism: (1) Si nanoparticles-mediated decrease in Cr accumulation, (2) decreased level of ROS and oxidative damage, (3) enhanced antioxidant defense system, (4) enhanced level of macro- as well as micronutrients, etc. [32].

## 11.4 Biosynthesis of Selenium and Silicon Nanoparticles

No doubt that several products containing nanoparticles became part of our daily life nowadays. Due to limited information regarding the fate and behavior of nanoparticles in the environment including their uptake, distribution, metabolism, and toxicity, there is an urgent need for nano-safety researches [185]. It is well known that a variety of plants and microorganisms including bacteria, fungi, algae, yeast, and actinomycetes can uptake and accumulate metals, but only a few groups can selectively reduce metal ions to produce nanoscale mineral phases [186, 187]. Biogenic synthesis of Se nanoparticles, for example, is frequently achieved by reduction of selenate/selenite in the presence of bacterial proteins or plant extracts containing phenols, alcohols, proteins, flavonoids amines, and aldehydes [188]. So, biogenic synthesis of nano-selenium could be mediated by plants, bacteria, and fungi [64, 65, 137].

### 11.4.1 Biosynthesis of Selenium Nanoparticles by Plants

By definition, particles having one dimension up to 100 nm could be called nanoparticles [174, 189]. However, it is important to note that Se sub-micronic particles of size more than 100 nm are also reported as Se nanoparticles in the literature. For example, *Duganella* sp. and *Agrobacterium* sp. can reduce selenite to elemental Se of size 140–200 nm and 185–190 nm, respectively [190]. There are few reports on biogenic Se nanoparticles in the range of 1–100 nm [9, 135, 191]. However, size of particles can be controlled by optimizing the physicochemical parameters during synthesis. Such studies are reported using silver, gold, and platinum nanoparticles via bacteria and plants [192–194]. On the other hand, a lot of plants are reported for nanoparticle synthesis [11, 18, 135, 195], such as leaf extract of *Capsicum annuum* that reduces $SeO_3^{-2}$ to Se nanoparticles indicating a red color [11]. Fruit extract (dried) of *Vitis vinifera* can synthesize Se nanoparticles in the range of 3–18 nm [9]. Also, polysaccharides extracted from *Undaria pinnatifida*, edible seaweed, enhance the stability of Se nanoparticles [195] as shown in Fig. 11.2.

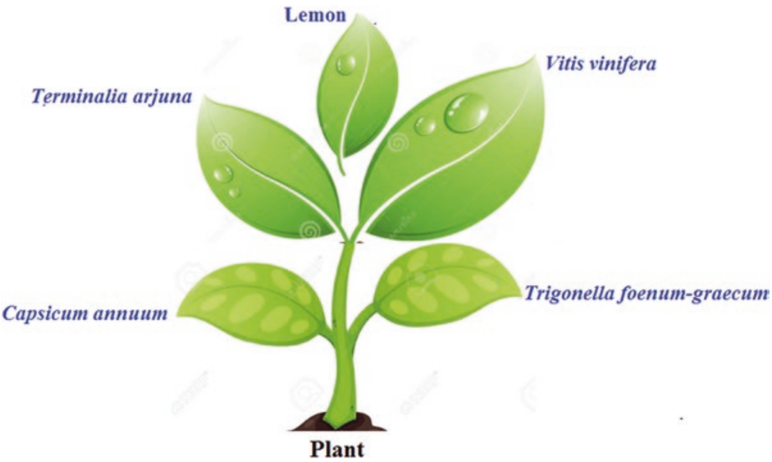


**Fig. 11.2** Some plants reported for synthesis of selenium nanoparticles, whereas *Trigonella foenum-graecum* (fenugreek) from seed extract reported by [10] *Capsicum annuum* from polygonal leaves extract reported by [11]; Lemon from leaves extract reported by [135]; *Terminalia arjuna* from polydispersed leaf extract reported by [191] and *Vitis vinifera* from spherical fruit extract [9]. Plant photo was extracted from the following link: http://www.theecologist.org/blogs_and_comments/commentators/other_comments/2150973/peak_soil_act_now_or_the_very_ground_beneath_us_will_die.html

Biosynthesis of Se nanoparticles is safe and inexpensive and employs eco-friendly nontoxic materials [189, 194, 196]. Furthermore, the biogenic Se nanoparticles can be found in a varying shape and size as well as stability due to natural coating of the organic molecules and do not aggregate with time, whereas external addition of stabilizing agents is required in chemical synthesis [197]. Se nanoparticles can be synthesized through physical methods, such as laser ablation, UV radiation, and hydrothermal techniques [198–200], and chemical synthesis is mediated by precipitation, acid decomposition, and catalytic reduction using ascorbic acid, glucose, sulfur dioxide, sodium dodecyl sulfate, etc. ([16, 152, 201]). However, these methods require high temperature, acidic pH, and harsh chemicals [198], which may render the nanoparticles unsafe for biomedical applications.

### *11.4.2 Biosynthesis of Selenium Nanoparticles by Bacteria*

Gerrard and his colleagues firstly observed Se deposits on the cell wall and cell membrane of *Escherichia coli* under electron microscopy. It has been suggested that the bacterial cells have the ability to reduce sodium selenite presented in the culture medium to elemental Se [202]. After that, many Gram-negative and Gram-positive bacteria have been reported to reduce selenate and selenite such as *Veillonella atypica* and *Pseudomonas* sp., and there are also two reports on Se nanoparticles synthesis by actinomycetes [203, 204]. In addition, several bacteria are known to exhibit Se resistance as shown in Table 11.1. Therefore, synthesis of Se nanoparticles is considered one of the Se detoxification mechanisms [213], as these nanoparticles can be extracellular, intracellular, or membrane bounded. Many bacteria (Table 11.1) such as *Bacillus cereus* [16], *Bacillus subtilis* [17], Se-respiring bacteria *Sulfurospirillum barnesii*, *Bacillus selenitireducens*, *Selenihalanaerobacter shriftii*, and *Shewanella sp*. HN-41 have been isolated, identified, and characterized for their ability to reduce selenate and selenite oxyanions to elemental nanoparticles [18].

### *11.4.3 Biosynthesis of Selenium Nanoparticles by Fungi*

Fungi are eukaryotic, spore-producing, achlorophyllous organisms that are usually filamentous, branched somatic structures with hyphae surrounded by cell walls. The myco-synthesis of nanomaterials, or myco-nanotechnology, is at the interface between mycology and nanotechnology and includes an exciting new applied interdisciplinary science with considerable potential due to the wide range and diversity of fungi [221]. As shown in Table 11.2, a few reports have described fungal mediated synthesis of Se-NPs [6, 20]. Gharieb et al. [222] showed that *Fusarium* sp. and *Trichoderma reesei* obtained red-colored colonies on Czapek-Dox agar containing sodium selenite. For extracellular production, monodispersed spherical Se nanoparticles have been reported in *Aspergillus terreus* which is isolated from soil and

**Table 11.1** Some bacteria are reported for synthesis of selenium nanoparticles

| Name of bacterium | Shape | Size (nm) | References |
|---|---|---|---|
| *Extracellular* | | | |
| *Pseudomonas aeruginosa* | Spherical | 140[a] | [16] |
| *Microbacterium* sp. | Spherical | 30–150[a] | [18] |
| *E. coli* K-12 DSMZ | Spherical | 24–122[a] | [205] |
| *Duganella* sp. | Spherical | 140–200[a] | [190] |
| *Agrobacterium* sp. | Spherical | 185–190[a] | [190] |
| *Zooglea ramigera* MTCC | Spherical | 30–150[a] | [138] |
| *Sulfurospirillum barnesii* | Spherical | 300[a] | [206] |
| *Selenihalanaerobacter shriftii* | Spherical | 300[a] | [206] |
| *Bacillus subtilis* | Spherical | 50–400[a] | [17] |
| *Bacillus mycoides* | Spherical | 50–400[a] | [207] |
| *Bacillus selenitireducens* | Spherical | 300[a] | [206] |
| *Intracellular* | | | |
| *Klebsiella pneumonia* | Spherical | 90–320[a] | [208] |
| *Pantoea agglomerans* | Spherical | 30–300[a] | [209] |
| *Pseudomonas alcaliphila* | Spherical | 50–500[a] | [210] |
| *Shewanella* sp. | Spherical | 11–20 | [211] |
| *Thauera selenatis* | Spherical | 150[a] | [212] |
| *Rhodospirillum rubrum* | Spherical | – | [213] |
| *Shewanella oneidensis* | Spherical | 1–100 | [214] |
| *Moraxella bovis* | – | – | [215] |
| *Pseudomonas fluorescens* | – | – | [216] |
| *Stenotrophomonas maltophilia* | – | – | [217] |
| *Bacillus* sp. MSh-1 | Spherical | 80–220[a] | [218] |
| *Bacillus cereus* | Spherical | 150–200[a] | [219] |
| *Lactobacillus acidophilus* | Spherical | – | [144] |
| *Lactobacillus plantarum* | – | >250[a] | [220] |

– not reported

*DSMZ* Deutsche Sammlung von Mikroorganismen und Zellkulturen culture collection, *MTCC* microbial type culture collection

[a] Size ≥ 100 nm but reported as nanoparticles

**Table 11.2** Some fungi are reported for synthesis of selenium nanoparticles

| Name of fungus | Source | Localization | Shape | Size (nm) | References |
|---|---|---|---|---|---|
| *Aspergillus terreus* | Soil | Extracellular | Spherical | 47 | [20] |
| *Alternaria alternate* | Leaf spot on *Stevia rebaudiana* | Extracellular | Spherical | 30–150[a] | [21] |
| *Fusarium* sp. | – | On the surfaces of hyphae and conidia | – | – | [222] |
| *Trichoderma reeii* | – | – | – | – | [222] |

– not reported

[a] Size ≥ 100 nm but reported as nanoparticles

*Alternaria alternate* which is isolated from leaf spot on *Stevia rebaudiana*, but edible *Lentinula edodes* synthesize Se nanoparticles intracellularly [6, 20].

### 11.4.4 Biosynthesis of Silicon Nanoparticles

Many organisms produce naturally optimized and hierarchical structures with higher efficiency and at lower cost than artificial methods. For this, they have great potential as environmentally friendly functional materials in bionanotechnology and micro-system fabrication [223]. For example, diatoms are unicellular and photosynthetic microalgae, which are widely distributed in seawater, freshwater, and even wet soils. They have almost 110,000 species ranging from 2 μm to 2 mm in size with thousands of different morphologies [224]. The rigid cell walls of diatoms [225, 226], called frustules, have nuanced structures made of amorphous silica [15]. They have well-arranged areolae on the outer or inner surfaces and a myriad of sieve pores measuring approximately 40 nm for exchanging nutrients and gases [227]. The porous structure of frustules also increases their surface area possibly reaching 200 $m^2/g$. Although biosilica frustules do not have excellent electrical conductivity and mechanical properties that carbon nanomaterials do, their multiple optical properties [228, 229] and good biocompatibility with unique porous architectures make them useful as core components in devices such as photoluminescence-based gas sensors [230], filters [231, 232], drug delivery systems [233–235], photovoltaic devices [236], and electrochemical/optical biomolecule diagnostic devices [237–239].

Although diatom biosilica has attracted wide interest, research, and applications in the past 10 years, biosilica are still in their infancy, considering the tremendous variety in their shapes and substructures. The silica structures formed, and their localization shows great variation between individual plant families. In the *Poaceae*, silica is deposited as a 2.5 mm layer immediately beneath the cuticle layer with a Si-cuticle double layer being found in the leaf blades of rice. The silicified structures in the *Poaceae* can be subdivided into silica cells, dumbbell-shaped cells located on vascular bundles, and silica bodies found on bulliform cells of rice leaves. However, the silicification of cells is not restricted to the leaf blades, and silicified cells are also found within the epidermis and vascular tissue of the stem, leaf sheath, and hull [240]. As already discussed, the amount of silica present in the different parts of the rice plant is very variable. The silicified structures of *Equisetum*, examined by a number of researchers [13, 241, 242], are found on the epidermal surface of the entire cell wall primarily as discrete knobs and rosettes, which are themselves covered in spicules. The thickness of this silica surface layer is dependent upon the location within the plant: thicknesses of 3–7 and 0.2–1.0 mm are observed in the stem and leaf, respectively. Characterization of the silica ultrastructures found in plant hairs from an example of the *Poaceae* [12], *Equisetaceae* [13], and nettle stinging hairs [14] revealed similar microstructural forms, including globular, fibrous, and sheet-like structures with the distribution of these ultrastructural motifs being dependent on the anatomical region studied. The silica formed in biological systems show a narrow particle size distribution for specific structural motifs.

#### 11.4.4.1 Biosynthesis of Silicon Nanoparticles by Bacteria

Microorganisms, especially bacteria, generate a large amount of inorganic nanoparticles in the environment, such as iron-, silicon-, and calcium-based minerals [70]. There are indeed several other such reports, and for brevity all have not been included here. In continuation of the synthesis of metal nanoparticles, there are reports regarding the synthesis of metal oxide nanoparticles using bacteria. Bharde et al. [243] have isolated, as a contaminant, a bacterium *Actinobacteria* sp. from a potassium ferrocyanide and potassium ferricyanide containing flask kept open for 1 week. Subsequently when a cultured part of this bacterium was challenged with a mixture of potassium ferrocyanide and potassium ferricyanide, the formation of crystalline iron oxide nanoparticles was observed. While it is clear that this bacterium must have developed resistance to the $Fe^{3+}/Fe^{2+}$ ion mixture in which it grew and hence the formation of $Fe_3O_4$ is expected to some extent, its response to other metal-/nonmetal containing precursors would be an interesting aspect to investigate. The impetus for such investigations comes from earlier reports where it is clearly shown that challenging the microorganisms by metal ion precursors that they have never encountered in their life cycle could lead to interesting products exemplifying the great potential of these methods [244]. Singh et al. [19] found that *Actinobacteria* sp. not only survived the exposure of $SiF_6^{2-}$ ions but led to the concurrent hydrolysis and reduction of this precursor finally forming silicon/silica ($Si/SiO_2$) nanocomposites. The synthesis is quite rapid (48 h) compared to other biosynthetic processes and is accomplished at room temperature, without use of any extreme situation and at relatively neutral pH. It is hypothesized that addition of the precursor ($K_2SiF_6$) to *Actinobacteria* sp. culture causes a stressful environment (ionic stress), and in order to nullify this stressed situation, the bacteria secrete some enzymes that hydrolyze the metal precursor to form $Si/SiO_2$ nanocomposites.

#### 11.4.4.2 Biosynthesis of Silicon Nanoparticles by Fungi

It is well known that fungi can be considered bio-manufacturing units in producing nanomaterials, where different species of fungi have the ability to synthesize the nanoparticles. This myco-synthesis has been used in production of nanoparticles for silver, gold, selenium, silica, and others [221, 245]. For the synthesis of metallic nanoparticles, living extracts have been utilized by researchers. They followed easy processes such as the procedures of reducing the metal ions. In doing so, they made use of biomass extracts as a basis of extracellular or intracellular reductants. Currently, microbial approaches in the production of nanomaterials of variable compounds are mostly restricted to metals, a few metal sulfide, and very little oxides. The exploration of the implication of fungi in nano-biotechnology is considered important. In this regard, fungi have attracted more attention regarding the research on biological production of metallic nanoparticles due to their toleration and metal bioaccumulation capability [246]. Moreover, a number of fungi species grow fast, and therefore culturing and keeping them in the laboratory are very simple [247]. High wall binding and intracellular metal uptake are the capacities of most fungi [248]. Fungi are able to produce metal nanoparticles/meso- and nanostructure via reducing enzyme intracellularly or extracellularly and the procedure of biomimetic mineralization [249, 250]. It is reported that hexafluorozirconate ions could be reacted with the fungus *Fusarium oxysporum* to yield zirconia nanoparticles of average size 7–8 nm [22]. For example, the reaction of the fungus *Fusarium oxysporum* with aqueous anionic complexes of $SiF_6^{2-}$ results in protein-mediated hydrolysis and room temperature formation of silica particles in which the fungus to the silicon complex induces the secretion of proteins of molecular weight 21 kDa. These proteins have been tested for hydrolytic activity and indeed transform the metal complex to silica [22].

## 11.5 Conclusion

The use of nanotechnology in agriculture has touched several fields including plant nano-nutrition, plant productivity, and plant protection. The need for a process to synthesis of nanoparticles in a reliable and green way is becoming more pressing. Current chemical and physical methods involve toxic chemicals and high temperatures and pressure that are not only dangerous to the environment but costly too. Biological systems have been investigated in an effort to provide a sustainable, resource-efficient, and cheap method of synthesis. Bacteria are relatively cheap to cultivate and have a high growth rate compared to other biological systems such as fungi or plants. Therefore, it gives them the advantage over plants and fungi as the chassis of choice for the near-term bio-production of nanoparticles. Many fungi have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate, which have mycelia that provide a much higher surface area than bacteria, and this area could be used to support the interaction of metal ions and fungal reducing agent. The plants have allowed for many different types of studies on them. Single components of plant extracts to whole plants have been utilized for the synthesis of nanoparticles. However before any industrial relevance can be attributed to the synthesis of nanoparticles by plants, many more examples must be identified, and, especially in the case of whole plant synthesis, the risks must be thoroughly assessed. Rapidly developing field of biosynthetic aims to create predictable, standardized systems, and with such new technologies directed toward the production of metallic nanoparticles, biogenic nanoparticle samples are likely to become more homogenous and more reproducible; therefore the environmental and health risks posed will be more easily and more reliably assessed.

There is a parallel in the recognition of the importance of selenium and silicon elements in agricultural sector. Similar biological roles of both selenium and silicon in agriculture could be noticed. These similar roles include the alleviated effects of abiotic stresses such as drought, salinity, metal toxicity, radiation damage, nutrient imbalance, high or low temperature, and plant senescence as well as biotic stress. Both selenium and silicon also have the same opportunity to be more than beneficial element for higher plants, and they may be essential nutrients. Day by day concerning the essentiality of both Se and Si, more and more evidences will be discovered. The combined effect of both Se and Si on higher plants may be needed for more investigations. Therefore, further studies regarding the biological role of Se and Si for higher plants alone or in combination are needed.

**Acknowledgments** Authors thank the outstanding contribution of STDF research teams (Science and Technology Development Fund, Egypt) and MBMF/DLR (the Federal Ministry of Education and Research of the Federal Republic of Germany) (Project ID 5310) for their help. Great support from this German-Egyptian Research Fund (GERF) is gratefully acknowledged. Also, authors would like to thank Tempus Public Foundation (TPF), Hungary, for co-financing this work and (Project ID: EFOP-3.6.2-16-2017-00001) research of complex rural economic and sustainable development, elaboration of its service networks in the Carpathian basin.

## References

1. Hussain I, Singh NB, Singh A, Singh H, Singh SC (2016) Green synthesis of nanoparticles and its potential application. Biotechnol Lett 38:545–560. https://doi.org/10.1007/s10529-015-2026-7
2. Mirzaei H, Darroudi M (2017) Zinc oxide nanoparticles: biological synthesis and biomedical applications. Ceram Int 43(Issue 1, Part B):907–914
3. Singh P, Kim Y-J, Zhang D, Yang D-C (2016) Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol 34(7):588–599
4. Hulkoti NI, Taranath T (2014) Biosynthesis of nanoparticles using microbes: a review. Colloids Surf B Biointerfaces 121:474–483
5. Zhou L, Wei XC, Ma ZJ, Mei B (2017) Anti-friction performance of FeS nanoparticle synthesized by biological method. Appl Surf Sci. https://doi.org/10.1016/j.apsusc.2017.02.182
6. Vetchinkina E, Loshchinina E, Kursky V, Nikitina V (2013) Reduction of organic and inorganic selenium compounds by the edible medicinal basidiomycete Lentinula edodes and the accumulation of elemental selenium nanoparticles in its mycelium. J Microbiol 51:829–835
7. Maruthupandy M, Zuo Y, Chen J-S, Song J-M, Shen Y-H (2017) Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) *via* biological template and their optical sensor applications. Appl Surf Sci 397:167–174
8. Salunke BK, Sawant SS, Lee S, Kim BS (2016) Microorganisms as efficient biosystem for the synthesis of metal nanoparticles: current scenario and future possibilities. World J Microbiol Biotechnol 32:88
9. Sharma S, Kaur N, Kaur S, Nayyar H (2014) Ascorbic acid reduces the phytotoxic effects of selenium on rice (*Oryza Sativa* L.) by up-regulation of antioxidative and metal-tolerance mechanisms. J Plant Physiol. https://doi.org/10.4172/2329-955X.1000128
10. Ramamurthy C, Sampath K, Arunkumar P, Suresh Kumar M, Sujatha V, Premkumar K, Thirunavukkarasu C (2013) Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells. Bioprocess Biosyst Eng. https://doi.org/10.1007/s00449-012-0867-1
11. Li S, Shen Y, Xie A, Yu X, Zhang X, Yang L, Li C (2007) Rapid, roomtemperature synthesis of amorphous selenium/protein composites using *Capsicum annuum* L extract. Nanotechnology 18:405101

12. Perry CC, Mann S, Williams RJP (1984) Structural and analytical studies of the silicified macrohairs from the lemma of the grass *Phalaris canariensis* L. Proc R Soc Lond B 222:427–438
13. Perry CC, Fraser MA (1991) Silica deposition and ultrastructure in the cell wall of *Equisetum arvense*: the importance of cell wall structures and flow control in biosilicification? Phil Trans R Soc Lond B 334:149–157
14. Hughes NP (1989) In vivo and in vitro studies of biomineralization processes. Ph.D. thesis, Oxford University
15. Sumper M, Brunner E (2006) Learning from diatoms: nature's tools for the production of nanostructured silica. Adv Funct Mater 16(1):17–26
16. Dwivedi C, Shah C, Singh K, Kumar M, Bajaj P (2011) An organic acidinduced synthesis and characterization of selenium. Nanopart J Nanotechnol. https://doi.org/10.1155/2011/651971
17. Wang T, Yang L, Zhang B, Liu J (2010) Extracellular biosynthesis and transformation of selenium nanoparticles and application in $H_2O_2$ biosensor. Colloids Surf B Biointerfaces 80:94–102
18. Prasad S, Vyas P, Prajapati V, Patel P, Selvaraj K (2012) Biomimetic synthesis of selenium nanoparticles using cell-free extract of *Microbacterium* sp. ARB05. Micro Nano Lett 8:11
19. Singh D, Kumar R, Kumar A, Rai KN (2008) Synthesis and characterization of rice husk silica, silica-carbon composite and $H_3PO_4$ activated silica. Ceramica 54:203–212
20. Zare B, Babaie S, Setayesh N, Shahverdi A (2012) Isolation and characterization of a fungus for extracellular synthesis of small selenium nanoparticles. Nanomed J 1:14–20
21. Sarkar J, Dey P, Saha S, Acharya K (2011) Mycosynthesis of selenium nanoparticles. Micro Nano Lett 6:599–602
22. Bansal V, Rautaray D, Ahmad A, Sastry M (2004) Biosynthesis of zirconia nanoparticles using the fungus *Fusarium oxysporum*. J Mater Chem 14:3303–3305
23. Khattab HI, Emam MA, Emam MM, Helal NM, Mohamed MR (2014) Effect of selenium and silicon on transcription factors *NAC5* and *DREB2A* involved in drought-responsive gene expression in rice. Biol Plant 58(2):265–273. https://doi.org/10.1007/s10535-014-0391-z
24. Tang H, Liu Y, Gong X, Zeng G, Zheng B, Wang D, Sun Z, Zhou L, Zeng X (2015) Effects of selenium and silicon on enhancing antioxidative capacity in ramie (*Boehmeria nivea* (L.) Gaud.) under cadmium stress. Environ Sci Pollut Res 22:9999–10008. https://doi.org/10.1007/s11356-015-4187-2
25. Shekari F, Abbasi A, Mustafavi SH (2015) Effect of silicon and selenium on enzymatic changes and productivity of dill in saline condition. J Saudi Soc Agric Sci. https://doi.org/10.1016/j.jssas.2015.11.006
26. Khan W-D, Aziz T, Maqsood MA, Sabir M, Ahmad HR, Ramzani PMA, Naseem M (2016) Silicon: a beneficial nutrient under salt stress, its uptake mechanism and mode of action. In: Hakeem KR et al (eds) Soil science: agricultural and environmental prospectives. Springer, Cham. https://doi.org/10.1007/978-3-319-34451-5_12
27. Imtiaz M, Rizwan MS, Mushtaq MA, Ashraf M et al (2016) Silicon occurrence, uptake, transport and mechanisms of heavy metals, minerals and salinity enhanced tolerance in plants with future prospects: a review. J Environ Manage 183(Part 3):521–529
28. Nawaz F, Naeem M, Ashraf MY, Tahir MN, Zulfiqar B, Salahuddin M, Shabbir RN, Aslam M (2016) Selenium supplementation affects physiological and biochemical processes to improve fodder yield and quality of maize (*Zea mays* L.) under water deficit conditions. Front Plant Sci 7:1438. https://doi.org/10.3389/fpls.2016.01438
29. Ma D, Sun D, Wang C, Qin H, Ding H, Li Y, Guo T (2016) Silicon application alleviates drought stress in wheat through transcriptional regulation of multiple antioxidant defense pathways. J Plant Growth Regul 35:1–10. https://doi.org/10.1007/s00344-015-9500-2
30. Handa N, Kohli SK, Thukral AK, Arora S, Bhardwaj R (2017) Role of Se(VI) in counteracting oxidative damage in Brassica juncea L. under Cr(VI) stress. Acta Physiol Plant 39(51). https://doi.org/10.1007/s11738-017-2352-6
31. Wu Z, Yin X, Bañuelos GS, Lin Z-Q, Liu Y, Li M, Yuan L (2016) Indications of selenium protection against cadmium and lead toxicity in oilseed rape (*Brassica napus* L.). Front Plant Sci 7:1875. https://doi.org/10.3389/fpls.2016.01875

32. Tripathi DK, Singh VP, Ahmad P, Chauhan DK, Prasad SM (2017) Silicon in plants: advances and future prospects. Taylor & Francis Group, LLC, Boca Raton, FL
33. Tripathi DK, Singh S, Singh VP, Prasad SM, Dubey NK, Chauhan DK (2017) Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. Plant Physiol Biochem 110(2017):70–81
34. Balal RM, Shahid MA, Javaid MM, Iqbal Z, Anjum MA, Garcia-Sanchez F, Mattson NS (2017) The role of selenium in amelioration of heat-induced oxidative damage in cucumber under high temperature stress. Acta Physiol Plant 38:158. https://doi.org/10.1007/s11738-016-2174-y
35. Soundararajan P, Sivanesan I, Jana S, Jeong BR (2014) Influence of silicon supplementation on the growth and tolerance to high temperature in Salvia splendens. Hort Environ Biotechnol 55(4):271–279. https://doi.org/10.1007/s13580-014-0023-8
36. Rahmat S, Hajiboland R, Sadeghzade N (2016) Selenium delays leaf senescence in oil seed rape plants. Photosynthetica 54:1–13. https://doi.org/10.1007/s11099-016-0643-6
37. Wu Z, Yin X, Bañuelos GS, Lin Z-Q, Zhu Z, Liu Y, Yuan L, Li M (2016) Effect of selenium on control of postharvest gray mold of tomato fruit and the possible mechanisms involved. Front Microbiol 6:1441. https://doi.org/10.3389/fmicb.2015.01441
38. Abdel-Haliem MEF, Hegazy HS, Hassan NS, Naguib DM (2017) Effect of silica ions and nano silica on rice plants under salinity stress. Ecol Eng 99:282–289
39. Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bharwana SA, Zia-ur-Rehman M, Qayyum MF, Abbas F (2015) Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. Environ Sci Pollut Res 22:15416–15431. https://doi.org/10.1007/s11356-015-5305-x
40. Liu J, Cai H, Mei C, Wang M (2015) Effects of nano-silicon and common silicon on lead uptake and translocation in two rice cultivars. Front Environ Sci Eng 9(5):905–911. https://doi.org/10.1007/s11783-015-0786-x
41. Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK (2016) Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultiver and hybrid differing in arsenate tolerance. Front Environ Sci 4:46. https://doi.org/10.3389/fenvs.2016.00046
42. Alsaeedi AH, El-Ramady H, Alshaal T, El-Garawani M, Elhawat N, Almohsen M (2017) Engineered silica nanoparticles alleviate the detrimental effects of Na+ stress on germination and growth of common bean (Phaseolus vulgaris). Environ Sci Pollut Res 24(27):21917–21928
43. Alsaeedi A, El-Ramady H, Alshaal T, El-Garawani M, Elhawat N, Al-Otaibi A (2018) Exogenous nanosilica improves germination and growth of cucumber by maintaining K+/Na+ ratio under elevated Na+ stress. Plant Physiol Biochem 125:164–171
44. Leopold FRA, Jan B-F, Regina MM, Fulvio U (2000) Selenium, the element of the Moon, in life on Earth. IUBMB Life 49:411–420
45. El-Ramady H, Abdalla N, Alshaal T, Domokos-Szabolcsy E, ElhawatN PJ, Sztrik A, Fari M, El-Marsafawy S, Shams MS (2015) Selenium in soils under climate change, implication for human health. Environ Chem Lett 13(1):1–19. https://doi.org/10.1007/s10311-014-0480-4
46. El-Ramady H, Abdalla N, Alshaal T, El-Henawy A, Faizy SE-DA, Shams MS, Shalaby T, Bayoumi Y, Elhawat N, Shehata S, Sztrik A, Prokisch J, Fari M, Pilon-Smits EA, Domokos-Szabolcsy E (2015) Selenium and its role in higher plants. In: Lichtfouse E et al (eds) Environmental chemistry for a sustainable world, vol 7. Springer, Dordrecht, pp 235–296. https://doi.org/10.1007/978-3-319-19276-5_6
47. El-Ramady H, Abdalla N, Fari M, Domokos-Szabolcsy E (2014) Selenium enriched vegetables as biofortification alternative for alleviating micronutrient malnutrition. Int J Hortic Sci 20(1–2):75–81. ISSN 1585-0404
48. El-Ramady H, Domokos-Szabolcsy E, Abdalla NA, Alshaal TA, Shalaby TA, Sztrik A, Prokisch J, Fari M (2014) Selenium and nano-selenium in agroecosystems. Environ Chem Lett 12(4):495–510. https://doi.org/10.1007/s10311-014-0476-0

49. Koller LD, Exon JH (1986) The two faces of selenium-deficiency and toxicity—are similar in animals and man. Can J Vet Res 50:297–306
50. Whanger P, Vendeland S, Park YC, Xia YM (1996) Metabolism of subtoxic levels of selenium in animals and humans. Ann Clin Lab Sci 26:99–113
51. Sakizadeh M, Sharafabadi FM, Shayegan E, Ghorbani H (2016) Concentrations and soil-to-plant transfer factor of selenium in soil and plant species from an arid area. IOP Conf Ser Earth Environ Sci 44:052027. https://doi.org/10.1088/1755-1315/44/5/052027
52. Andersson LO, Berg G (1969) Hydrolysis of disulfide bonds in weakly alkaline media I oxidized glutathione. Biochim Biophys Acta 192:534–536
53. Li LS, Kim H, Rhee H, Kim SH, Shin DH, Chung KY, Park KS, Paik YK, Chang J (2004) Proteomic analysis distinguishes basaloid carcinoma as a distinct subtype of nonsmall cell lung carcinoma. Proteomics 4:3394–3400
54. Nuttall KL, Allen FS (1984) Hydrogen selenide ion and colloidal selenium in the catalytic oxidation of thiols. Inorg Chim Acta 93:85
55. Fourniere d'Albe EE (1925) The Moon-element. T. Fisher Unwin Ltd., London
56. Winkel LHE, Vriens B, Jones GD, Schneider LS, Pilon-Smits E, Bañuelos GS (2015) Selenium cycling across soil-plantatmosphere interfaces: a critical review. Nutrients 7:4199–4239. https://doi.org/10.3390/nu7064199
57. El-Ramady H, Alshaal T, Abdalla N, Prokisch J, Sztrik A, Fári M, Domokos-Szabolcsy É (2016) Selenium and nano-selenium biofortified sprouts using micro-farm systems. In: Bañuelos GS, Lin Z-Q, Guilherme LRG, dos Reis AR (eds) Proceedings of the 4th international conference on selenium in the environment and human health, Sao Paulo, Brazil, 18–21 Oct 2015. CRC, Taylor & Francis Group, London, pp 189–190. https://doi.org/10.13140/RG.2.1.1065.9925
58. El-Ramady H, Abdalla N, Taha HS, Alshaal T, El-Henawy A, Faizy SE-DA, Shams MS, Youssef SM, Shalaby T, Bayoumi Y, Elhawat N, Shehata S, Sztrik A, Prokisch J, Fári M, Domokos-Szabolcsy É, Pilon-Smits EA, Selmar D, Haneklaus S, Schnug E (2016) Selenium and nano-selenium in plant nutrition. Environ Chem Lett 14(1):123–147. https://doi.org/10.1007/s10311-015-0535-1
59. Li Z, Liang D, Peng Q, Cui Z, Lin Z (2017) Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: a review. Geoderma 295:69–79
60. Tan LC, Nancharaiah YV, van Hullebusch ED, Lens PNL (2016) Selenium: environmental significance, pollution, and biological treatment technologies. Biotechnol Adv 34(5):886–907
61. Tan Y, Yao R, Wang R, Wang D, Wang G, Zheng S (2016) Reduction of selenite to Se(0) nanoparticles by filamentous bacterium Streptomyces sp. ES2-5 isolated from a selenium mining soil. Microb Cell Fact 15:157. https://doi.org/10.1186/s12934-016-0554-z
62. Reeder RJ, Schoonen MAA, Lanzirotti A (2006) Metal speciation and its role in bioaccessibility and bioavailability. In: Sahai N, Schoonen MAA, Skinner HCW (eds) The emergent field of medical mineralogy and geochemistry. Mineralogical Society of America and the Geochemical Society, Tucson, AZ, pp 59–113
63. Matos RP, Lima VMP, Windmöller CC, Nascentes CC (2017) Correlation between the natural levels of selenium and soil physicochemical characteristics from the Jequitinhonha Valley (MG), Brazil. J Geochem Explor 172:195–202
64. Wang J, Li H, Yang L, Li Y, B Wei JY, Feng F (2017) Distribution and translocation of selenium from soil to highland barley in the Tibetan Plateau Kashin–Beck disease area. Environ Geochem Health 39:221–229. https://doi.org/10.1007/s10653-016-9823-3
65. Wang X, Zhang D, Pan X, Lee D-J, Gadd GM (2017) Aerobic and anaerobic biosynthesis of nano-selenium for remediation of mercury contaminated soil. Chemosphere 170:266–273
66. Cabanero AI, Madrid Y, Camara C (2007) Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. Biol Trace Elem Res 119:195–211
67. Yadav SK, Singh I, Sharma A, Singh D (2008) Selenium status in food grains of northern districts of India. J Environ Manage 88:770–774. https://doi.org/10.1016/j.jenvman.2007.04.012

68. Charya LS (2017) Selenium pollution in the marine environment and marine bacteria in selenium bioremediation. In: Naik MM, Dubey SK (eds) Marine pollution and microbial remediation. Springer, Singapore. https://doi.org/10.1007/978-981-10-1044-6_14
69. El-Ramady H, Domokos-Szabolcsy E, Shalaby TA, Prokisch J, Fari M (2015) Selenium in agriculture: water, air, soil, plants, food, animals and nanoselenium. In: Lichtfouse E (ed) Environmental chemistry for a sustainable world, Vol. 5., $CO_2$ sequestration, biofuels and depollution. Springer, Berlin, pp 153–232. https://doi.org/10.1007/978-3-319-11906-9_5
70. Sharma G, Jasuja ND, Kumar M, Ali MI (2015) Biological synthesis of silver nanoparticles by cell-free extract of *Spirulina platensis*. J Nanotechnol 2015:1–6
71. Schiavon M, Pilon-Smits EAH (2017) The fascinating facets of plant selenium accumulation – biochemistry, physiology, evolution and ecology. New Phytol 213:1582–1596. https://doi.org/10.1111/nph.14378
72. Yasin M, El Mehdawi AF, Jahn CE, Anwar A, Turner MFS, Faisal M, Pilon-Smits EAH (2015) Seleniferous soils as a source for production of selenium-enriched foods and potential of bacteria to enhance plant selenium uptake. Plant and Soil 386:385–394. https://doi.org/10.1007/s11104-014-2270-y
73. Kabata-Pendias E (2011) Trace elements in soils and plants, 4th edn. CRC Press Taylor & Francis Group, Boca Raton, FL
74. Di Gregorio S (2008) Selenium: a versatile trace element in life and environment. In: Prasad AS (ed) Trace elements in human health and disease, vol 2. Academic, New York, pp 593–622
75. Kroflič A, Germ M, Mechora Š, Stibilj V (2016) Selenium and its compounds in aquatic plant Veronica anagallis-aquatica. Chemosphere 151:296–302
76. Pilon-Smits EAH (2015) Selenium in plants. In: Luettge U, Beyschlag W (eds) Progress in botany, vol 76. Springer International Publishing, Cham, pp 93–107. https://doi.org/10.1007/978-3-319-08807-5_4
77. Reilly C (2006) Selenium in food and health, 2nd edn. Springer, Dodretch
78. Kapolna E, Fodor P (2006) Speciation analysis of selenium enriched green onions (Allium fistulosum) by HPLC–ICP-MS. Microchem J 84:56–62
79. Bierla K, Szpunar J, Lobinski R (2016) Biological Selenium species and Selenium speciation in biological samples. In: Hatfield DL et al (eds) Selenium. Springer, New York, NY. https://doi.org/10.1007/978-3-319-41283-2_35
80. Liu X, Yang Y, Deng X, Li M, Zhao Z (2017) Effects of sulfur and sulfate on selenium uptake and quality of seeds in rapeseed (Brassica napus L.) treated with selenite and selenate. Environ Exp Bot 135:13–20
81. Graham RR, Cragg LH (1960) The carbon family. The essentials of chemistry. Holt, Rinehart, and Winston, Inc., New York, NY, pp 408–423
82. Bowen HJ (1966) Trace elements in biochemistry. New York, NY, Academic
83. Condie KC (1974). Abundance in igneous rocks and in the crust. In: Wedepohl KH (ed) Handbook geochemistry. 2. Springer, Berlin, pp. 14-E-l to 14-E-4.
84. Arnon DI, Stout PR (1939) The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol 14:371–375
85. Epstein E (1999) Silicon. Annu Rev Plant Physiol Plant Mol Biol 50:641–664
86. Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci U S A 91:11–17
87. Liang Y, Nikolic M, Bélanger R, Gong H, Song A (2015) Silicon in agriculture: from theory to practice. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-9978-2
88. Liang YC, Chen Q, Liu Q, Zhang WH, Ding RX (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J Plant Physiol 160:1157–1164
89. Ma JF, Goto S, Tamai K, Ichii M (2001) Role of root hairs and lateral roots in silicon uptake by rice. Plant Physiol 127:1773–1780
90. Savant NK, Snyder GH, Datnoff LE (1997) Silicon management and sustainable rice production. Adv Agron 58:151–199
91. Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London, pp 7–78
92. Lanning FC, Eleuterius LN (1989) Silica deposition in some C3 and C4 species of grasses, sedges and composites in the USA. Ann Bot 63:395–410

93. Klotzbücher T, Marxen A, Jahn R, Vetterlein D (2016) Silicon cycle in rice paddy fields: insights provided by relations between silicon forms in topsoils and plant silicon uptake. Nutr Cycl Agroecosyst 105:157–168. https://doi.org/10.1007/s10705-016-9782-1
94. Alexandre A, Meunier J-D, Colin F, Koud J-M (1997) Plant impact on the biogeochemical cycle of silicon and related weathering processes. Geochim Cosmochim Acta 61:677–682
95. Runge F (1999) The opal phytolith inventory of soils in central Africa — quantities, shapes, classification, and spectra. Rev Palaeobot Palynol 107:23–53
96. Zhang X, Song Z, McGrouther K, Li J, Li Z, Ru N, Wang H (2016) The impact of different forest types on phytolith-occluded carbon accumulation in subtropical forest soils. J Soil Sediment 16:461–466. https://doi.org/10.1007/s11368-015-1259-3
97. Dal Corso M, Nicosia C, Balista C, Cupito M, Longa ED, Leonardi G, Kirleis W (2017) Bronze age crop processing evidence in the phytolith assemblages from the ditch and fen around Fondo Paviani, northern Italy. Veg Hist Archaeobotany 26:5–24. https://doi.org/10.1007/s00334-016-0573-z
98. Bartoli F (1983) The biogeochemical cycle of silicon in two temperate forest ecosystems. Ecol Bull 35:469–476
99. Li Z, Song Z, Li B (2013) The production and accumulation of phytolith-occluded carbon in Baiyangdian reed wetland of China. Appl Geochem 37:117–124
100. Norris AR, Hackney CT (1999) Silica content of a mesohaline tidal marsh in North Carolina. Estuar Coast Shelf Sci 49:597–605
101. Song Z, McGrouther K, Wang H (2016) Occurrence, turnover and carbon sequestration potential of phytoliths in terrestrial ecosystems. Earth Sci Rev 158:19–30
102. Keller C, Guntzer F, Barboni D, Labreuche J, Meunier J-D (2012) Impact of agriculture on the Si biogeochemical cycle: input from phytoliths studies. C R Geosci 344(11–12):739–746
103. Song Z, Wang H, Strong PJ, Li Z, Jiang P (2012) Plant impact on the coupled terrestrial biogeochemical cycles of silicon and carbon: implications for biogeochemical carbon sequestration. Earth Sci Rev 115(4):319–331
104. Struyf E, Smis A, Van Damme S, Meire P, Conley DJ (2009) The global biogeochemical silicon cycle. Silicon 1:207–213. https://doi.org/10.1007/s12633-010-9035-x
105. Hartmann J, Jansen N, Dürr HH, Harashima A, Okuba K, Kempe S (2009) Predicting riverine dissolved silica fluxes to coastal zones from a hyperactive region and analysis of first-order controls. Int J Earth Sci. https://doi.org/10.1007/s00531-008-0381-5
106. Knoll MA, James WC (1987) Effect of the advent and diversification of vascular land plants on mineral weathering through geologic time. Geology 15:1099–1102
107. Penido ES, Bennett AJ, Hanson TE, Seyfferth AL (2016) Biogeochemical impacts of silicon-rich rice residue incorporation into flooded soils: implications for rice nutrition and cycling of arsenic. Plant and Soil 399:75–87. https://doi.org/10.1007/s11104-015-2682-3
108. Epstein E, Bloom AJ (2003) Mineral nutrition of plants: principles and perspectives, 2nd edn. John Wiley & Sons, New York
109. Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci Plant Nutr 50:11–18
110. Zhu Y, Gong H (2014) Beneficial effects of silicon on salt and drought tolerance in plants. Agron Sustain Dev 34:455–472. https://doi.org/10.1007/s13593-013-0194-1
111. Jarvis SC (1987) The uptake and transport of silicon by perennial ryegrass and wheat. Plant and Soil 97:429–438
112. Jones LHP, Handreck KA (1967) Silica in soils plants and animals. Adv Agron 19:107–149
113. Yamaji N, Ma JF (2007) Spatial distribution and temporal variation of the rice silicon transporter Lsi1. Plant Physiol 143:1306–1313
114. Takahashi E, Hino K (1978) Silica uptake by plant with special reference to the forms of dissolved silica. Jpn J Soil Sci Manure 49:357–360
115. Takahashi E, Ma JF, Miyake Y (1990) The possibility of silicon as an essential element for higher plants. Comments Agric Food Chem 2:99–122
116. Liang YC, Si J, Römheld V (2005) Silicon uptake and transport is an active process in *Cucumis sativus* L. New Phytol 167:797–804. https://doi.org/10.1111/j.1469-8137.2005.01463.x

117. Datnoff LE, Deren CW, Snyder GH (1997) Silicon fertilization for disease management of rice in Florida. Crop Prot 16:525–531
118. Miyake Y, Takahashi E (1982) Effect of silicon on the growth of solution-cultured cucumber plants, Part 16. Comparative studies on silica nutrition in plants. Jpn J Soil Sci Plant Nutr 53:15–22
119. Miyake Y, Takahashi E (1982) Effect of silicon on the growth of solution-culutured cucumber plants, Part 17. Comparative studies on silica nutrition in plants. Jpn J Soil Sci Plant Nutr 53:23–29
120. Marxen A, Klotzbücher T, Jahn R, Kaiser K, Nguyen VS, Schmidt A, Schädler M, Vetterlein D (2016) Interaction between silicon cycling and straw decomposition in a silicon deficient rice production system. Plant and Soil 398:153–163. https://doi.org/10.1007/s11104-015-2645-8
121. Ma JF, Takahashi E (2002) Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam
122. Shimoyama S (1958) Effect of calcium silicate application to rice plants on the alleviation of lodging and damage from strong gales. Studies in the improvement of ultimate yields of crops by the application of silicate materials. Jpn Assoc Adv Sci 56:57–99
123. Liang Y, Nikolic M, Bélanger R, Gong H, Song A (2015) Silicon uptake and transport in plants: physiological and molecular aspects. In: Liang Y et al (eds) Silicon agriculture. Springer, Dordrecht, pp 69–82. https://doi.org/10.1007/978-94-017-9978-2_4
124. Gao XY, Zhang JS, Zhang LD (2002) Hollow sphere selenium nanoparticles: their in vitro anti hydroxyl radical effect. Adv Mater 14:290–293
125. White PJ, Bowen HC, Parmaguru P, Fritz M, Spracklen WP, Spiby RE, Meacham MC, Mead A, Harriman M, Trueman LJ, Smith BM, Thomas B, Broadley MR (2004) Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. J Exp Bot 55:1927–1937. https://doi.org/10.1093/jxb/erh192
126. Huang B, Zhang J, Hou J, Chen C (2003) Free radical scavenging efficiency of nano-Se *in vitro*. Free Radic Biol Med 35(7):805–813. https://doi.org/10.1016/S0891-5849(03)00428-3
127. Pelyhe C, Mézes M (2013) Myths and facts about the effects of nanoselenium in farm animals-mini review. Eur Chem Bull 2:1049–1052
128. Wang Q, Webster TJ (2013) Short communication: inhibiting biofilm formation on paper towels through the use of selenium nanoparticles coatings. Int J Nanomedicine 8:407–411
129. Amin KA, Hashem KS, Alshehri FS, Awad ST, Hassan MS (2017) Antioxidant and hepatoprotective efficiency of selenium nanoparticles against acetaminophen-induced hepatic damage. Biol Trace Elem Res 175:136–145. https://doi.org/10.1007/s12011-016-0748-6
130. Wadhwani SA, Shedbalkar UU, Singh R, Chopade BA (2016) Biogenic selenium nanoparticles: current status and future prospects. Appl Microbiol Biotechnol 100:2555–2566. https://doi.org/10.1007/s00253-016-7300-7
131. Nuttall KL (1985) Elemental selenium and glutathione reductase. Med Hypotheses 16:155–158
132. Zhang J, Wang X, Xu T (2008) Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. Toxicol Sci 101(1):22–31. https://doi.org/10.1093/toxsci/kfm221
133. Zhang L, Feng C, Chen Z et al (2008) Superaligned carbon nanotube grid for high resolution transmission electron microscopy of nanomaterials. Nano Lett 8(8):2564–2569
134. Peng D, Zhang J, Liu Q, Taylor EW (2007) Size effect of elemental selenium nanoparticles (Nano-Se) at supranutritional levels on selenium accumulation and glutathione S-transferase activity. J Inorg Biochem 101(10):1475–1563
135. Prasad KS, Patel H, Patel T, Patel K, Selvaraj K (2013) Biosynthesis of Se nanoparticles and its effect on UV-induced DNA damage. Colloids Surf B Biointerfaces 103:261–266. doi:10.1016/j. colsurfb.2012.10.029
136. Zhang J-S, Gao X-Y, Zhang L-D, Bao Y-P (2001) Biological effects of a nano red elemental selenium. Biofactors 15:27–38

137. Chaudhary S, Umar A, Umar A, Mehta SK (2016) Selenium nanomaterials: an overview of recent developments in synthesis, properties and potential applications. Progr Mater Sci 83:270–329. https://doi.org/10.1016/j.pmatsci.2016.07.001
138. Srivastava N, Mukhopadhyay M (2013) Biosynthesis and structural characterization of selenium nanoparticles mediated by Zooglea ramigera. Powder Technol. https://doi.org/10.1016/j.powtec.2013.03.050
139. Bhattacharjee A, Basu A, Biswas J, Sen T, Bhattacharya S (2017) Chemoprotective and chemosensitizing properties of selenium nanoparticle (Nano-Se) during adjuvant therapy with cyclophosphamide in tumor-bearing mice. Mol Cell Biochem 424:13–33. https://doi.org/10.1007/s11010-016-2839-2
140. Bhattacharjee A, Basu A, Sen T, Biswas J, Bhattacharya S (2016) Nano-Se as a novel candidate in the management of oxidative stress related disorders and cancer. Nucleus. https://doi.org/10.1007/s13237-016-0183-2
141. Fu L, Yan X, Ruan X, Lin J, Wang Y (2014) Differential protein expression of Caco-2 cells treated with selenium nanoparticles compared with sodium selenite and selenomethionine. Nanoscale Res Lett 9:589
142. Benko I, Nagy G, Tanczos B, Ungvari E, Sztrik A, Eszenyi P, Prokisch J, Banfalvi G (2012) Subacute toxicity of nano-selenium compared to other selenium species in mice. Environ Toxicol Chem 31:2812–2820
143. Hu CH, Li YL, Xiong L, Zhang HM, Song J, Xia MS (2012) Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. Anim Feed Sci Technol 177:204–210
144. Domokos-Szabolcsy E, Marton L, Sztrik A, Babka B, Prokisch J, Fari M (2012) Accumulation of red elemental selenium nanoparticles and their biological effects in *Nicotinia tabacum*. Plant Growth Regul 68:525–531
145. Srivastava N, Mukhopadhyay M (2015) Biosynthesis and structural characterization of selenium nanoparticles using *Gliocladium roseum*. J Clust Sci. https://doi.org/10.1007/s10876-014-0833-y
146. Prasad KS, Vaghasiya JV, Soni SS, Patel J, Patel R, Kumari M, Jasmani F, Selvaraj K (2015) Microbial selenium nanoparticles (SeNPs) and their application as a sensitive hydrogen peroxide biosensor. Appl Biochem Biotechnol 177:1386–1393. https://doi.org/10.1007/s12010-015-1814-9
147. Wang Q, Webster TJ (2012) Nanostructured selenium for preventing biofilm formation on polycarbonate medical devices. J Biomed Mater Res A 100:3205–3210
148. Kong L, Yuan Q, Zhu H, Li Y, Guo Q, Wang Q, Bi X, Gao X (2011) The suppression of prostate LNCaP cancer cells growth by selenium nanoparticles through Akt/Mdm2/AR controlled apoptosis. Biomaterials 32:6515–6522
149. Gu Y, Cui R, Zhang Z, Xie Z, Pang D (2012) Ultra-small nearinfrared Ag2Se quantum dots with tunable fluorescence for in vivo imaging. J Am Chem Soc 134:79–82
150. Robel I, Subramanian V, Kuno M, Kamat PV (2006) Quantum dot solar cells. Harvesting light energy with CdSe nanocrystals molecularly linked to mesoscopic TiO2 films. J Am Chem Soc 128:2385–2393
151. Niu Y, Qin A, Song W, Wang M, Gu X, Zhang Y, Yu M, Zhao X, Dai M, Yan L, Li Z, Fan Y (2012) Biocompatible single-crystal selenium nanobelt based nanodevice as a temperature-tunable photosensor. J Nanomater 2012:1–6
152. Zhang Y, Wang J, Zhang L (2010) Creation of highly stable selenium nanoparticles capped with hyperbranched polysaccharide in water. Langmuir 26(22):17617–17623. https://doi.org/10.1021/la1033959
153. Mastronardi E, Tsae P, Zhang X, Monreal C, De Rosa MC (2015) Strategic role of nanotechnology in fertilizers: potential and limitations. In: Rai M et al (eds) Nanotechnologies in food and agriculture. Springer, Cham, p 25. https://doi.org/10.1007/978-3-319-14024-7_2
154. Bindraban PS, Dimkpa C, Nagarajan L, Roy A, Rabbinge R (2015) Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. Biol Fertil Soils. https://doi.org/10.1007/s00374-015-1039-7

155. Servin A, Elmer W, Mukherjee A, De La Torre-Roche R, Hamdi H, White JC, Bindraban PS, Dimkpa CO (2015) A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J Nanopart Res 17:92
156. Domokos-Szabolcsy E (2011) Biological effect and fortification possibilities of inorganic selenium forms in higher plants. Ph.D. dissertation, Debrecen University
157. Premarathna HL, McLaughlin MJ, Kirby Jason K, Hettiarachchi GM, Beak D, Stacey S, Chittleborough DJ (2010) Potential availability of fertilizer selenium in field capacity and submerged soils. Soil Sci Soc Am J 74:1589–1596. https://doi.org/10.2136/sssaj2009.0416
158. Haghighi M, Abolghasemi R, Teixeira da Silva JA (2014) Low and high temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment. Sci Hortic 178:231–240. https://doi.org/10.1016/j.scienta.2014.09.006
159. Domokos-Szabolcsy E, Abdalla N, Alshaal T, Sztrik A, Márton L, El-Ramady H (2014) In vitro comparative study of two Arundo donax L. ecotypes' selenium tolerance. Int J Horti Sci 20(3–4):119–122
160. Pavlidou S, Papaspyrides CD (2008) A review on polymer–layered silicate anocomposites. Prog Polym Sci 33:1119–1198
161. Choi Y, Lee S, Ryu S (2009) Effect of silane functionalization of montmorillonite on epoxy/montmorillonitenano composite. Polym Bull 63:47–55
162. Xanthos M (2005) Functional fillers for plastics. Wiley, Weinheim
163. Aso O, Eguiazabal JI, Nazabal J (2007) The influence of surface modification on the structure and properties of a nanosilica filled thermoplastic elastomer. Compos Sci Technol 67:2854–2863
164. Abdelghany AM, Morsi MA, Abdelrazek A, Ahmed MT (2016) Role of silica nanoparticles on structural, optical and morphological properties of poly(vinyl chloride-co-vinyl acetate-co-2-hydroxypropyl acrylate) copolymer. Silicon. https://doi.org/10.1007/s12633-016-9483-z
165. Gharehbash N, Shakeri A (2015) Preparation and thermal and physical properties of nano-silica modified and unmodified. Orient J Chem 31:207–212. https://doi.org/10.13005/ojc/31.Special-Issue1.25
166. Parvinzadeh M, Moradian S, Rashidi A, Yazdanshenas ME (2010) Surface characterization of polyethylene terephthalate/silica nanocomposites. Appl Surf Sci 256:2792–2802
167. Singh A, Singh S, Prasad SM (2017) Silicon and nanotechnology: role in agriculture and future perspectives. In: Tripathi DK, Singh VP, Ahmad P, Chauhan DK, Prasad SM (eds) Silicon in plants: advances and future prospects. Taylor & Francis Group, LLC, Boca Raton, FL, pp 101–116
168. Datnoff LE Snyder GH, Korndorfer GH (2001) Silicon in agriculture, studies in plant science, 8, Elsevier, New York, NY.
169. Tubaña BS, Heckman JR (2015) Silicon in soils and plants. In: Rodrigues FA, Datnoff LE (eds) Silicon and plant diseases. Springer, Cham. https://doi.org/10.1007/978-3-319-22930-0_2
170. Wang S, Wang F, Gao S (2015) Foliar application with nano-silicon alleviates Cd toxicity in rice seedlings. Environ Sci Pollut Res 22:2837–2845. https://doi.org/10.1007/s11356-014-3525-0
171. Wang S, Wang F, Gao S, Wang X (2016) Heavy metal accumulation in different rice cultivars as influenced by foliar application of nano-silicon. Water Air Soil Pollut 227:228. https://doi.org/10.1007/s11270-016-2928-6
172. Tossi V, Lombardo C, Cassia R, Lamattina L (2012) Nitric oxide and flavonoids are systemically induced by UV-B in maize leaves. Plant Sci 193-194:103–109
173. Singh VP, Kumar J, Singh S, Prasad SM (2014) Dimethoate modifies enhanced UV-B effects on growth, photosynthesis and oxidative stress in mung bean (*Vigna radiata* L.) seedlings: implication of salicylic acid. Pest Biochem Physiol 116:13–23
174. Singh VP, Singh S, Kumar J, Prasad SM (2015) Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbateeglutathione cycle: possible involvement of nitric oxide. J Plant Physiol 181:20–29
175. Singh VP, Srivastava PK, Prasad SM (2013) Nitric oxide alleviates arsenic induced toxic effects in ridged Luffa seedlings. Plant Physiol Biochem 71:155–163

176. Jansen MAK, Gaba V, Greenberg BM (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135
177. Kakani VG, Reddy KR, Zhao D, Mohammed AR (2003) Effects of ultraviolet-B radiation on cotton (Gossypium hirsutum L.) morphology and anatomy. Ann Bot 91:817–826
178. Liu C, Li F, Luo C, Liu X, Wang S, Liu T, Li X (2009) Foliar application of two silica sols reduced cadmium accumulation in rice grains. J Hazard Mater 161:1466–1472
179. Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol 167:1248–1252
180. Yao X, Chu J, Cai K, Liu L, Shi J, Geng W (2011) Silicon improves the tolerance of wheat seedlings to ultraviolet-B stress. Biol Trace Elem Res 143:507–517
181. Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK, Rai AK (2015) Silicon-mediated alleviation of Cr (VI) toxicity in wheat seedlings as evidenced by chlorophyll florescence, laser induced breakdown spectroscopy and anatomical changes. Ecotoxicol Environ Saf 113:133–144
182. Xing W, Li D, Liu G (2010) Antioxidative responses of Elodea nuttallii (Planch.) H. St. John to short-term iron exposure. Plant Physiol Biochem 48:873–878
183. Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK (2015) Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in *Pisum sativum* (L.) seedlings. Plant Physiol Biochem 96:189–198
184. Genty B, Harbinson J, Briantais JM, Baker NR (1990) The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves. Photosynth Res 25:249–257
185. Higashisaka K, Nagano K, Yoshioka Y, Tsutsumi Y (2017) Nano-safety research: examining the associations among the biological effects of nanoparticles and their physicochemical properties and kinetics. Biol Pharm Bull 40(3):243–248. https://doi.org/10.1248/bpb.b16-00854
186. Ingale AG, Chaudhari AN (2013) Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach. J Nanomed Nanotechol 4(165):1–7. https://doi.org/10.4172/2157-7439.1000165
187. Iravani S (2011) Green synthesis of metal nanoparticles using plants. Green Chem 13(10):2638–2650
188. Husen A, Siddiqi KS (2014) Plants and microbes assisted selenium nanoparticles: characterization and application. J Nanobiotechnol 12:28
189. Shedbalkar U, Singh R, Wadhwani S, Gaidhani S, Chopade BA (2014) Microbial synthesis of gold nanoparticles: current status and future prospects. Adv Colloid Interface Sci 209:40–48
190. Bajaj M, Schmidt S, Winter J (2012) Formation of Se (0) nanoparticles by Duganella sp. and Agrobacterium sp. isolated from Se-laden soil of north-east Punjab, India. Microb Cell Fact 11:64
191. Prasad S, Selvaraj K (2014) Biogenic synthesis of selenium nanoparticles and their effect on as(III)-induced toxicity on human lymphocytes. Biol Trace Elem Res 157:275–283
192. Gaidhani S, Singh R, Singh D, Patel U, Shevade K, Yeshvekar R, Chopade BA (2013) Biofilm disruption activity of silver nanoparticles synthesized by *Acinetobacter calcoaceticus* PUCM 1005. Mater Lett 108:324–327
193. Gaidhani S, Yeshvekar RV, Shedbalkar US, Bellare JH, Chopade BA (2014) Bio-reduction of hexachloroplatinic acid to platinum nanoparticles employing *Acinetobacter calcoaceticus*. Process Biochem 49:2313–2319
194. Wadhwani S, Shedbalkar U, Singh R, Karve M, Chopade B (2014) Novel polyhedral gold nanoparticles: green synthesis, optimization and characterization by environmental isolate of Acinetobacter spSW30. World J Microbiol Biotechnol 30:2723–2731
195. Chen T, Wong Y, ZhengW BY, Huang L (2008) Selenium nanoparticles fabricated in Undaria pinnatifida polysaccharide solutions induce mitochondria-mediated apoptosis in A375 human melanoma cells. Colloids Surf B Biointerfaces 67:26–31

196. Salunke GR, Ghosh S, Santosh Kumar RJ, Khade S, Vashisth P, Kale T, Chopade S, Pruthi V, Kundu G, Bellare JR, Chopade BA (2014) Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant *Plumbago zeylanica* and their application in biofilm control. Int J Nanomedicine 9:2635–2653
197. Nancharaiah YV, Lens PNL (2015) Ecology and biotechnology of selenium-respiring bacteria. Microbiol Mol Biol Rev 79:61–80
198. Iranifam M, Fathinia M, Sadeghi T, Hanifehpour Y, Khataee A, Joo S (2013) A novel selenium nanoparticles-enhanced chemiluminescence system for determination of dinitrobutylphenol. Talanta 107:263–269
199. Overschelde O, Guisbiers G, Snyders R (2013) Green synthesis of selenium nanoparticles by excimer pulsed laser ablation in water. Appl Mater 1:042114
200. Quintana M, Haro-Poniatowski E, Morales J, Batina N (2002) Synthesis of selenium nanoparticles by pulsed laser ablation. Appl Surf Sci 195:175–186
201. Hong Lin Z, Chu Lin F, Wang C (2004) Observation in the growth of selenium nanoparticles. J Chin Chem Soc 51:239–242
202. Gerrard T, Telford J, Williams H (1974) Detection of selenium deposits in *Escherichia coli* by electron microscopy. J Bacreriol 119:1057–1060
203. Ahmad MS, Yasser MM, Sholkamy EN, Ali AM, Mehanni MM (2015) Anticancer activity of biostabilized selenium nanorods synthesized by Streptomyces bikiniensis strain Ess_amA-1. Int J Nanomedicine 10:3389–3401
204. Forootanfara H, Zare B, Fasihi-Bam H, Amirpour-Rostami S, Ameri A, Shakibaie M, Torabi Nami M (2014) Biosynthesis and characterization of selenium nanoparticles produced by terrestrial actinomycete *Streptomyces microflavus* strain FSHJ31. Res Rev J Microbiol Biotechnol 3(1):47–53
205. Dobias J, Suvorova EI (2011) Role of proteins in controlling selenium nanoparticle size. Nanotechnology 22:195605
206. Oremland R, Herbe M, Blum J, Langley S, Beveridge T, Ajayan P, Sutto PT, Ellis AV, Curran S (2004) Structural and spectral features of selenium nanospheres produced by se-respiring bacteria. Appl Environ Microbiol 70:52–60
207. Lampis S, Zonaro E, Bertolini C, Bernardi P, Butler C, Vallini G (2014) Delayed formation of zero-valent selenium nanoparticles by *Bacillus mycoides* SeITE01 as a consequence of selenite reduction under aerobic conditions. Microb Cell Fact 13:35
208. Kazempour Z, Hossein M, Yazdi F, Shahverdi A (2013) Sub-inhibitory concentration of biogenic selenium nanoparticles lacks post antifungal effect for *Aspergillus niger* and *Candida albicans* and stimulates the growth of *Aspergillus niger*. Iran J Microbiol 5:81–85
209. Torres K, Campos V, Leon C, Rojas S, Guez-Llamazares S, Gonza'lez M, Smith C, MA M (2012) Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity. J Nanopart Res:14–1236
210. Zhang J, Spallholz J (2011) Toxicity of selenium compounds and nanoselenium particles. Gen Appl Syst Toxicol. https://doi.org/10.1002/9780470744307
211. Tam K, Ho C, Lee J, Lai M, Chang C, Rheem Y, Chen H, Hur N, Myung V (2010) Critical evaluation of nanoparticle tracking analysis (NTA) by nanosight for the measurement of nanoparticles and protein aggregates. Biosci Biotechnol Biochem 74:696–700
212. Debieux C, Dridge E, Mueller C, Splatt P, Paszkiewicz K, Knight I, Florance R, Love J, Titball RW, Lewis RJ, Richardson DJ, Butler CS (2011) A bacterial process for selenium nanosphere assembly. Proc Natl Acad Sci 108:–13481
213. Kessi J, Ramuz M, Wehrli E, Spycher M, Bachofen R (1999) Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium *Rhodospirillum rubrum*. Appl Environ Microbiol 65:4734–4740
214. Bo Li D, Cheng Y, Wu C, Li W, Li N, Yang Z, Tong Z, Yu H (2014) Selenite reduction by *Shewanella oneidensis* MR-1 is mediated by fumarate reductase in periplasm. Sci Rep 4:3735

215. Biswas K, Barton L, Tsui W, Shuman K, Gillespie J, Eze C (2011) A novel method for the measurement of elemental selenium produced by bacterial reduction of selenite. J Microbiol Methods 86:140–144
216. Garbisu C, Ishii T, Leighton T, Buchanan BB (1996) Bacterial reduction of selenite to elemental selenium. Chem Geol 132:199–204
217. Gregorio S, Lampis S, Vallini G (2005) Selenite precipitation by a rhizospheric strain of *Stenotrophomonas* sp. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective. Environ Int 31:233–241
218. Shakibaie M, Khorramizadeh M, Faramarzi M, Sabzevari O, Shahverdi A (2010) Biosynthesis and recovery of selenium nanoparticles and the effects on matrix metalloproteinase-2 expression. Biotechnol Appl Biochem 15:7–15
219. Dhanjal S, Cameotra S (2010) Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil. Microb Cell Fact 9:52
220. Yazdi M, Mahdavi M, Setayesh N, Esfandyar M, Shahverdi A (2013) Selenium nanoparticle-enriched *Lactobacillus brevis* causes more efficient immune responses *in vivo* and reduces the liver metastasis in metastatic form ofmouse breast cancer. DARU J Pharm Sci 21:33
221. Moghaddam AB, Namvar F, Moniri M, Tahir PM, Azizi S, Mohamad R (2015) Nanoparticles biosynthesized by fungi and yeast: a review of their preparation, properties, and medical applications. Molecules 20:16540–16565. https://doi.org/10.3390/molecules200916540
222. Gharieb M, Wilkinson S, Gadd G (1995) Reduction of selenium oxyanions by unicellular, polymorphic and filamentous fungi: cellular location of reduced selenium and implications for tolerance. J Ind Microbiol 14:300–311
223. Zhou H, Fan T, Zhang D (2011) Biotemplated materials for sustainable energy and environment: current status and challenges. Chem Sustain Chem 4(10):1344–1387
224. Round FE, Crawford RM, Mann DG (1990) The diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge
225. Hamm CE, Merkel R, Springer O, Jurkojc P, Maier C, Prechtel K, Smetacek V (2003) Architecture and material properties of diatom shells provide effective mechanical protection. Nature 421(6925):841–843
226. Losic D, Short K, Mitchell JG, Lal R, Voelcker NH (2007) AFM nanoindentations of diatom biosilica surfaces. Langmuir 23(9):5014–5021
227. Losic D, Rosengarten G, Mitchell JG, Voelcker NH (2006a) Pore architecture of diatom frustules: potential nanostructured membranes for molecular and particle separations. J Nanosci Nanotechnol 6(4):982–989
228. De Stefano L, Rea I, Rendina I, De Stefano M, Moretti L (2007) Lensless light focusing with the centric marinediatom *Coscinodiscus walesii*. Opt Express 15(26):18082–18088
229. Fuhrmann T, Landwehr S, El-Rharbi-Kucki M, Sumper M (2004) Diatoms as living photonic crystals. Appl. Phys. Appl Phys B Lasers O 78(3–4):257–260
230. Lettieri S, Setaro A, De Stefano L, De Stefano M, Maddalena P (2008) The gas-detection properties of light-emitting diatoms. Adv Funct Mater 18(8):1257–1264
231. Losic D, Mitchell JG, Voelcker NH (2009) Diatomaceous lessons in nanotechnology and advanced materials. Adv Mater 21(29):2947–2958
232. Yang W, Lopez PJ, Rosengarten G (2011) Diatoms: self assembled silica nanostructures, and templates for bio/chemical sensors and biomimetic membranes. Analyst 136(1):42–53
233. Aw MS, Simovic S, Yu Y, Addai-Mensah J, Losic D (2012) Porous silica microshells from diatoms as biocarrier for drug delivery applications. Powder Technol 223:52–58
234. Gordon R, Losic D, Tiffany MA, Nagy SS, Sterrenburg FAS (2009) The glass menagerie: diatoms for novel applications in nanotechnology. Trends Biotechnol 27(2):116–127
235. Losic D, Yu Y, Aw MS, Simovic S, Thierry B, Addai-Mensah J (2010) Surface functionalisation of diatoms with dopamine modified iron-oxide nanoparticles: toward magnetically guided drug microcarriers with biologically derived morphologies. Chem Commun 46(34):6323–6325
236. Jeffryes C, Campbell J, Li H, Jiao J, Rorrer G (2011) The potential of diatom nanobiotechnology for applications in solar cells, batteries, and electroluminescent devices. Energ Environ Sci 4(10):3930–3941

237. Gale DK, Gutu T, Jiao J, Chang C-H, Rorrer GL (2009) Photoluminescence detection of biomolecules by antibody-functionalized diatom biosilica. Adv FunctMater 19(6):926–933
238. Lin K-C, Kunduru V, Bothara M, Rege K, Prasad S, Ramakrishna BL (2010) Biogenic nanoporous silica-based sensor for enhanced electrochemical detection of cardiovascular biomarkers proteins. Biosens Bioelectron 25(10):2336–2342
239. De Stefano L, Rotiroti L, De Stefano M, Lamberti A, Lettieri S, Setaro A, Maddalena P (2009) Marine diatoms as optical biosensors. Biosens Bioelectron 24(6):1580–1584
240. Prychid CJ, Rudall PJ, Gregory M (2004) Systematics and biology of silica bodies in monocotyledons. Bot Rev 69:377–440
241. Holzhüter G, Narayanan K, Gerber T (2003) Structure of silica in *Equisetum arvense*. Anal Bioanal Chem 376:512–517
242. Kaufman PB, Bigelow WC, Schmid R, Ghosheh NS (1971) Electron microprobe analysis of silica in epidermal cells of Equisetum. Am J Bot 58:309–316
243. Bharde A, Wani A, Shouche Y, Joy PA, Prasad BLV, Sastry M (2005) Bacterial aerobic synthesis of nanocrystalline magnetite. J Am Chem Soc 127:9326–9327
244. Bansal V, Rautaray D, Bharde A, Ahire K, Sanyal A, Ahmad A, Sastry M (2005) Fungus-mediated biosynthesis of silica and titania particles. J Mater Chem 26:2583–2589
245. Alghuthaymi MA, Almoammar H, Rai M, Said-Galiev E, Abd-Elsalam KA (2015) Myconanoparticles: synthesis and their role in phytopathogens management. Biotechnol Biotechnol Equip 29(2):221–236. https://doi.org/10.1080/13102818.2015.1008194
246. Sastry M, Ahmad A, Islam Khan M, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85:162–170
247. Castro-Longoria E, Vilchis-Nestor AR, Avalos-Borja M (2011) Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus Neurospora crassa. Colloids Surf B Biointerfaces 83:42–48
248. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250
249. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V, Sastry M (2003) Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. Nanotechnology 14:824–828
250. Durán N, Marcato PD, Alves OL, de Souza GIH, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol 3. https://doi.org/10.1186/1477-3155-3-8

# Part VI

# Specialized Plant Species for Phytoremediation

# Chapter 12
# Dendroremediation: The Role of Trees in Phytoextraction of Trace Elements

**Mirosław Mleczek, Monika Gąsecka, Janina Kaniuczak, Piotr Goliński, Małgorzata Szostek, Zuzanna Magdziak, Paweł Rutkowski, and Sylwia Budzyńska**

## 12.1 Physical-Chemical Characteristics of Substrates and Metal Uptake Efficiency

Contamination of soil with trace elements is a serious problem worldwide. Trace elements accumulated in the soil can penetrate other components of the environment, entering and progressing up the food chain, until finally affecting the human diet. One of the methods for reclamation of trace metal-contaminated areas is phytoextraction. Its effectiveness depends primarily on the bioavailability of trace elements, which in turn is influenced by a number of external factors related to the soil and internal determinants associated with the properties of the tree species used in this process.

Bedrock, atmospheric dust or precipitation, and decomposed biological material are a natural source of trace elements in soils. However, over recent decades, environmental contamination with trace elements has increased tremendously [1–4]. Average levels of Zn, Cu, Pb, Ni, Cd, and Cr in soils range from 0.0001% to 0.065%, and content of Fe and Mn is 10% and 0.002%, respectively [5, 6]. With the exception of Fe, all elements present at a level exceeding 0.1% become toxic to plants [7]. Unlike other contaminants, trace elements (typically) are not decomposed and transformed into harmless forms through microbiological and chemical transformations; thus, they persist in the environment forever [1, 8]. Deposited in soils, they

---

M. Mleczek (✉) · M. Gąsecka · P. Goliński · Z. Magdziak · S. Budzyńska
Department of Chemistry, Poznań University of Life Sciences, Poznań, Poland
e-mail: miroslaw.mleczek@up.poznan.pl

J. Kaniuczak · M. Szostek
Department of Soil Science, Environmental Chemistry and Hydrology, University of Rzeszów, Rzeszów, Poland

P. Rutkowski
Department of Forest Sites and Ecology, Poznań University of Life Sciences, Poznań, Poland

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_12

undergo various transformations (e.g., insoluble sediments, speciation, or chelation), depending on composition and soil properties [6, 9]. Therefore, understanding the behavior of trace elements in soils is one of the major tasks in environmental sciences [10, 11].

A criterion for application of phytoextraction techniques is the presence of soluble and exchangeable fractions of trace elements to ensure their bioavailability [6, 12–15]. The bioavailability of trace elements is influenced by various factors and their mutual interactions (Fig. 12.1). Thus, the effectiveness of phytoextraction depends on soil processes and plant physiological processes [3, 16]. The mobility and bioavailability of trace elements depends on, e.g., soil pH; organic matter content; soil granulation; content of iron (Fe), manganese (Mn), and aluminum (Al) oxides; cation exchange capacity; water properties; the type of metal; soil salinity; and soil biological properties [10, 16–19]. Compounds secreted by tree roots, such as organic acids, induce changes in soil composition and properties, e.g., pH or redox potential, thereby influencing the mobility and bioavailability of trace elements to plants and their degradation [20]. Therefore, to assess the effectiveness of phytoextraction of trace elements by trees, it is essential to recognize the soil properties that affect their mobility and bioavailability and determine the efficacy of the process.

### *12.1.1 Soil Granulation*

Soil granulation has an impact on the physical and chemical properties of soil. It determines soil permeability as well as water and air relations, and the colloidal fraction (below 0.002 mm) determines soil sorption capacity [21]. Soil contamination with trace elements and their content in plant tissues is one of the most important determinants [6, 12, 22, 23]. In the majority of soil types, trace elements are mainly associated with the clay fraction. Clay minerals are hydrated aluminosilicates—components of the colloidal fraction of soils, sediments, and water. Clay minerals are constituents of the soil sorption complex involved in cation and anion

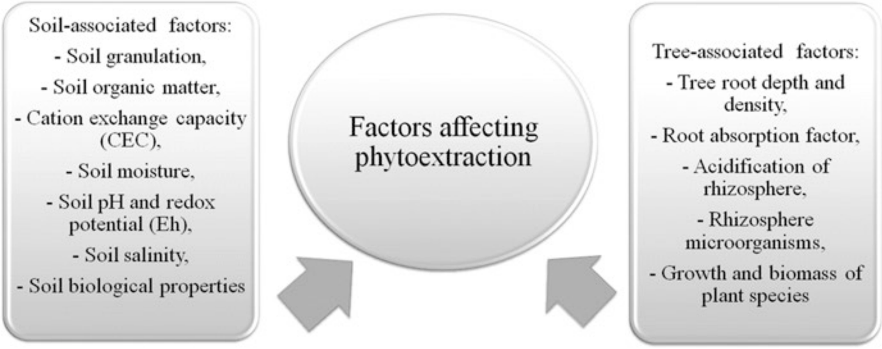


**Fig. 12.1** Factors affecting phytoextraction (Modified according to [16])

exchange and adsorption (in the case of trace elements as well); hence, they play an important role in the environment [15].

The clay fraction content can exert a potent effect on the amounts of plant-available trace metals and influence the uptake and accumulation of these elements in plants [6, 12]. In general, a higher level of trace elements has been detected in soils with fine fractions (clays, silt) in their granulometric composition, while coarse soils (sands) are characterized by a lower level of trace elements [16, 23–25]. An inverse correlation has been observed between the release of Cd and the content of silt and clay particles, which suggests the strong retention of this element in finer soil fractions [26]. Most frequently, the content of Fe, Mn, Zn, Cu, Cd, and Pb exhibits the strongest correlation with the level of floatable soil fractions [6]. Sandy soils have a poorly developed sorption complex; therefore, they are easily acidified and trace elements are rarely retained. Most trace elements in sandy soils leach into surface water and groundwater [6, 17, 23]. Furthermore, soils dominated by sandy fractions are poor in organic carbon, whereas humic substances bind to finer fractions, thus forming stable complexes affecting the mobility and absorption of trace elements [27].

### *12.1.2 Soil Organic Matter*

Organic matter is an important component of soil which exerts an impact on its physical, chemical, and biological properties [6, 27–30]. The content of organic matter in soils in combination with other factors significantly effects the level and mobility of trace elements [10, 24, 31]. All reactions between organic matter and trace elements lead to the formation of water-soluble or insoluble complexes [6]. The influence of organic matter on the mobility of trace elements strongly depends on its properties, e.g., the degree of humification or the ratio between soluble low-molecular organic acids and high-molecular components [32]. Generally, a high content of organic matter in soil and pH close to neutral values have an effect on the transformation of trace elements into biologically inactive forms [33]. Soils that are heavily contaminated with trace elements often exhibit a deficit of organic matter [34]. Therefore, the processes of reclamation of such soils involve the addition of various types of organic matter which reduce the bioavailability of As, Zn, Pb, and Cd and the uptake of these elements by plants, as demonstrated by several investigations [35–37].

Organic matter consists of humic and non-humic substances. Non-humic substances are composed of sugars, amino acids, fats, etc. [18]. Humic substances (HS), which are regarded as the most widespread organic materials on the Earth's surface, are the main components of organic matter [10, 38]. HS are formed through biological transformations related to microbial activity and biochemical conversion of organic compounds. In terms of solubility, humic substances have been divided into the following fractions: fulvic acids (FA), soluble in water; humic acids (HA), insoluble in acidic conditions; and humins, insoluble over the entire pH range [39]. Humic acids are highly important for interactions with inorganic contaminants and constitute the dominant fraction among humic substances [10]. In general, binding of trace elements by humic acids increases with a growing pH value [11]; however, it has been shown that these compounds are efficient in trace element binding even at pH = 0.5 [32]. The high content of various functional groups in humic and fulvic acids facilitates ion exchange and formation of stable complexes with ions of many metals [38, 40]; hence, higher bioavailability of trace metals is observed in soils characterized by a low content of humic and fulvic acids [19]. The complexation ability of humic substances, stability of the complex of humic substances with metals, and interactions of this complex with other soil components determine the solubility and mobility of trace elements [41].

An important factor modifying the mobility and bioavailability of trace elements in soils is reactions with dissolved organic matter (DOC)—the most mobile fraction of organic matter with an essential role in the soil environment in, e.g., physical and chemical binding of trace elements and other contaminants and in their transport into the soil profile [42]. Organic matter influences both the accumulation and release of trace elements in soil; therefore, it serves a key function in phytoextraction processes.

### *12.1.3 Soil Cation Exchange Capacity (CEC)*

The soil cation exchange capacity mainly depends on the type and proportion of clay minerals and the content of humic substances in soils [6, 21]. Higher values of CEC are observed, e.g., in clayey soils, which reduce the mobility of the cationic forms of trace elements, thus affecting their availability. Although CEC by definition involves cations, much higher levels of anionic metal forms are retained in soils characterized by high CEC values than in soils with a low value of this parameter [22]. Soils with a high cation exchange capacity, i.e., soils with a high content of clay minerals, in particular from the montmorillonite and illite groups [21], and soils with a high level of organic matter have great potential to bind trace elements strongly and retain them in the topsoil [6]. The binding of trace element cations increases with their valence, atomic mass, and ionic potential; their affinity for clay minerals has been ranked as follows: $Cu^{2+} > Cd^{2+} > Fe^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+}$. Trace element binding by clay minerals can take place inside or on the surface, depending on the structure of the mineral [23]. The cation exchange capacity of organic matter is usually substantially higher than that of clay minerals and depends on its properties and degree of humification as well as the pH value, which is related to the presence of variable charges [43]. Similarly, iron (Fe), manganese (Mn), and aluminum (Al) oxides exhibit a high capacity for trace element sorption, and their content in soils depends primarily on weathering processes [6, 18, 34]. Adsorption of cationic elements on Fe and Mn oxides increases with growing pH values, whereas anionic forms exhibit an opposite tendency [44]. Sorption and desorption reactions are the dominant processes controlling the bioavailability of trace elements in soils and, together with other factors, determine the effectiveness of the phytoextraction process [45].

### 12.1.4 Soil Moisture

Soil moisture exerts a dominant effect on all processes occurring in the soil and has a decisive influence on the conditions of plant growth and development [46]. Moisture conditions prevailing in the soil largely determine its thermal and mechanical properties, which regulate the temperature in the soil profile as well as the conditions and effectiveness of mechanical interactions in the soil. Water is the basic determinant of such factors as pH and Eh, which affect the mobility of trace elements in soils and—as a consequence—their availability to plants [47]. Therefore, knowledge of the hydrophysical soil properties is necessary for the interpretation, prediction, and modeling of all physical, chemical, and biological processes occurring in soils [46].

Soil water content influences the bioavailability of trace metals, whose greatest mobility has been found in water-saturated soils [47]. In investigations of the effect of soil moisture on growth and Ni accumulation in three plant species, i.e., *Alyssum murale*, *Berkheya coddii*, and *Thlaspi caerulescens*, Angle et al. [48] found that these plants were able to grow even below the average soil moisture content. However, the biomass of all the analyzed species was considerably higher at a higher soil moisture level, while growth was inhibited at lower values of this factor.

Low water capacity and/or low content of nutrients (P, N) increase the toxicity of trace metals in soils [7]. At higher soil moisture, plants take up greater quantities of trace metals and produce larger amounts of biomass, which in turn contributes to higher efficiency of phytoextraction of these elements from soil [48].

### 12.1.5 Soil pH and Redox Potential (Eh)

Soil pH is one of the most important determinants of trace element uptake by plants [6, 22, 34, 49, 50]. It not only determines the mobility and bioavailability of trace elements but also largely influences the uptake thereof by plant roots [16]. The mobility and bioavailability of trace metals in soils depends on pH as this parameter influences the balance between sorption and desorption of $H^+$ cations and trace element cations, e.g., from Mn, Fe, and Al oxides [12, 15, 44, 51]. At low soil pH values, the solubility of trace elements, mainly in cationic forms, increases but is low in the range of neutral and alkaline pH values [12, 34]. Soil acidification may lead to slow dissolution of hydrated Fe, Al, and Mn oxides or even primary and secondary minerals and to the release of trace elements, e.g., Fe, Cu, Mn, B, and Zn, to the soil solution [6, 16, 51]. Low soil pH contributes to an increase in the content of the mobile forms of trace elements and enhances their bioaccumulation rate in plants [37]. An increase in pH results in reduced mobility of the majority of trace elements [19, 52]. Nevertheless, some of them, mainly those present in anionic forms in soil solution (As, Cr, Mo, Se), which can exhibit increased solubility in an alkaline environment as well, are also associated with the potential formation of complexes between these elements and organic compounds [6, 12, 51].

Similarly to changes in pH value, redox reactions have an impact on the dynamics and mobility of trace elements, especially in soils undergoing drastic changes in redox conditions [44]. The redox potential is a physicochemical parameter characterizing the soil oxygenation state. The value of Eh, which is a measure of electron activity in soil solution, provides information about the soil oxygenation state. This parameter clearly reflects changes in hypoxic soils. Soils with good air relations exhibit Eh values above +300 mV. The oxygenation status of the porous soil system is variable and depends on a number of factors, such as the prevailing physical conditions, in particular the water-air and thermal status as well as the chemical composition. Upon drastic redox changes, the dynamics of trace elements are largely regulated by Fe and Mn oxides, which can act as both electron donors and acceptors [44]. Most frequently, the mobility of trace elements (Cd, Cu, Cr, Zn) increases under lower soil oxygenation, while Cd solubility increases with a rise in the Eh value to +200 mV [6]. As shown in some studies, in As-contaminated soils, changes in the solubility of this element in waterlogged soils and, hence, reduction of the Eh value result from the overlapping of complex physicochemical and microbiological processes which lead to increased mobilization of the element on the one hand and secondary immobilization thereof on the other [53]. The solubility of trace elements is often defined as a function of soil pH and redox potential (Eh); therefore, along with the other factors, these parameters have the greatest effect on the phytoextraction process [6].

### 12.1.6 Soil Salinity

Soil salinity is a result of excessive accumulation of easily soluble inorganic salts in the soil solution via both natural and anthropogenic processes [54–56]. Excessive salinity is responsible for excessively high levels of $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, and $K^{+}$ cations and $NO_3^{-}$, $SO_4^{2-}$, $Cl^{-}$, and $(COO)_2^{2-}$ anions, which exceed plant requirements [55]. Soil salinity limits proper plant growth and development [56]. A high content of $Cl^{-}$ and $Na^{+}$ ions can considerably limit the uptake of biogenic components such as $K^{+}$, $Ca^{2+}$, and $NO_3^{-}$ by plants, thereby affecting normal plant growth and development [54, 57]. Elevated levels of sodium ions affect the soil structure by increasing soil dispersion and swelling capacity which reduces its permeability and capillary water absorption, causes alkalization of soil, and affects soil biochemical processes [56]. Moreover, soil salinity has a substantial impact on water availability to plants. Due to the interaction between water and salt ions, the water potential in a strongly saline environment is low. In such conditions, plants have a limited ability to uptake water from the substrate and osmotic stress impedes plant growth [54, 57].

It has been shown that a high accumulation of soluble inorganic salts in the soil can determine the content of trace elements and affect their mobility [24, 26, 58, 59]. As demonstrated by Manusaki et al. [59], cadmium uptake by *Tamarix smyrnensis* increases with rising substrate salinity level, which greatly influences Cd translocation from the roots to the aboveground parts of the plant [59]. In turn, Filipović et al. [58] have shown that substrate salinity has an impact on Cd speciation in soil solution by formation of $CdCl_n^{2-n}$ complexes; nevertheless, no increase in Cd phytoavailability was observed in these investigations. The authors suggest that the chlorine complex can serve as a Cd carrier between different soil fractions and may cause only the transfer of this element between individual solid phases of soils rather than an increase in its bioavailability. Hence, soil salinity increasing the mobility of Pb, Cd, Zn, and Cu in soils is additionally determined by other mechanisms, in particular competition with calcium for sorption sites, complexation with chlorides, complexation with sulfates, and competition with Mg and/or Ca [26, 58]. Since soil salinity largely affects normal plant growth and development and promotes mobilization of trace elements in soils, this factor should be regarded as a potent determinant of phytoextraction processes.

### *12.1.7 Soil Biology*

Microbial biomass in soils is a significant part of organic matter and serves as the parent material for humus formation. There are microorganisms that are able to survive even at strong trace element contamination of the soil environment [60]. Soil biological activity is characterized by enzymatic activity, microbial biomass, respiration activity ($CO_2$ emission, $O_2$ uptake), microbial composition and abundance, and specific activity for specific soil conditions accompanied by redox processes or changes in pH [61]. Soil enzymatic activity primarily depends on microbial diversity [18]. Therefore, biological soil indicators are used to assess the function of soil ecosystems and the influence of anthropo-pressure. A low content of soil organic matter and a high content of bioavailable forms of trace elements contribute to a reduction of the enzymatic activity of soils [33]. High concentrations of copper or zinc in soils reduce the activity of such enzymes as dehydrogenase, urease, acid phosphatase, alkaline phosphatase, etc. [61, 62]. They also inhibit the proliferation of microorganisms in soils, in particular actinomycetes [62].

Enzymatic reactions in soils result in an increase in soil pH, thus inducing changes in the bioavailability of trace elements, e.g., Ni, Cu, Pb, Co, Zn, and Cd [63], for trees. A key influence on the behavior of trace elements in soils is exerted by microorganisms responsible for the processes of methylation, mainly of such elements as Hg, As, Se, Te, Tl, Pb, and In [6]. Although methylation of some metals proceeds through chemical processes as well, biological methylation is assigned a dominant role in both soil and aquatic environments [64]. Soil biological indicators, e.g., enzymatic activity and microbial communities, can have a direct effect on the biological availability and mobility of trace elements [15, 18], thus affecting the efficiency of the tree phytoextraction process.

## 12.2 Trees: Plants with Morphological Traits Important in Phytoextraction of Pollution

The basic purpose of each species is to survive. In trees, the most long-lived organisms in the world, adaptation mechanisms responsible for survival in extreme conditions are especially important. Such extreme conditions include places with a high concentration of substances harmful to plants. In nature, only a small fraction of plants that exhibit metal tolerance or super-accumulative capacity (hyperaccumulators) can survive in toxic metal-contaminated soils, and the majority are herbaceous species with low biomass [65]. It seems natural that most are herbaceous species because the degree of metal accumulation in a plant is determined by its genus, species, and anatomical parts, as well as the time of exposure to environmental conditions [66]. Thus, trees, organisms of considerable longevity, are particularly suitable for exposure to contamination.

For any description of morphological traits in phytoextraction of pollution, it is important to define what phytoextraction is. According to the ITRC [67], phytoextraction is the "use of plants to extract contaminants (e.g. metals) from the environment (especially soil). When the plants are saturated with contaminants they are harvested." Alkorta et al. [68] defined phytoextraction as "The use of plants to remove pollutants (mostly, metals) from soils," while Sarma [69] describes this process as follows: "plants absorb metals from soil through the root system and translocate them to harvestable shoots where they accumulate." Therefore, it is important to clarify whether this process involves only extraction from soil, or also from water or air, and only metals, or also, for example, dust, and whether the absorbed metals should be accumulated only in shoots or in other parts of trees. If phytoextraction is understood according to the ITRC [67], as the state in which plants become saturated with contaminants after which are harvested, the state of saturation could be equated with the dying of a tree. If the final stage of phytoextraction is cutting the tree, the fact that the tree would have died would not have much significance. However, it would be significant if saturation and tree death occurred simultaneously. Moreover, the relationship between the age of a saturated and dying tree to its natural lifespan, which, depending on species, could be from several dozen (willows, poplars,), several hundred (Scots pine, sessile and pedunculate oak, beech), or over 1000 years (*Taxus baccata*, *Pinus aristata*, *Picea abies*), should also be considered. If the lifespan is short, from the point of view of phytoextraction, fast-growing species, as described by Pajević et al. [70], are better. If, however, the tree should live for a long time, it requires either a relatively unpolluted environment or must have the ability to neutralize toxic substances. Therefore, it would seem natural for trees to transport and accumulate toxic substances in those parts of plants where they can be quickly removed, such as leaves or fruits. Nevertheless, research conducted on apples by Bednarek et al. [71] and Tošić et al. [72] does not confirm this. The results of the Bošković-Rakočević [73] study on plums indicate, however, that the soil-to-fruit transfer factor (TF) shows large differences between metals. The TF for Cd, Pb, Mn, and Zn indicates no potential risk to human health, whereas for Fe and Cu, it suggests that plums can accumulate Fe and Cu. Clearly, more research is needed before morphological traits in phytoextraction of pollution in fruit trees can be fully understood.

The accumulation of toxic substances in leaves could be an effective extraction process, but it should be discussed from the point of view of the goal of phytoextraction. To ensure that toxic elements do not return to the soil during the decomposition of litter, leaves would have to be removed regularly—in deciduous trees and some conifers at least once a year. It is not difficult to remove fallen leaves, but in this way, the soil loses nutrients which return to the soil during litter decomposition, the upper soil layer loses an important protective layer, the soil loses moisture more quickly, the temperature of the upper layer of soil rises more rapidly, and the plant-soil-soil microorganism balance is destroyed. Therefore, it is essential to monitor what happens to the contaminants after phytoextraction when they are accumulated in leaves.

Trees can inactivate toxins in their roots, but if the mechanism of phytoextraction was to make sense, it would be necessary to clear and remove the roots, which is laborious and expensive and greatly interferes with the soil cover. For some types of pollutants, bark plays an important absorbing role [74], but it is difficult to strip the bark of a tree during its life. On the other hand, leaving toxic substances on the bark leads to their flushing from the trunk and their transfer with rainfall or dust into the soil. From a practical point of view, the definition of phytoextraction given by Alkorta et al. [68], to "remove pollutants from soils and translocate them to harvestable shoots," seems the most relevant.

Knowledge of the adaptive mechanism of trees growing on contaminated soils and the use of long-growing trees for phytoremediation is thus still an open topic. However, phytoremediation can bring considerable benefits. For fruit trees, knowledge of morphological traits would be important from the point of view of the health of fruit-eating organisms (not only in relation to humans), while forest trees can create a balanced, rich ecosystem, with both natural and functional qualities. Forest stands have a retention function, regulating the water regime, they can act as barriers to noise, and, of course, they are aesthetically pleasing and attractive.

The use of long-growing trees for phytoextraction purposes, however, is not without complication. Different species have their own limitations. The main limiting factors are climatic conditions, available water, and the type of soil substrate. There is no single tree species able to grow in every environment, and even if such a species did exist, phytoextraction properties are significantly different. When selecting trees for phytoextraction purposes, it is important to decide whether we are looking for the most universal species or for a species for a given location with strictly defined requirements and habitat restrictions. Pine (*Pinus sylvestris*), despite growing in a wide geographical range (in varied altitude conditions, in very diverse habitat conditions, from dry to swampy soil), being a coniferous species, is sensitive to environmental pollution, although pine needles can be treated as bioindicators in the assessment of urban environmental contamination with heavy metals [75]. Therefore, it would be better to choose species with phytoextraction properties adapted to specific environmental conditions. In addition, conducting research on native plants with phytoremediation potential is a particularly important strategy, as indigenous plants are often more dependent in terms of survival, growth, and reproduction under environmental stress than exotic plants [76].

The fact that the mechanism of phytoextraction in trees is relatively poorly described is largely due to many factors that affect this process. For example, Pinto et al. [77] say that soils with larger concentrations of trace elements may be less toxic than those with smaller concentrations, depending on many of the previously mentioned factors. Parzych and Jonczak [78] showed that the content of Zn, Cu, Fe, Mn, and Zn in the needles of *Pinus sylvestris* varied within a growing season. The impact on the shape of accumulation of heavy metals in the needles of *Pinus sylvestris* is particularly dependent on the age of the needles, sum of rainfall, and humidity of the solum. It should also be noted that Parzych and Jonczak [78] conducted their studies in one of the cleanest regions of Poland, where the concentration of metals is very low. It is possible that with a low concentration of metals in the soil, the trees do not accumulate but only utilize it for current needs in their physiological processes.

To sum up, the physiological and morphological features of trees are a reflection of the adaptation of the tree to living under given conditions, and the tree must first fulfill its basic ecological requirements. This naturally leads to a consideration of phytoextraction potential. It is difficult to compare the features of the fast-growing Nepalese alder (*Alnus nepalensis*), a species native to the eastern Himalayas [65], or fast-growing willows [79, 80] to those of long-living oaks [81], *Ulmus laevis* [82], or *Fraxinus excelsior* [93] growing in wet flooded sites of the temperate climate region to species of humid tropical lowlands, like *Acacia mangium* [83]. Thus, general attributes of species of trees with a potential for phytoextraction cannot be clearly defined because the features of roots, stems, and leaves will always in the first place be determined by environmental conditions. Hence it is very important to test different tree species in different site conditions, which make the subject considerably more interesting.

## 12.3 Tree Species in Phytoextraction of Toxic Elements

When comparing literature data presented in numerous databases, there is a great deal of information concerning hyperaccumulators or many other plants, usually those with low biomass [84]. In the case of trees, however, information on their phytoremediative potential is limited. This is probably due to the difficulties involved in such kinds of study (relatively large size of seedlings, difficulty in the collection of proper representative samples, low homogeneity of material as regards differences in cellulose, holocellulose, or lignin content). Additionally, selection of the most promising tree species, potentially useful in phytoextraction/phytostabilization of trace elements present in polluted substrate is difficult with respect to their ontogenic traits, usually specific environmental requirements but also their interactions with other plants. In spite of these limitations, interest in woody plants is growing due to their perennial character, highly developed root systems, great upper branches that determine lower or higher transpiration, as well as the ease with which they are able to adapt to new unfavorable environmental conditions.

Phytostabilization is only one of several phytoremediation mechanisms, i.e., how plants can affect trace elements present in polluted substrates. In this case, data about dendroremediation are limited to a small number of studies with clear information on the positive traits of trees necessary for the effective phytostabilization process [85]. Analysis of this process in the case of mature and large trees is generally difficult; therefore younger and fast-growing tree and bush species such as poplar and willow species are more suitable for analysis [86]. Evangelou et al. [87] recommend that plants used in phytostabilization should be characterized by a high biomass crop, which in the case of selected willow species is particularly fitting. Authors have shown that interactions between metals (their presence and concentrations) in contaminated substrate can be a factor that has an influence on the use of trees for this process. A proper interpretation of studies on phytostabilization is not always straightforward because there are some data where bioconcentration factor (BCF) and translocation factor (TF) values $<1$ have shown this process to have occurred. However, no uptake was evident of this process as both in trees, but also in the majority of other plants, there are two possible paths: precipitation of metals in the rhizosphere and/or their accumulation in roots only [88]. In our experience, phytostabilization using selected tree species is generally an effective process for metal immobilization in the rhizosphere, a factor that clearly decreases the bioavailability of trace elements.

The potential of trees and bushes is mainly related to their ability to uptake elements from soil along with the possible transport of accumulated metals/metalloids to aerial plant parts [89]. Unfortunately, phytoextraction with trees is clearly limited to selected elements only. The most frequently studied metals are cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), in both model [90–92] and field experiments (Yankun et al. 2004; [93, 94]). Other metals have also been studied in tree species organs among which are chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), and selenium (Se) [95–97]. Among metalloids, according to available literature data, only arsenic (As) concentration has been studied widely [98, 99], for both its total concentration and its organic and inorganic forms [82]. Analysis of the phytoextraction of toxic elements has been described for many different tree and bush species, although the majority of papers have concentrated on *Acacia* [98], *Acer* [100], *Betula* [101], *Pinus* [102], *Populus* [103], *Quercus* [104], *Ulmus* [105], and *Salix* [97]. Additionally, many other less known species such as *Anadenanthera peregrina* [106], *Eucalyptus rostrata* [91], *Prosopis juliflora* [107], *Rhizophora racemosa* [108], *Hopea odorata*, or *Intsia palembanica* [109] have been studied.

Trees and bushes have been subjected to hydroponic, pot, and field experiments. In the case of hydroponic experiments, plants were grown in solutions enriched with particular trace element salts [110], while in pot experiments, substrates were originated from different sites, e.g., a gold (Au) mining area [99], Cu postindustrial wastes [81], or other mine tailings [104]. Analysis of trees growing in field experiments has been decidedly more common. This is largely due to some of the characteristics of tree and bush species, such as their size, distribution, or the possibility to obtain representative samples of experimental materials—usually a significant problem in hydroponic and pot experiments with the necessary age limitation of the plants used in these trials. Generally, trees have been grown in highly polluted areas, contaminated with particular metals such as ex-tin mining [109], or Pb-Zn [111] or mercury (Hg) mine areas [112], where soil was contaminated with metal salts [94] or sewage sludges [93], although areas contaminated by crude oil during exploration have also been investigated [96]. Studies of trees in the environment can also be problematic in terms of their methodological aspects and the lack of any homogenous structure of trees [113], which is particularly important to estimate the average concentration of metals in the whole tree biomass.

The efficiency of trace element phytoextraction differs and is often characteristic of particular tree species. On the other hand, the ability of trees to effectively uptake metals from polluted substrate depends on numerous environmental factors (pH, electrical conductivity, redox potential, or organic matter content) [114]. Not without significance is the presence of selected microorganisms in the rhizosphere of tree roots [115] or specific interactions between metals with selective phytoextraction ability (particular elements only) [116]. A simple comparison of tree species and their ability to uptake trace elements is not possible because there must be full data concerning the age of plants, their origin, possible growth on the same substrate, and also similar development of seedlings or mature trees. The general tendency, however, is always the same; a higher concentration of bioavailable metal forms in substrate corresponds to a higher concentration of this metal in trees, whose biomass is usually lower [94]. On the other hand, this was not the case described by Wang and Jia [94], for *Populus canadensis* Moench and *Larix olgensis* Henry during a 2-year field experiment. One possible way to compare tree species is to consider the bioconcentration factor (BCF). Usually below 1, it suggests the general exclusion of elements [93, 95, 102]. However, Yanqun et al. [111] described *Salix cathayana* with a BCF > 1 for Cd, Cu, and Zn. Moreover, Dmuchowski et al. [101] found *Betula pendula* Roth to be highly effective in phytoextraction and translocation of Zn from roots to leaves, similarly to hyperaccumulators. The same observations were described by Goliński et al. [117], where *S.* × *rubens* (*S. purpurea* × *triandra* × *viminalis*) growing in a hydroponic experiment was characterized by a concentration of Zn of up to 5, 1.3, and 2.3 g/kg dry weight (DW) in roots, shoots, and leaves, respectively, with translocation factor (TF) values >1. This factor (describing transport of elements from roots to aerial tree parts) is particularly important. It leads directly to the total efficiency of trees in the uptake of elements from soil and determines their potential for practical usage in phytoremediation in terms of biomass crop.

The aspect of element distribution in tree species has been widely discussed. The general tendency in transport of metals to particular tree organs, where they accumulate, is as follows: roots > leaves > stem, although this is not affixed relationship [109]. Vertical distribution of metals in trees depends both on the kind of element and tree species, as confirmed by Rosselli et al. [93], who observed the transport of Cd and Zn to aerial plant parts of *Betula* and *Salix*, while in the case of *Alnus*, *Fraxinus*, and *Sorbus*, transport was limited to the root system. Transport of elements depends on several internal plant factors which can modulate the physiological response of trees and finally alter the translocation of elements. This may lead to situations contrary to those present for the majority plants, e.g., as described by Zhao et al. [118], where concentration of Pb was higher in the leaves of *Broussonetia papyrifera* (L.) Vent. and *Paulownia fortunei* (Seem.) Hemsl. than in roots. Values of over 1000 mg/kg persuaded the authors to consider them as hyperaccumulators, particularly as the trend of metal accumulation was as follows: leaves > branches > trunks. Differences in the concentration of particular elements observed in tree and bush species are not the same for the same species/varieties. Moreover, various distribution patterns are also observed for different forms of the same elements. In trees, many atypical distributions of particular forms of one element are observed, which suggests that there are different mechanisms of transport of metals/metalloids and their forms to aerial tree parts [82].

As hyperaccumulators of toxic elements, the previously mentioned trees could offer an interesting solution to the critical ecological problem of highly polluted postindustrial wastes and degraded soils. On the other hand, negative opinion suggests that such hyperaccumulators produce high biomass polluted with metals, which limits their practical use. It is the long duration of tree growth related with the temporal polluted area reservation that is the real limitation of phytoextraction [94]. The production of biomass that is highly contaminated can be, but does not necessarily have to be, a serious problem [119, 120]. It should be remembered that Vervaeke et al. [116], who analyzed *Salix viminalis* L. 'Orm,' described the limitation of the prospects for phytoextraction of metals in the case of the limited efficiency of this process. This rather encourages a search for new tree and bush species that can be analyzed for their phytoremediative potential for eventual cultivation in locations that are highly polluted with metals. Additionally, data on the ability of selected tree species to break down organic compounds points to a promising role for these plants in the reduction of many highly toxic substances such as polycyclic aromatic hydrocarbons (PAHs) or explosives [116, 121, 122].

## 12.4 Adaptation and Physiological Response of Trees

The main limitation to using trees for phytoremediative practice is the temporary reservation of a polluted area. In the case of trees, the plants solely occupy polluted areas, and additional activities are not possible and/or forbidden by law.

### *12.4.1 Phenolic Compounds*

In phytoremediation of metal-polluted soil, the selection of suitable plants that are able to grow, develop, and accumulate metals decides the effectiveness of the process. Thus, new species, especially hyperaccumulators, are introduced and investigated. Many of them are herbaceous, but their use in polluted soil is limited by low biomass production or slow growth. In recent years, various tree species have been examined for their potential in phytoremediation. Research on the defense mechanisms and tolerance of different species undergoing metal stress is particularly relevant in the light of their application to the phytoremediation process. Heavy metal stress induces reactive oxygen species (ROS) ($O_2{\cdot}^-$and $H_2O_2$) formation in plants [123–125], which can affect chlorophyll, proteins, and lipids, cause DNA damage and cell death, and consequently damage the whole plant when the ROS production exceeds the capacity of antioxidant and detoxification mechanisms [126, 127]. Increased ROS production elevates the content of antioxidants in different parts of plants [124, 128, 129].

Phenolics, well-known plant antioxidants, play an important role in allowing plants to adapt to variable environmental conditions, in defense mechanisms as substrates for different peroxidases and by direct scavenging of excess ROS and in chelating heavy metals by hydroxyl and carboxyl groups [130–133]. The role of phenolic compounds as antioxidants is related to their structure, and the phenolic antioxidant system is an analogue to the ascorbic acid system. Phenolic acids, flavonoids, and lignin precursors synthesized and accumulated under stress conditions are important antioxidants and play a major role in scavenging mechanisms as electron donors [131, 133]. Some phenolic compounds, like salicylic acid (stress signaling molecule), catechol, and catechin, participate in response mechanisms in plants under metal excess [134–136], while other phenolic compounds, such as epicatechin, rutin, and derivatives of cinnamic acid, participate in metabolism under metal stress [137]. Phenolics in plant cells are also able to act as prooxidants [133].

The exudation of some phenolic compounds is triggered by specific mechanisms which participate in tolerance, so it is important to use some species of trees in phytoremediation of metal-polluted soil. It has been found that apiin (apigenin-7-O-apiosyl-glucoside) and quercetin-3-dirhamnosyl-galactos are the main phenolic compounds of root exudates of *Silene vulgaris* [138]. Apiin concentration significantly increases under Cr(VI) treatment. This is probably because of its ability to scavenge free radicals and act as a metal chelator [139, 140]. Other studies have identified quercetin and its derivatives as important chelators of Cr in plant tissue suggesting that the maintenance of a suitable concentration of quercetin in the root exudates of *S. vulgaris* could be significant for growth in Cr-polluted soils [138], while in As-treated *Ulmus laevis*, plant quercetin was accumulated in roots and leaves under DMA(V)-treatment. Trees are able to accumulate soluble phenolics in different organs under metal stress [124, 129, 141, 142]. Accumulation of phenolics was observed in the roots of Scots pine under Cd treatment, and newly formed phenolic compounds were localized in the cytosol root tips (elongation zone), a process that was faster than lignification [129]. Accumulation of phenolics has been observed in *Populus* species as a response to Cd exposure in different parts of plants in comparison to a control, i.e., up to 47% in roots of *P. deltoides*, in the range of 38% and 168% in wood of *P.* × *euramericana*, and up to 47% in leaves of *P. nigra* and *P. popularis* [124]. However, the presence of heavy metals in a growing medium can also modify phenolic content in different ways. A decrease of total polyphenols in root exudates was noted in *S. vulgaris* under Cr(III) and at a low concentration of Cr(VI) treatment [138]. Some authors have pointed to the significant role of carboxylic acid groups of the cell wall in chelation of metals (Cr, Ni) [138, 143, 144]. Detoxification of Cd in trees is linked to phenolic metabolism [145, 146]. A study on the mangrove plant *Aegiceras corniculatum* Blanco [145] found that polyphenolic compounds play a significant role in the detoxification of Cd in plant tissues. The elevation of the total content of phenolics and condensed tannins in the roots and leaves of the mangrove plant was linked to its ability to chelate metal ions with hydroxyl and carboxyl functional groups [147], and as a consequence their further transfer to vacuoles [148]. However, a high level of Cd can depress the synthesis of polyphenolic compounds, and the authors suggest a tolerance limit of *A. corniculatum* for Cd concentration [145]. Changes in phenolic content under Cd exposure were also confirmed in different tissues of *Populus* × *canescens* with the highest accumulation of the secondary metabolites in bark, roots, and leaves, but no changes in wood [124]. The authors suggest that phenolics may be particularly important as ROS scavengers because their concentrations increased most strongly in the tissues with the greatest Cd accumulation.

Zafari et al. [125] studied the effect of Pb stress on the phenylpropanoid pathway in *Prosopis farcta. Prosopis* trees and shrubs are known as resistant species to heavy metals and are recognized as indicators of soil pollution [149]. One study [125] showed not only an increase of total phenolic content in shoots of Pb-treated *Prosopis* plants but also some phenolic acids and flavonoids, thereby confirming the close relationship between phenolic compounds and response to Pb stress. An increase of salicylic, ferulic, and cinnamic acids, daidzein, vitexin, resveratrol, and myricetin was observed, although the time of increase ranged between 12 and 96 h when compared to a control. Falls and increases in the content of luteolin and diosmin, caffeic acid, and naringenin were noticed, while kaempferol content decreased. No changes were observed for quercetin.

Alterations in the phenolic profile have also been detected in leaves [128, 142, 150, 151]. Among the phenolic compounds that significantly increased in the leaves of *Betula pubescens* from a polluted area were chlorogenic acid, catechin, derivatives of myricetin, some gallic acid derivatives, and quercetin derivatives, associated with an impact on shikimate and phenylpropanoid pathways [128, 151].

Environmental studies and pot experiments on *Salix* response to metal stress reveal an increase in total phenolic and flavonoid content in leaves exposed to different metal stresses (Cu, Ni, Pb, Zn) [141, 142, 152]. The phenolic profile of *Salix* leaves reveals the presence of vanillic, chlorogenic, p-coumaric, ferulic, sinapic, t-cinnamic, and benzoic acids (only in environmental study), myricetin, and quercetin [142, 152]. The elevation in the content of most of the phenolic components after Zn treatments, and in polluted areas, indicates their role in the detoxification mechanism [142, 152]. Moreover, in conjunction with their elevated level in polluted areas [142] have suggested that they can be an indicator of metal stress in *Salix* species growing in contaminated soil. Phenolic and flavonoid synthesis and accumulation under metal treatment can be modified by the ratio of Ca/Mg, temperature, or $CO_2$ [153–155]. A study on *Robinia pseudoacacia* under Cd and Pb treatments suggests that temperature could stimulate a prevention mechanism via enhanced synthesis of some secondary metabolites including phenolics [155]. Total phenolic content was significantly increased by a change in the ratio of Ca/Mg in Cu-treated plants [153]. Research on the phenolic profile of *U. laevis* under As treatments revealed a considerable increase of phenolics in photosynthetic tissue and also found that arsenic forms strongly influenced the content of some phenolic compounds. The profile of the roots of the control consisted only of protocatechuic, syringic, and vanillic acids, while the leaves of the control were richer in phenolic components (*trans*-cinnamic, gallic, p-hydroxybenzoic, protocatechuic, syringic, and vanillic acids). As treatments induced changes in the content and composition of phenolic compounds; in roots the content of the abovementioned acids was elevated, while a reduction of these acids under As(III), As(V), DMA(V), and some combination of As forms was observed in leaves. Additionally, the greatest diversity of phenolics found in the roots of *U. laevis* was in plants treated with dimethylarsinic acid DMA(V), As(III), and As(III) + As(V) while in leaves under As(V), As(III) + As(V), As(III) + DMA(V), and As(III) + As(V) + DMA(V). The profile of roots of As-treated plants consisted of phenolic acids and flavonoids with a strong increase of 2,5-dihydroxybenzoic acid, p-hydroxybenzoic acid, and protocatechuic acids. The last of these exhibits antioxidant and chelating properties [135] which correlates with As accumulation [150]. The As treatments modified both the content and composition of the leaf profile with a concentration decrease of the abovementioned acid under As(III), As(V), DMA(V), and some combination of As forms. Caffeic, chlorogenic, p-coumaric, ferulic, and sinapic acids occurred in leaves under As stress. Among phenolic compounds, salicylic acid and its derivatives play an important role as biomarkers of oxidative stress induced by heavy metals [141]. An elevation of their content was confirmed in leaves of *Salix* and *U. laevis* and was correlated with metal content ([143, 150, 153]).

### *12.4.2 Low-Molecular-Weight Organic Acids*

Proper homeostasis of plant tissue is critical for growth and development. Under stress conditions (nutrient deficiency, salinity, metal toxicity), plants have to balance the uptake, utilization, and storage of elements through absorption, translocation, compartmentation, and secretion. In the case of higher plants, especially when growing under metal stress conditions, roots exudate more metabolites, including different complex compounds (namely, enzymes, mono- and oligosaccharides), carboxylic acids, alcohols, phenolics, amino acids, and proteins [156, 157] which regulate growth, while the metal toxicity is relieved.

Low-molecular-weight organic acids (LMWOAs) represent a significant component in root exudate profiles [157, 158]. In soil, they play an important role in a number of rhizospheric processes, including pH regulation, nutrient acquisition by increasing nutrient solubility (e.g., Fe, $PO_4^{3-}$) [156, 159, 160], and reducing the adverse effect of anaerobic conditions on plants. In addition, they are a source of easily available and rapidly mineralized organic matter [161], as well as a critical factor for the distribution and bioavailability of pollutants, primarily including metal detoxification and then phytoextraction (e.g., Al, Zn, Cd, and Pb) [157, 162, 163]. LMWOAs form complexes with metals, especially with citric, malic, and oxalic acids, which affect their mobility and availability [160]. Acid-induced changes are of ecophysiological significance, and consequently, the analysis of LMWOAs is necessary for a good understanding of the ecophysiology of plant tissues in their physiological response and adaptation mechanisms [164]. This is particularly because LMWOAs in the rhizosphere are also assumed to be a critical factor for the distribution and bioavailability of metal pollutants, although their determination in the case of trees is yet to be fully investigated. For this reason, research on the subject of acidic phytoextraction should be developed as trees provide excellent plant material for soil cleaning (significant biomass, divergent root system) and at the same time secrete elevated amounts of LMWOAs into the rhizosphere under metal stress, as indicated in some literature data [165, 166].

Haoliang et al. [166] found that Cd induced root exudates of *Kandelia candel* (L.) Druce (*K. candel*). Total concentration of LMWOAs under different Cd stresses showed high variation. For studied systems with the addition of Cd, a higher content of analyzed acids was observed in comparison to the control. The dominant root exudates of *K. candel* consisted of the following acids, acetic, lactic, malic, and citric, and the total amount of LMWOAs in the *K. candel* root exudates studied here ranged from 13.09 to 39.05 μmol $g^{-1}$ DW roots [166]. Interestingly, high exudation of malic acids was interpreted as a complexation organic ligand and detoxifying mechanism of Cd in *K. candel* [166]. Similar results were presented by Xie et al. [160], where *Kandelia obovata* (S., L.) Yong (*K. obovata*) was tested in order to investigate the stress response of tree roots and whether root exudation could act as a defense mechanism against heavy metal toxicity. The results obtained in a greenhouse experiment showed that the total concentration of LMWOAs during Cd exposure, for every Cd treatment group, was higher than in the control. Oxalic, acetic, L-malic, and tartaric acids were dominant, and the content of dicarboxylic acids was significantly higher than mono- and tricarboxylic acids (~fourfold) [160]. In the case of *K. obovata*, oxalic acid concentration was higher than the control for every Cd-treated plant, which—according to previous studies—may be a result of the adverse conditions of Cd presence and species differences.

In recent years, research on the adaptation and physiological response of *Salix* taxa has clearly pointed to their phytoextraction ability [167]. *Salix* is well known as a good accumulator of metals, but there is only limited information on the physiological and morphological parameters of this plant's response to metal stress. The adaptation and physiological response of *Salix viminalis* L. (*S. viminalis*) was described by Magdziak et al. [168], where the presence of metals in solution had a significant impact on the profile and amount of the studied acids. Under Cd, Cu, Pb, and Zn conditions, and at a physiological ratio Ca/Mg (4:1 Ca/Mg), the total concentration of LMWOAs was 97.0, 55.6, 122.4, 291.4, and 175.9 μM 100 $g^{-1}$ DM, respectively, while the total concentration of LMWOAs under a 1:10 Ca/Mg ratio was 99.8, 62.0, 61.8, 77.7, and 95.9 μM 100 $g^{-1}$ DM [168]. For both studied systems, lactic, succinic, malonic, and formic acids were dominant, and total concentration of LMWOAs was always lower than in the control. An exception was only recorded in the system where Zn was at a level of 0.5 mM and Ca/Mg ratio was 4:1. The authors surmised that such a situation was a consequence of the LMWOAs the plants use for metal chelation, detoxification, and translocation into the aerial parts. Oxalic acid was detected only for the physiological ratio Ca/Mg and only for the control. A different physiological response for the same *Salix* taxa was obtained in relation to increasing Ni and Cu concentration [143, 169]. Under increasing Ni concentration, the dominant acids were acetic, citric, formic, lactic, oxalic, and succinic [143], while under Cu treatment, the presence of malonic acid was determined instead of succinic acid [169]. However, in both independent experiments, the tested *S. viminalis* exudated the highest concentration of oxalic and acetic acids. For these acids, and for the total concentration of LMWOAs in *Salix* rhizosphere, a significant exponential relationship of their exudation to Ni and Cu concentration in the cultivation medium was observed. When Cd and Pb were added to nutritional solution, the acetic, citric, formic, malonic, and lactic acids were dominant in *S. viminalis* rhizosphere [170]. Importantly, acetic acid composed almost 90% of all detected molecules. Under Cd and Pb, acetic acids probably play a crucial role in maintaining adaptation and homeostasis. However, for *S. viminalis* taxa, the presence—at higher concentration—of acetic acid or both acetic and oxalic acids together can be explained by the reaction of willow to metal stress, which activated a defense mechanism to tolerate the presence of toxic elements, i.e., a metal complexation reaction and then transport via xylem followed by immobilization in the vacuole [143, 169, 171]. It should be noted that the type and amount of the separated acid strictly depends on the physicochemical character of the metal in the medium and the specific plant reaction.

The abovementioned study provides clear evidence that metals induce variation in root exudates of LMWOAs in the investigated tree species. The dissociated carboxylic acids have one or more negative charges which interact with dissolved metal cations and form metal-carboxylic acid complexes through their carboxyl groups ($-COO^-$). Therefore, through their complexing capacity, organic acids reduce toxic trace elements, influence metal solubility, mobilization, and then uptake by plants. Free metal ions are known to be more toxic compared with metal-organic molecules.

Equally, LMWOAs released into the rhizosphere are also widely distributed in plant tissue—root, shoot, and leaves. Organic acids (citric, fumaric, malic, malonic, oxalic, tartaric, and succinic) have an important function in plant metabolism, such as the maintenance of internal pH and ionic balance, energy and respiration generation, photosynthesis, amino acid synthesis, and formation of a metal-organic acid complex in the vacuole for metal detoxification [162, 172]. Studies of crop plants, grassy hyperaccumulators, or herbs are quite common in the literature [173–177]. However, to date there are probably only two works that have focused on tree tissue under metal exposure. Goliński et al. [117] showed that under Zn exposure, roots biosynthesize higher amounts of LMWOAs when compared to a control, where malic, malonic, and citric acids were dominant acids. The total amounts of analyzed acids were higher than amount exudated into the rhizosphere. Moreover, leaves were characterized by a higher amount of LMWOAs than the rhizosphere and roots, where malic, succinic, and citric acids were highest. It should also be mentioned that the total content of LMWOAs formed in leaf tissue under Zn stress showed a decreasing trend in comparison to the control system. However, the higher Zn accumulation potential of *S* × *rubens* is accompanied by higher concentrations of acids in roots and in leaves which may indicate that the studied LMWOAs might be closely involved in Zn translocation and accumulation in roots and leaves. Analysis of the results suggests that this retention may be attributable to sequestration and/or complexation with organic ligands in the tissue symplast and possibly in the vacuole.

Magdziak et al. [162] described results obtained from analysis of LMWOAs in nine *Salix* taxa grown in two experimental areas in different environmental conditions. Both areas differed, especially in the concentration of Cu, Pb, and Zn elements in the soil (Area 1 had a markedly lower concentration of these elements in comparison to Area 2). This study revealed that the total concentrations of LMWOAs in the rhizosphere, roots, and leaves were higher in Area 2, where metal pollution was much more pronounced (especially in the case of Cu) than Area 1. A significant increase was observed for oxalic, malic, acetic, and citric acids in the rhizosphere and also roots in Area 2, while succinic acid was also detected in leaves. The authors suggest that the higher levels of mainly oxalic and acetic acids might be explained as a specific mechanism of *Salix* taxa to the physiological stress generated by the high concentration of metals present in the soil of Area 2. Other environmental studies have confirmed results of a previous hydroponic experiment on *S. viminalis* L., where the willow exudated higher amounts of oxalic and/or acetic acids. Moreover, this study complemented the previous research with an analysis of LMWOAs in the roots and leaves, demonstrating their important role in the plant tissues, mainly the fact that oxalic, malonic, and citric acids are listed as molecules that are involved in the transport of metal through the xylem and vascular metal sequestration [178, 179]. The determined acids were present at a considerably higher content in willow organs. Evidence of the significance of the abovementioned participation can be observed in the healthy growth (with no symptoms of necrosis) and high efficiency of Cu phytoextraction in the roots and leaves of all the studied *Salix* taxa.

Currently, information in literature about trees and the role of LMWOAs exuded into the rhizosphere and created in the tissue under metal stress fragmentary. Nevertheless, the published literature indicates that dendroremediation may have great potential for the remediation of soils contaminated by metals. Based on the available literature data, it seems clear that metal toxicity increases the concentrations of LMWOAs and the rate of metal ion uptake, thereby altering the metal transfer factor and subcellular distribution. The significant correlations that occur between metal concentration and the concentrations of LMWOAs in the rhizosphere, roots, and leaves of the studied trees species after metal treatment may indicate that LMWOAs could play a role in maintaining metal homeostasis to resist metal stress. However, these studies require further research, and it may be necessary to implement dendroremediation successfully at a particularly mixed contaminated site.

## 12.5 Conclusion

The following conclusions, tips, and suggestions should be very helpful in further studies on efficient environmental phytoremediation:

- A criterion for application of phytoextraction techniques is the presence of soluble and exchangeable fractions of trace elements, i.e., their bioavailability.
- The effectiveness of phytoextraction of trace elements by trees depends on soil properties affecting ion mobility and bioavailability.
- Soil granulation has an impact on the physical and chemical properties of soil.
- Organic matter is an important component of soil.
- Sorption and desorption reactions are the dominant processes controlling the bioavailability of trace elements in soil.
- Soil water content salinity and pH influence the bioavailability of trace metal uptake by plants.
- Soil biological indicators, e.g., enzymatic activity and microbial communities, can directly influence the availability and mobility of trace elements.
- The efficiency of trace element phytoextraction differs for particular tree species.
- The defense mechanism and tolerance to metal stress are important indicators for plants in their application to the phytoremediation process.
- The exudation of some phenolic compounds is important in metal tolerance and in phytoremediation.
- LMWOAs (presence and exudation) in the rhizosphere, roots, and leaves indicate that these compounds are important in homeostasis and plant resistance to metal stress.

## References

1. Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004) Role of assisted natural remediation in environmental cleanup. Geoderma 122:121–142
2. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals – concepts and applications. Chemosphere 91:869–881
3. Baldantoni D, Cicatelli A, Bellino A, Castiglione S (2014) Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. J Environ Manag 146:94–99
4. Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Hussain S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721
5. Ernst WHO (2006) Evolution of metal tolerance in higher plants. For Snow Landsc Res 80:251–274
6. Kabata-Pendias A (2001) Trace elements in soils and plants, 3rd edn. CRC Press, London
7. Bothe H, Słomka A (2017) Divergent biology of facultative heavy metals plants. J Plant Physiol 219:45–61

8. Ahmad W, Najeeb U, Zia MH (2015) Soil contamination with metals: sources, types and implications. In: Soil remediation and plants. Elsevier, London, pp 37–61
9. Alvarez A, Catalano SA, Amoroso MJ (2013) Heavy metal resistant strains are widespread along Streptomyces phylogeny. Mol Phylogenet Evol 66:1083–1088
10. Barančíková G, Makovníková J (2003) The influence of humic acid quality on the sorption and mobility of heavy metals. Plant Soil Environ 49:565–571
11. Pourret O, Davranche M, Gruau G, Dia A (2007) Rare earth elements complexation with humic acid. Chem Geol 243:128–141
12. Kabata-Pendias A (2004) Soil-plant transfer of trace elements – an environmental issue. Geoderma 122:143–149
13. Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res 2:1–25
14. Xiao L, Guan D, Peart MR, Chen Y, Li Q, Dai J (2017) The influence of bioavailable heavy metals and microbial parameters of soil on the metal accumulation in rice grain. Chemosphere 185:868–878
15. Xu Y, Liang X, Xu Y, Qin X, Huang Q, Wang L, Sun Y (2017) Remediation of heavy metal-polluted agricultural soils using clay minerals: a review. Pedosphere 27:193–204
16. Sheoran V, Sheoran AS, Poonia P (2016) Factors affecting phytoextraction: a review. Pedosphere 26:148–166
17. Jović M, Šljivić-Ivanović M, Dimović S, Marković J, Smičiklas I (2017) Sorption and mobility of Co(II) in relation to soil properties. Geoderma 297:38–47
18. Kushwaha A, Hans N, Kumar S, Rani R (2018) A critical review on speciation, mobilization and toxicity of lead in soil microbe-plant system and bioremediation strategies. Ecotox Environ Safe. 147:1035–1045
19. Wyszkowska J, Borowik A, Kucharski M, Kucharski J (2003) Effect of cadmium, copper and zinc on plants, soils microorganisms and soil enzymes. J Elem 18:769–796
20. Gerhardt KE, Gerwing PD, Greenberg BM (2017) Opinion: taking phytoremediation from proven technology to accepted practice. Plant Sci 256:170–185
21. Skiba S (2002) Gleba w środowisku przyrodniczym [Soil in the natural environment]. In: Barabasz W (ed) Aktywność drobnoustrojów w różnych środowiskach [Microbial activity in various environments]. Wydawnictwo AR w Krakowie, Drukrol, pp 157–170 (in Polish)
22. Antoniadis V, Levizou E, Shaheen SM, Ok YS, Sebastian A, Baum C et al (2017) Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation – a review. Earth-Sci Rev 171:621–645
23. Fijałkowski K, Kacprzak M, Grobelak A, Placek A (2012) The influence of selected soil parameters on the mobility of heavy metals in soils. Inżynieria i Ochrona Środowiska 15:81–92
24. Bartkowiak A, Lemanowicz J, Hulisz P (2017) Ecological risk assessment of heavy metals in salt-affected soils in the Natura 2000 area (Ciechocinek, north-central Poland). Environ Sci Pollut Res 24:27175–27187
25. Li H, Ji H, Shi C, Gao Y, Zhang Y, Xu X, Ding H, Tang L, Xing Y (2017) Distribution of heavy metals and metalloids in bulk and particle size fractions of soils from coal-mine brownfield and implications on human health. Chemosphere 172:505–515
26. Acosta JA, Jansen B, Kalbitz K, Faz A, Martinez-Martinez S (2011) Salinity increases mobility of heavy metals in soils. Chemosphere 85:1318–1324
27. Dziamski A, Gonet SS, Debska B (2005) Properties of humus in reaction to soil particle size fraction. Scientific papers, vol 692. University of Lativia, Latvia, pp 29–37
28. Dexter AR (2004) Soil physical quality part I. Theory, effects of soil texture, density and organic matter, and effects on root growth. Geoderma 120:201–214
29. Dexter AR, Richard G, Czyż EA, Jolivet C, Duval O (2008) Complexed organic matter controls soil physical properties. Geoderma 144:620–627

30. Ilek A, Kucza J, Szostek M (2017) The effect of the bulk density and the decomposition index of organic matter on the water storage capacity of the surface layers of forest soil. Geoderma 285:27–34
31. Hajduk E, Kaniuczak J (2014) Microelements in soils and in leaves of selected tree species in an industrial urban area. J Elem 19:1001–1020
32. Coles CA, Yong RN (2006) Humic acid preparation, properties and interactions with metals lead and cadmium. Eng Geology 85:26–32
33. Gucwa-Przepióra E, Nadgórska-Socha A, Fojcik B, Chmura D (2016) Enzymatic activities and arbuscular mycorrhizal colonization of *Plantago lanceolata* and *Plantago major* in a soil root zone under heavy metal stress. Environ Sci Pollut Res 23:4742–4755
34. Antoniadis V, Golia EE (2015) Sorption of Cu and Zn in low organic matter-soils as influenced by soil properties and by the degree of soil weathering. Chemosphere 138:364–369
35. Kantek K, Karczewska A (2012) Effect of sewage sludge on the solubility of arsenic in soils contaminated by the arsenic industry in Złoty Stok. Zesz Nauk UP Wroc, Rol CIII 589:135–146
36. Karczewska A, Galka B, Dradrach A, Lewińska K, Molczan M, Cuske M, Gersztyn L, Litak K (2017) Solubility of arsenic and its uptake by ryegrass from polluted soils amended with organic matter. J Geochem Explor 182:193–200
37. Kwiatkowska-Malina J, Maciejewska A (2011) The uptake of heavy metals by plants at differentiated soil reaction and content of organic matter. Ochrona Środowiska i Zasobów Naturalnych 49:43–51
38. Alverez-Puebla RA, Valezuela-Calahorro C, Garrido JJ (2004) Cu(II) retention on a humic substance. J Colloid Interf Sci 270:47–55
39. Kononowa M (1968) Soil organic substances, their structure, properties and test methods. Państwowe Wydawnictwo Rolnicze i Leśne (in Polish)
40. Wang S, Mulligan CN (2009) Enhanced mobilization of arsenic and heavy metals from mine tailings by humic acid. Chemosphere 74:274–279
41. Özkaraova Güngör EB, Bekbölet M (2010) Zinc release by humic and fulvic acid as influenced by pH, complexation and DOC sorption. Geoderma 159:131–138
42. Gonet SS, Dębska B, Pakuła J (2002) The content of dissolved organic carbon in soils and organic fertilizers. Polish Humic Substances Society, Wrocław (in Polish)
43. Cuske M, Karczewska A (2016) Influence of organic matter on the solubility of heavy metals in contaminated soils – a review of literature. Inżynieria Środowiska 42:39–59 (in Polish)
44. Suda A, Makino T (2016) Functional effects of manganese and iron oxides on the dynamics of trace elements in soils with a special focus on arsenic and cadmium: a review. Geoderma 270:68–75
45. Caporale AG, Violante A (2016) Chemical processes affecting the mobility of heavy metals and metalloids in soil environments. Curr Pollut Rep 2:15–27
46. Walczak R, Ostrowski J (2004) Parameterization and spatial characteristics of hydrophysical properties of mineral soil in Poland. Water Environ Rural Areas 4:175–184 (in Polish)
47. Nowińska K, Adamczyk Z (2013) Mobilność pierwiastków towarzyszących odpadom hutnictwa cynku i ołowiu w środowisku. [The mobility of accompanying elements to wastes from metallurgy of the zinc and the leadon in the environment]. Górnictwo i Geologia 8:77–87 (in Polish)
48. Angle JS, Baker AJM, Whiting SN, Chaney RL (2003) Soil moisture effects on uptake of metals by *Thlaspi Alyssum*, and *Berkheya*. Plant Soil 256:325–332
49. Gronflaten LK, Steinnes E (2005) Comparison of four extraction methods to assess plant availability of some metals in organic forest soils. Commun Soil Sci Plan 36:2699–2718
50. Zeng F, Ali S, Zhang H, Ouyang Y, Qiu B, Wu F, Zhang G (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159:84–91

51. Karczewska A (2002) Metale ciężkie w glebach zanieczyszczonych emisjami hut miedzi – formy i rozpuszczalność[dissertation] [Heavy metals in soils polluted by emissions from copper smelters – forms and solubility]. Agriculture University in Wroclaw (in Polish)
52. Kaniuczak J, Hajduk E, Właśniewski S (2011) Effect of liming and mineral fertilization on cadmium content in grain of spring barley (*Hordeum vulgare* L.) and winter wheat (*Triticum aestivum* L.) cultivated on loessial soil. J Elem 16:535–542
53. Krysiak A, Karczewska A (2011) Effects of soil flooding on arsenic mobility in soils in the area of former gold and arsenic mining in Złoty Stok. Roczniki Gleboznawcze 62:240–248
54. Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
55. Siddikee MA, Tipayno SC, Kim K, Chung J, Sa T (2011) Influence of varying degree of salinity–sodicity stress on enzyme activities and bacterial populations of coastal soils of Yellow Sea, South Korea. J Microbiol Biotechnol 21(4):341–346
56. Telesiński A (2012) The effect of salinity on some biochemical indices of soil fertility. Water Environ Rural Areas 12:209–217
57. Nouri H, Chavoshi Borujeni S, Nirola R, Hassanli A, Beecham S, Alaghmand S, Saint C, Mulcahy D (2017) Application of green remediation on soil salinity treatment: a review on halophytoremediation. Process Safe Environ Prot 107:94–107
58. Filipović L, Romić M, Romić D, Filipović V, Ondrašek G (2018) Organic matter and salinity modify cadmium soil (phyto)availability. Ecotox Environ Safe 147:824–831
59. Manousaki E, Kadukova J, Papadantonakis N, Kalogerakis N (2008) Phytoextraction and phytoexcretion of Cd by the leaves of *Tamarix smyrnensis* growing on contaminated non-saline and saline soils. Environ Res 106:326–332
60. Mohammadian E, Ahari AB, Arzanlou M, Oustan S, Khazaei SH (2017) Tolerance to trace elements in filamentous fungi isolated from contaminated mining soils in the Zanjan Province, Iran. Chemosphere 185:290–296
61. Kucharski J, Wieczorek K, Wyszkowska J (2011) Changes in the enzymatic activity in sandy loam soil exposed to zinc pressure. J Elementol 16:577–589
62. Lenart-Boroń A, Boroń T, Banach T (2013) The effect of the selected heavy metals on the growth and proliferation of *Streptomyces* spp. Isolated from soils. Inżynieria i Ochrona Środowiska 16:81–91
63. Li M, Cheng X, Guo H (2013) Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int Biodeter Biodegr 76:81–85
64. Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize?J Hazard Mater 266:141–166
65. Jing Y, Cui H, Li T, Zhao Z (2014) Heavy metal accumulation characteristics of Nepalese alder (*Alnus nepalensis*) growing in a lead-zinc spoil heap, Yunnan, Southwestern China. IForest 7:204–208
66. Figurska-Ciura D, Łoźna K, Styczyńska M (2007) Cadmium, lead, zinc and copper contents in selected vegetables and fruit from garden allotments of the South – Western Poland, vol 57, pp 137–143
67. Interstate Technology and Regulatory Cooperation Work Group (ITRC) (1999) Phytoremediation decision tree. www.itrcweb.org
68. Alkorta I, Hernández-Allica J, Becerril JM, Amezaga I, Albizu IGC (2004) Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev Lit Arts Am 3:71–90
69. Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138
70. Pajević S, Borišev M, Nikolić N, Arsenov DD, Orlović S, Župunski M (2016) Phytoextraction of heavy metals by fast—growing trees: a review. In: Ansari AA, Gill SS, Gill R, Lanza G, Newman L (eds) Phytoremediation. Management of environmental contaminants. Springer, Cham, pp 29–64

71. Bednarek W, Tkaczyk P, Dresler S (2007) Contents of heavy metals as a criterion for apple quality assessment and soil properties. Polish J Soil Sci 40:47–56
72. Tošić S, Alagić S, Dimitrijević M, Pavlović A, Nujkić M (2016) Plant parts of the apple tree (Malus spp.) as possible indicators of heavy metal pollution. Ambio 45:501–512
73. Bošković-Rakočević L, Milivojević J, Milošević T, Paunović G (2014) Heavy metal content of soils and plum orchards in an uncontaminated area. Water Air Soil Pollut 225:2199
74. Chrabąszcz M, Mróz L (2017) Tree Bark, a valuable source of information on air quality. Polish J Environ Stud 26:453–466
75. Parzych A, Jonczak J (2014) Pine needles (*Pinus sylvestris* L.) as bioindicators in the assessment of urban environmental contamination with heavy metals. J Ecol Eng 15:29–38
76. Yoon J, Cao XD, Zhou QX, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ 368:456–464
77. Pinto AP, de Varennes A, Lopes ME, Teixeira DM (2016) Biological approaches for remediation of metal-contaminated sites. In: Ansari AA, Gill SS, Gill R, Lanza G, Newman L (eds) Phytoremediation. Management of environmental contaminants. Springer, Cham, pp 65–112
78. Parzych A, Jonczak J (2013) Content of heavy metals in needles of scots pine (*Pinus sylvestris* L.) in selected pine forests in Słowiński National Park. Arch Environ Prot 39:41–51
79. Mleczek M, Rissmann I, Rutkowski P, Kaczmarek Z, Golinski P (2009) Accumulation of selected heavy metals by different genotypes of *Salix*. Environ Exp Bot 66(2):289–296
80. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees – a review. Environ Int 29:529–540
81. Mleczek M, Goliński P, Krzesłowska M, Gąsecka M, Magdziak Z, Rutkowski P, Budzyńska S, Waliszewska B, Kozubik T, Karolewski Z, Niedzielski P (2017) Phytoextraction of potentially toxic elements by six tree species growing on hazardous mining sludge. Environ Sci Pollut Res 24:22183–22195
82. Budzyńska S, Mleczek M, Goliński P, Rutkowski P, Niedzielski P (2017) The influence of As forms in substrate on the phytoextraction of this metalloid in *Ulmus laevis* Pall organs – pot experiment. Microchem J 132:333–340
83. Majid NM, Islam MM, Mathew L (2012) Heavy metal uptake and translocation by mangium (*Acacia mangium*) from sewage sludge contaminated soil. Aust J Crop Sci 6:1228–1235
84. Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362:319–334
85. Van Nevel L, Martens J, Staelens J, De Schrijver A, Tack FMG, De Neve S, Meers E, Verheyen K (2011) Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. Ecol Eng 37:1072–1080
86. Hu Y, Nan Z, Su J, Wang N (2013) Heavy metal accumulation by poplar in calcareous soil with various degrees of multi-metal contamination: implications for phytoextraction and phytostabilization. Environ Sci Pollut Res Int 20:7194–7203
87. Evangelou MWH, Robinson BH, Günthardt-Goerg MS, Schulin R (2013) Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. Int J Phytoremediation 15:77–90
88. Sylvain B, Mikael MH, Floride M, Emmanuel J, Marilyne S, Sylvain B, Domenico M (2016) Phytostabilization of As, Sb and Pb by two willow species (*S. viminalis* and *S. purpurea*) on former mine technosols. Catena 136:44–52
89. Mertens J, Vervaeke P, Schrijver AD, Luyssaert S (2004) Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilization. Sci Total Environ 326:209–215
90. Majid NM, Islam MM, Justin V, Abdu A, Ahmadpour P (2011) Evaluation of heavy metal uptake and translocation by *Acacia mangium* as a phytoremediator of copper contaminated soil. Afr J Biotechnol 10:8373–8379
91. Qados AMSA (2015) Phytoremediation of Pb and Cd by native tree species grown in the Kingdom of Saudi Arabia. Int Res J Agri Sci Soil Sci 6:8–21

92. Shi X, Chen YT, Wang SF, Pan HW, Sun HJ, Liu CX et al (2016) Phytoremediation potential of transplanted bare-root seedlings of trees for lead/zinc and copper mine tailings. Int J Phytoremediation 18:1155–1163
93. Rosselli W, Keller C, Boshi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272
94. Wang X, Jia Y (2010) Study on adsorption and remediation of heavy metals by poplar and larch in contaminated soil. Environ Sci Pollut Res 17:1331–1338
95. Čudić V, Stojiljković D, Jovović A (2016) Phytoremediation potential of wild plants growing on soil contaminated with heavy metals. Arch Hig Rada Toxicol 67:229–239
96. Erakhrumen AA (2015) Assessment of in-situ natural dendroremediation capability of *Rhizophora racemosa* in a heavy metal polluted mangrove forest, River State, Nigeria. J Appl Sci Environ Manage 19:21–27
97. Yu XZ, Gu JD, Xing LQ (2008) Differences in uptake and translocation of hexavalent and trivalent chromium by two species of willows. Ecotoxicology 17:747–755
98. Alcantara-Martinez N, Guizar S, Rivera-Cabrera F, Anicacio-Acevedo BE, Buendia-Gonzalez F, Volke-Sepulveda T (2016) Tolerance, arsenic uptake, and oxidative stress in *Acacia farnesiana* under arsenate-stress. Int J Phytoremediation 18:671–678
99. Anawar HM, Damon P, Rengel Z, Jasper D, Tibbett M (2016) Alleviating arsenic toxicity to plants in a simulated cover system with phosphate placement in topsoil and subsoil. In: Fourie AB, Tibbett M (eds) Proceedings of the 11th international conference on mine closure; 2016. Australian Centre for Geomechanics, Crawley, pp 555–565
100. Kirkey FM, Matthews J, Ryser P (2012) Metal resistance in populations of red maple (*Acer rubrum* L.) and white birch (*Betula papyrifera* Marsh.) from a metal-contaminated region and neighbouring non-contaminated regions. Environ Pollut 164:53–58
101. Dmuchowski W, Gozdowski D, Brągoszewska P, Baczewska AH, Suwara I (2014) Phytoremediation of zinc contaminated soil using silver birch (*Betula pendula* Roth). Ecol Eng 71:32–35
102. Bergqvist C, Greger M (2012) Arsenic accumulation and speciation in plants from different habitats. Appl Geochem 27:615–622
103. Hassinen V, Vallinkoski VM, Issakainen S, Tervahauta A, Kärenlampi S, Servomaa K (2009) Correlation of foliar MT2b expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula x tremuloides*) grown in contaminated soil. Environ Pollut 157:922–930
104. Shi X, Wang S, Sun H, Chen Y, Wang D, Pan H et al (2017) Comparative of *Quercus* spp. and *Salix* spp. for phytoremediation of Pb/Zn mine tailings. Environ Sci Pollut Res 24:3400–3411
105. Venturas M, Fernández V, Nadal P, Guzmán P, Lucena JJ, Gil L (2014) Root iron uptake efficiency of *Ulmus laevis* and *U. minor* and their distribution in soils of the Iberian Peninsula. Front Plant Sci 5:104
106. Gomes MP, Duarte DM, Miranda PL, Barreto LC, Matheus MT, Garcia QS (2012) The effects of arsenic on the growth and nutritional status of *Anadenanthera peregrina*, a Brazilian savanna tree. J Plant Nutr Soil Sci 175:466–473
107. Makgalaka-Matlala NS, Flores-Tavizón E, Castillo-Michel H, Peralta-Videa JR, Torresdey JD (2008) Toxicity of arsenic (III) and (V) on plant growth, element uptake, and total amylolytic activity of mesquite (*Prosopis Juliflora x P. Velutina*). Int J Phytoremediation 10:47–60
108. Erakhrumen AA (2014) Potentials of *Rhizophora racemosa* for bio-indication and dendroremediation of heavy metal contamination in a mangrove forest, Ondo State. Nig J Agric Food Environ 10:1–5
109. Ang LH, Tang LK, Ho WM, Hui TF, Theseira GW (2010) Phytoremediation of Cd and Pb by four tropical timber species grown on an ex-tin min in Peninsular Malaysia. Int J Environ Ecol Eng 4:70–74
110. Zacchini M, Pietrini F, Bianconi D, Iori V, Congiu M, Mughini G et al (2011) Physiological and biochemical characterisation of Eucalyptus hybrid clones treated with cadmium in hydroponics: perspectives for the phytoremediation of polluted waters. In:Book of Abstracts 5th European Bioremediation Conference. Technical University of Creta, Chania

111. Yanqun Z, Yuan L, Schvartz C, Langlade L, Fan L (2004) Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead-zinc mine area, China. Environ Int 30:567–576
112. Larios R, Fernández-Martínez R, LeHecho I, Rucandio I (2012) A methodological approach to evaluate arsenic speciation and bioaccumulation in different plant species from two highly polluted mining areas. Sci Total Environ 414:600–607
113. Cheng Z, Buckley BM, Katz B, Wright W, Bailey R, Smith KT et al (2007) Arsenic uptake by trees at highly contaminated site. Sci Total Environ 376:324–334
114. Madejón P, Lepp NW (2007) Arsenic in oils and plants of woodland regenerated on an arsenic-contaminated substrate: a sustainable natural remediation? Sci Total Environ 379:256–262
115. Shin MN, Shim J, You Y, Myung H, Bang KS, Cho M et al (2012) Characterization of lead resistant endophytic *Bacillus sp*. MN3-4 and its potential for promoting lead accumulation in metal hyperaccumulator *Alnus firma*. J Hazard Mater 199-200:314–320
116. Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack FMG, Lust N (2003) Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ Pollut 126:257–282
117. Goliński P, Mleczek M, Magdziak Z, Gąsecka M, Borowiak K, Dąbrowski J et al (2015) Efficiency of Zn phytoextraction, biomass yield and formation of low-molecular-weight organic acids in *S* × *rubens* – a hydroponic experiment. Chem Ecol 31:345–364
118. Zhao X, Liu J, Xia X, Chu J, Wei Y, Shi S et al (2014) The evaluation of heavy metal accumulation and application of a comprehensive bio-concentration index for woody species on contaminated sites in Hunan, China. Environ Sci Pollut Res 21:5076–5085
119. Delplanque M, Collet S, Del Gratta F, Schnuriger B, Gaucher R, Robinson B et al (2013) Combustion of *Salix* used for phytoextraction: the fate of metals and viability of the processes. Biomass Bioenergy 49:160–170
120. 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
121. Schoenmuth BW, Pestemer W (2004a) Dendroremediation of trinitrotoluene (TNT). Part 1: literature overview and research concept. Environ Sci Pollut Res 11:273–278
122. Schoenmuth BW, Pestemer W (2004b) Dendroremediation of trinitrotoluene (TNT). Part 2: fate of radio-labelled TNT in trees. Environ Sci Pollut Res 11:331–339
123. Ali B, Mwamba TM, Gill GRA, Yang C, Ali S et al (2014) Improvement of element uptake and antioxidative defense in *Brassica napus* under lead stress by application of hydrogen sulfide. Plant Growth Regul 74:261–273
124. He J, Ma C, Hong YM, Kang LJ, Liu T, Polle A et al (2013) Cadmium tolerance in six poplar species. Environ Sci Pollut Res 20:10163–10174
125. Zafari S, Sharifi M, Chashmi NA, Mur LAJ (2016) Modulation of Pb-induced stress in Prosopis shoots through an interconnected network of signaling molecules, phenolic compounds and amino acids. Plant Physiol Biochem 99:11–20
126. Diáz J, Bernal A, Pomar F, Merino F (2001) Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annuum* L.) seedlings in response to copper stress and its relation to lignification. Plant Sci 161:179–188
127. Mittova V, Volokita M, Guy M, Tal M (2000) Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol Plant 10:42–51
128. Loponen J, Ossipov V, Lempa K, Haukioja E, Pihlaja K (1998) Concentrations and among-compound correlations of individual phenolics in white birch leaves under air pollution stress. Chemosphere 37:1445–1456
129. Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL et al (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in scots pine roots. Plant Physiol 127:887–898
130. Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: Location and functional significance. Plant Sci 196:67–76

131. Posmyk MM, Kontek K, Janas KM (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicol Environ Safe 72:596–602
132. Sakihama Y, Cohen MF, Grace SC, Yamasaki H (2002) Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177(1):67–80
133. Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35:985
134. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13:572–584
135. Kováčik J, Klejdus B, Hedbavny J, Bačkora M (2010) Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). Plant Sci 178:307–311
136. Poschenrieder C, Tolrà R, Barceló J (2006) Can metals defend plants against biotic stress? Trends Plant Sci 11:288–295
137. Márquez-García B, Fernández MA, Córdoba F (2009) Phenolics composition in *Erica* sp. differentially exposed to metal pollution in the Iberian Southwestern Pyritic Belt. Bioresour Technol 100:446–451
138. Pradas del Real AE, Silvan JM, del Pascual-Teresa S, Guerrero A, García-Gonzalo P, Lobo MC et al (2017) Role of the polycarboxylic compounds in the response of *Silene vulgaris*. Environ Sci Pollut Res 24:5746–5756
139. Kasthuri J, Rajendiran N (2009) Functionalization of silver and gold nanoparticles using amino acid conjugated bile salts with tunable longitudinal plasmon resonance. Colloid Surface B 73:387–393
140. Mikhaeil BR, Badria F, Maatooq G, Amer M (2004) Antioxidant and immunomodulatory constituents of henna leaves. Z Naturforsch C 59:468–476
141. Drzewiecka K, Mleczek M, Gąsecka M, Magdziak Z, Budka A, Chadzinikolau T, Kaczmarek Z, Goliński P (2017) Copper and nickel co-treatment alters metal uptake and stress parameters of *Salix purpurea*×*viminalis*. Plant Physiol 216:125–134
142. Gąsecka M, Mleczek M, Jutrzenka A, Goliński P, Stuper-Szablewska K (2017) Phenolic compounds in leaves of *Salix* species and hybrids growing under different soil conditions. Chem Ecol 33:196–212
143. Drzewiecka K, Mleczek M, Gąsecka M, Magdziak Z, Goliński P (2012) Changes in *Salix viminalis* L. cv. 'Cannabina' morphology and physiology in response to nickel ions – hydroponic investigations. J Hazard Mater 217-218:429–438
144. Pradas del Real AE, Pérez-Sanz A, Lobo MC, McNear DH Jr (2014) The chromium detoxification pathway in the multimetal accumulator *Silene vulgaris*. Environ Sci Technol 48:11479–11486
145. Jiang S, Weng B, Liu T, Su Y, Liu J, Lu H et al (2017a) Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac). Ecotoxicol Environ Safe 141:290–297
146. Kováčik J, Klejdus B (2008) Dynamics of phenolic acids and lignin accumulation in metal-treated *Matricaria chamomilla* roots. Plant Cell Rep 27:605–615
147. Lavid N, Schwartz A, Yarden O, Tel-Or E (2001) The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (*Nymphaeaceae*). Planta 212:323–331
148. Wang J, Evangelou BP, Nielsen MT, Wagner GJ (1992) Computer, simulated evaluation of possible mechanisms for sequestering metal ion activity in plant vacuoles: II. Zinc. Plant Physiol 99:621–626
149. Usha B, Venkataraman G, Parida A (2009) Heavy metal and abiotic stress inducible metallothionein isoforms from *Prosopis juliflora* (SW) D.C. show differences in binding to heavy metals in vitro. Mol Gen Genomics 281:99–108

150. Drzewiecka K, Gąsecka M, Rutkowski P, Magdziak Z, Goliński P, Mleczek M (2018) Arsenic forms and their combinations induce differences in phenolic accumulation in *Ulmus laevis* Pall. J Plant Physiol 220:34–42
151. Loponen J, Lempa K, Ossipov V, Kozlov MV, Girs A, Hangasma K et al (2001) Patterns in content of phenolic compounds in leaves of mountain birches along a strong pollution gradient. Chemosphere 45:291–301
152. Borowiak K, Gąsecka M, Mleczek M, Dąbrowski J, Chadzinikolau T, Magdziak Z et al (2015) Photosynthetic activity in relation to chlorophylls, carbohydrates, phenolics and growth of a hybrid *Salix purpurea* × *triandra* × *viminalis* 2 at various Zn concentrations. Acta Physiol Plant 37:155
153. Drzewiecka K, Mleczek M, Gąsecka M, Magdziak Z, Goliński P, Chadzinikolau T (2014) Copper phytoextraction with *Salix purpurea* × *viminalis* under various Ca/Mg ratios. Part 2. Effect on organic acid, phenolics and salicylic acid contents. Acta Physiol Plant 36:903–913
154. Jia X, Zhao Y, Liu T, Huang S (2016) Elevated $CO_2$ affects secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soils. Chemosphere 160:199–207
155. Zhao YH, Jia X, Wang WK, Liu T, Huang SP, Yang MY (2016) Growth under elevated air temperature alters secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soils. Sci Total Environ 565:586–594
156. Adeleke R, Nwangburuka C, Oboirien B (2017) Origins, roles and fate of organic acids in soils: a review. S Afr J Bot 108:393–406
157. Jiang S, Xie F, Lu H, Liu J, Yan C (2017) Response of low-molecular-weight organic acids in mangrove root exudates to exposure of polycyclic aromatic hydrocarbons. Environ Sci Pollut Res 24:12484–12493
158. Martin BC, George SJ, Price CA, Shahsavari E, Ball AS, Tibbett M et al (2016) Citrate and malonate increase microbial activity and alter microbial community composition in uncontaminated and diesel-contaminated soil microcosms. Soil 2:487–498
159. Nardi S, Concheri G, Pizzeghello D, Sturaro A, Rella R, Parvoli G (2000) Soil organic matter mobilization by root exudates. Chemosphere 41:653–658
160. Xie X, Weiss DJ, Weng B, Liu J, Lu H, Yan C (2013) The short-term effect of cadmium on low molecular weight organic acid and amino acid exudation from mangrove (*Kandelia obovata* (S., L.) Yong) roots. Environ Sci Pollut Res 20:997–1008
161. Xiao M, Wu F (2014) A review of environmental characteristics and effects of low-molecular weight organic acids in the surface ecosystem. J Environ Sci 26:935–954
162. Magdziak Z, Mleczek M, Rutkowski P, Goliński P (2017) Diversity of low-molecular weight organic acids synthesized by *Salix* growing in soils characterized by different Cu, Pb and Zn concentrations. Acta Physiol Plant 39:137
163. Wang Y, Fang L, Lin L, Luan T, Tam NFY (2014) Effects of low molecular-weight organic acids and dehydrogenase activity in rhizosphere sediments of mangrove plants on phytoremediation of polycyclic aromatic hydrocarbons. Chemosphere 99:152–159
164. Wang S, ChQ F, Wang P (2015) Determination of ultra-trace organic acids in Masson pine (*Pinus massoniana* L.) by accelerated solvent extraction and liquid chromatography–tandem mass spectrometry. J Chromatogr B 981-982:1–8
165. Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56
166. Haoliang L, Chongling Y, Jingchun L (2007) Low-molecular-weight organic acids exuded by Mangrove (Kandelia *candel* (L.) Druce) roots and their effect on cadmium species change in the rhizosphere. Environ Exp Bot 61:159–166
167. Kersten GJ (2015) Phytoremediation of metal contamination using *Salix* (willows) [dissertation]. University of Denver
168. Magdziak Z, Kozłowska M, Kaczmarek Z, Mleczek M, Chadzinikolau T, Golinski P, Drzewiecka K (2011) Influence of Ca/Mg ratio on phytoextraction properties of *Salix*

*viminalis*. II. Secretion of low molecular weight organic acids to the rhizosphere. Ecotoxicol Environ Safe 74:33–40

169. Gąsecka M, Mleczek M, Drzewiecka K, Magdziak Z, Rissmann I, Chadzinikolau T et al (2012) Physiological and morphological changes in *Salix viminalis* L. as a result of plant exposure to copper. J Environ Sci Health A 74:33–40
170. Magdziak Z, Mleczek M, Kaczmarek Z, Golinski P (2013) Influence of Ca/Mg ratio and $Cd^{2+}$ and $Pb^{2+}$ elements on low molecular weight organic acid secretion by *Salix viminalis* L. roots into the rhizosphere. Trees 27:663–673
171. Nigam R, Srivastava S, Prakash S, Srivastava M (2001) Cadmium mobilization and plant availability-the impact of organic acids commonly exuded from roots. Plant Soil 230:107–113
172. Dresler S, Hanaka A, Bednarek W, Maksymiec W (2014) Accumulation of low-molecular-weight organic acids in roots and leaf segments of *Zea mays* plants treated with cadmium and copper. Acta Physiol Plant 36:1565–1575
173. Adeniji BA, Budimir-Hussey MT, Macfie SM (2010) Production of organic acids and adsorption of Cd on roots of durum wheat (*Triticum turgidum* L. var. Durum). Acta Physiol Plant 32:1063–1072
174. 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
175. Liao YC, Chang Chien S, Wang M, Shen Y, Seshaiah K (2007) Relationship between lead uptake by lettuce and water-soluble low-molecular-weight organic acids in rhizosphere as influenced by transpiration. J Agric Food Chem 55:8640–8649
176. Meier S, Alvear M, Borie F, Aguilera P, Ginocchio R, Cornejo P (2012) Influence of copper on root exudate patterns in some metallophytes and agricultural plants. Ecotoxicol Environ Safe 75:8–15
177. Muhammad D, Chen F, Zhao J, Zhang G, Wu F (2009) Comparison of EDTA- and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. Int J Phytoremediation 11:558–574
178. Parisová M, Navrátil T, Šestáková I, Dytrtová JJ, Mareček V (2013) Influence of low molecular weight organic acids on transport of cadmium and copper ions across model phospholipid membranes. Int J Electrochem Sci 8:27–44
179. Ueno D, Ma JF, Iwashita T, Zhao FJ, McGrath SP (2005) Identification of the form of Cd in the leaves of a superior Cd-accumulating ecotype of *Thlaspi caerulescens* using 113Cd-NMR. Planta 221:928–936

# Chapter 13
# The Possibility of Use of Oil Seed Plants and Grasses for Phytoremediation

**Saule Atabayeva**

## 13.1 Introduction

The ecological aspect of Kazakhstan's sustainable development presupposes the preservation of the environment and the rational use of natural resources, the conservation of biological diversity, and the solution of the problem of man-made waste. The most important factor affecting the health of the population of the country is the state's steps to prevent diseases by reducing the objects polluting the environment [1]. The term "phytoremediation" means a large number of methods and technologies, in particular phytoextraction, phytoimmobilization, phytostabilization, and phytovolatilization [2, 3]. After application of phytotechnology, soils do not lose their natural properties; therefore, these technologies are soil-preserving, environmentally safe, and economically profitable.

Heavy metals that enter the soil through various ways due to human economic activity are classified as dangerous environmental pollutants. The amount of heavy metals accumulated in this way can exceed many times their natural content in the soil. Dissemination of technogenic pollution of heavy metals in the atmosphere has acquired a global character. The main sources of copper, lead, cadmium, and zinc in the environment are the mining, metallurgical and chemical industries, heat-power engineering, vehicles and chemical pesticides, and household waste.

Pollution of the atmosphere, soil, plants, and water with heavy metals in the vicinity of large industrial centers has become one of the most pressing environmental problems. In soils near industrial enterprises, the content of heavy metals exceeds the

---

S. Atabayeva (✉)
Al-Farabi Kazakh National University, Research Institute of Ecology Problems,
Almaty, Kazakhstan
e-mail: sauleat@yandex.kz

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_13

background content in similar soils by a factor of tens and hundreds of times [4]. The high concentration of a number of heavy metals in the soil adequately reflects the yield and quality of plant products grown within the boundaries of industrial centers in the horticultural areas. In a significant part of plant samples, the content of heavy metals exceeds the allowable concentration by 2–3.5 times [1, 4]. Excessive concentration of heavy metals in plants disrupts the natural course of the physiological and biochemical processes, suppresses the growth and development of the plant organism, and reduces the quality of the products obtained. Thus, the increasing technogenic contamination of the medium of heavy metals, migrating through the trophic bonds, leads to various unfavorable consequences in living organisms.

It is known from the literature that heavy metals adversely affect the physiological and biochemical processes of plants: change the properties of membranes [5, 6], the activity of enzymes, cause oxidative stress [7, 8, 9, 10]. The consequences of this effect are an inhibition of growth processes, delay in the onset of phenological phases, decrease of yield. In response to the negative effect of heavy metals on plants, a number of protective mechanisms are activated, such as an increase in the synthesis of metallothioneins (phytochelatins), organic acids, polyamines and antioxidant enzyme activity [11, 12, 13], aimed at reducing the toxic effect of heavy metals and maintaining homeostasis. But in different types of plants, protective mechanisms are developed to varying degrees. Different species, and even populations within a single species, can differ in their sensitivity to heavy metals and in the degree of accumulation in their organs, which may be the basis for the formation of a metallophyte flora [14]. The use of such qualities of plants as resistance to heavy metals and high metal-accumulating activity formed the basis for the technology of phytoremediation of contaminated soils, which is defined as the technology of cleaning the environment from chemical pollutants with the help of plants [15, 16].

One of the necessary steps to prevent the toxic effect of heavy metals on animals and humans is soil purification. The most effective way is at present phytoremediation of soils, i.e., cleaning of soils with the help of plant hyperaccumulators of heavy metals. Compared with physical and chemical methods, this method is less expensive, effective, and safe [3, 13]. According to some estimates, depending on the soil conditions and metal concentration, the cost of cleaning with plants (using only solar energy) can be only 5% of the costs required for other methods of restoring ecosystems contaminated with metals [17].

## 13.2 Heavy Metal Toxicity and Ways to Prevent It by Using Phytoremediation Technology

The term “heavy metals,” which characterizes a wide group of pollutants, has recently gained considerable popularity. As the membership criteria, numerous characteristics are used: atomic mass, density, toxicity, prevalence in the natural environment, and degree of involvement in natural and man-made cycles. Heavy metals belong to the elements of the periodic system Mendeleev with an atomic mass of more than 50 atomic units and a density of >7 g/cm$^3$ [18].

An important role in the definition of the term “heavy metals” is played by the following conditions: their high toxicity for living organisms in relatively low concentrations, as well as the ability to bioaccumulate [19]. Heavy metals belong to pollutants having anthropogenic origin, which are characterized by high toxic, mutagenic and carcinogenic effects. An important feature of heavy metals is that they belong to the class of nonspecific substances that are present in the “biosphere” in contrast to specific pollutants, like pesticides. Another difference between heavy metals and other pollutants is that in principle, the concept of selfpurification is not applicable to heavy metals. As a result of all the processes of migration and scattering, an irreversible increase in concentration in water, soil, air, and food takes place. There is a pollution of natural environments and biota. First of all, those metals that pollute the atmosphere to the greatest extent because of their use in significant volumes in production activity and as a result of accumulation in the external environment are of great danger from the point of view of their biological activity, and toxic properties are of interest. The most common metals that pollute the territory around metallurgical plants are zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd).

Almost all the metals falling under this definition (with the exception of lead, mercury, cadmium, and bismuth, a biological role, which are not currently detected) are actively involved in biological processes and are part of many enzymes [20].

Since many trace elements are heavy metals, soil contamination by them is essentially the accumulation of a large number of essential trace elements (Zn, Mn, Cu, Ni) or metals that can act as their counterparts (Cd, Pb, Hg). The biophilicity and toxicity of chemical elements are two sides of one phenomenon: the more amount of element is required for a living substance, the less toxic it is. It follows that trace elements are strong toxicants [14]. Hence, it follows that trace elements are strong toxicants.

Heavy metals, like Cu and Zn, are essential elements for growth of the body, as they are part of many enzymes and other proteins. Cu is the key component that provides the functioning of a number of enzymes, such as cytochrome oxidase, ascorbate oxidase, and a number of nonenzymatic proteins. It is included in the formation of the plastocyanin—a component of the electron transport chain of photosynthesis. It plays an important role in the life of organisms: it strengthens oxidative processes and promotes the formation of chlorophyll [20–22].

Zinc (Zn) is essential for the growth and normal development of most organisms. It is an important component of protoplasm, because it is associated with enzymes, regulators of cellular metabolism. Zinc participates in the synthesis of chlorophyll, prevents it from decay, affects nitrogen assimilation by plants, activates enzymes of carbohydrate and energy metabolism, and participates in the construction of a number of enzymes (some phosphatases) [20].

At high concentrations of these microelements, they have a toxic effect on plants. Excess amounts of them lead to symptoms of toxicity and suppression of plant growth, as a result of their binding to sulfhydryl groups of proteins, which leads to inhibition of activity and destruction of their structure [23–27].

Cadmium (Cd) and lead (Pb) are among the most common environmental pollutants. Cadmium is a heavy metal, usually present in soil in trace amounts. Nevertheless, human industrial activities and agricultural practices increase the level of cadmium in the soil. Everywhere used fertilizers and pesticides can contain large amounts of this metal, which for a long time enters the soil along with fertilizers [28]. Most of the Cd, contained in soil, is available for plants, since the soluble fraction reaches up to 35% of the total amount of urea [29]. Cadmium is characterized by high toxicity, possessing high mobility. There is also greater availability of Cd compared to other heavy metals, such as Zn, Cu, and Pb, which have a higher biological absorption coefficient [30]. Cadmium remains in the human body for many years, so eating food with the contents of this metal can induce chronic toxicity [31, 32]. Cadmium is a calcium antagonist. Even in soils that are considered to be uncontaminated or poorly polluted as a result of cadmium contamination coming from fertilizers or the atmosphere, some crops such as hard wheat, flax, sunflower, and potatoes can accumulate Cd in amounts exceeding the existing maximum level for consumption [28].

Lead is one of the most dangerous pollutants. The main way to enter the environment is anthropogenic pollution. Its widespread use as a liquid fuel antidetonator is one of the main reasons for increasing the content in terrestrial and aquatic ecosystems. If there are detergents in urban sewage waters, lead compounds are dissolved by these substances (polyphosphates, aminopolycarboxylic acids) [33]. Lead compounds containing a toxic anion, for example, orthoarsenates, chromates, and azide, are particularly toxic [34]. One of the necessary steps to prevent toxic effects of heavy metals on animals and humans is soil purification. To reduce global environmental pollution by technogenic pollutants, phytoremediation technology has been successfully applied worldwide. Phytoremediation is defined as the technology of using plants to clean contaminated soils, being economically advantageous and safe in comparison with other physicochemical methods of purification. In this regard, the study of metal-accumulating activity of natural species of Kazakhstan is particularly relevant and timely, and the use of the most suitable species for phytoremediation of contaminated soils is the most promising direction.

Compared with physical and chemical methods, this method is less expensive, effective, and safe. The term “over-accumulator” refers to plant species that accumulate 10–100 times more metals than conventional plants. These plants can be used to extract toxicants from the soil and thus can contribute to the restoration of the fertility of contaminated land. The accumulation of metals by plants in nontoxic form is one of the strategies used by plants to survive in conditions of severe environmental contamination [15]. One of the necessary steps to prevent toxic effects of heavy metals on animals and humans is soil purification. The most effective way at present is phytoremediation of soils, i.e., cleaning of soils with the help of plant hyperaccumulators of heavy metals. Compared to physical and chemical methods, this method is less expensive, effective, and safe [2, 15].

According to the literature data, the cost of conservative methods (chemical and physical methods) of soil purification is from $30 to $350 per hectare, and the cost of treating soils with plants is about $160 per hectare [35]. According to other estimates, depending on the soil conditions and metal concentration, the cost of cleaning with plants (using only solar energy) can be only 5% of the costs required for other methods of restoring ecosystems contaminated with metals [36]. The technology of phytoremediation has various directions. Phytoremediation technology includes phytoextraction (use of plants to extract metals from the soil), phytovolatilization (use of plants for volatilization of chemical elements), rhizofiltration (use of plant roots to extract metals from running water), and phytostabilization (use of plants to transfer metals to less toxic forms, but not extracting them from the soil) [36, 37]. For the phytoextraction of heavy metals from the soil, the use of plant hyperaccumulators of heavy metals is most beneficial. The term "over-accumulator" refers to plant species that accumulate 10–100 times more metals than conventional plants. Hyperaccumulators are of considerable interest from the point of view of phytoremediation [38], phytoextraction [39], and biofortification (improvement) in agricultural crops [40, 41].

These plants can be used to extract toxicants from the soil and thus can contribute to the restoration of the fertility of contaminated soil. Plant hyperaccumulators are endemic for those soils that are contaminated with heavy metals and do not compete with other species on unpolluted soils. Accumulation of metals by plants in nontoxic form is one of the strategies used by plants to survive in conditions of severe environmental contamination. The most well-known plant hyperaccumulators of heavy metals are *Ambrosia artemisiifolia* L. (ragwort ragweed), *Thlaspi rotundifolium* L., and *Thlaspi caerulescens* L., absorbing a significant amount of Zn, Cd, and Pb. Hyperaccumulators of Ni include *Alyssum* L. and *Arabidopsis* L. Currently, the definition of R. Brooks [42] is generally accepted, according to which those plants that accumulate zinc (Zn) >10,000, lead (Pb) >1000, and cadmium (Cd) >100 μg/g. are considered as hyperaccumulators of heavy metals. Plants-non-accumulators of heavy metals should accumulate on unpolluted soil - Zn < 100 μg/g, Pb < 10 μg/g, and Cd < 1 μg/g, respectively, and on contaminated soil - Zn < 1000 μg/g, Pb < 100 μg/g, and Cd < 10 μg/g.

The authors draw the attention of researchers to some important points in the study of plant hyperaccumulators. McGrath [43] considers that when comparing the hyperaccumulative ability of plants of different species, it is necessary to take into account not only the concentration of metal in plants (the content of metal per unit of plant weight) but also the amount of metal extracted from a given area. So, if one species strongly suppresses the accumulation of biomass of the aerial organs and the other to a lesser degree, the concentration of metal in the aerial organs of the latter may be lower than in the first due to the dilution effect. The absolute value of the metal content in plants in terms of a certain area will give a more correct picture for assessing the hyperaccumulation activity of plants in a comparative analysis [43]. Another important point is the ratio of the content of metals in the aerial plant organs to the content in the soil. As a rule, this value (up to 40 or more) is great for plant hyperaccumulators [44]. The most accurate determination of the status of hyperaccumulators can be established, the authors believe, only on a hydroponic medium, where the ability of plants to tolerate large concentrations of metals is manifested [45]. Phytoextraction is a fairly long duration of phytoremediation technology. Therefore, for the productive use of contaminated areas, it is necessary to use an economically viable and socially acceptable method for cleaning contaminated land. Technical crops, "energy crops," possessing phytoextraction potential may be candidates for biofuel production [46].

The main disadvantage of the plants hyperaccumulators of heavy metals is due to the low growth and low biomass of these plants. If we try to imagine what an ideal plant should be, from an ecological point of view, then the plant would obviously look like this: having a long, well-developed root system and a strong transpiration current, such a plant must intensively form biomass, and this plant biomass should be characterized by tolerance to organic and inorganic toxic compounds. In addition, such a plant must necessarily quickly form conjugates and have the appropriate potential (capacity) for storing them in cellular structures and the apoplast [34]. The use of "energy" crops as phytoremediants will reduce the level of pollution from one side and on the other hand increase the productive value of contaminated soils.

Since it is not always possible to use plant hyperaccumulators, some wild grasses, as well as oil plants like sunflower, can be successfully used to clean up areas around metallurgical plants. The best candidates for use in phytoremediation are plants such as sunflower plants (Helianthus annuus L.), castor oil plants (Ricinus communis L.), and white mustard (Sinapis alba L.) [46].

As a phytoextractant, technical crops such as sunflower can be used. Sunflower is a plant that accumulates huge biomass and has the ability to store heavy metals in large amounts.

It is known from the literature that sunflower plants can accumulate large amounts of Pb, Zn, and Cd in their organs. The low bioavailability of some heavy metals in experiments was eliminated by the addition of synthetic metal chelating agents such as EDTA (0.1, 1, 3, 5, 7, and 10 mM/kg soil) and citric acid (0.01, 0.05, 0.25, 0.442, and 0.5 M/kg soil). After the use of metal chelating agents, the concentration in plant tissues increased, and, consequently, the removal of heavy metals by plant biomass increased [47]. Plants of castor oil (*Ricinus communis* L.), from which castor oil is extracted, also have a high potential for phytoextraction of metals from the soil.

When grown on a hydroponic medium that contained lead in amounts of 0, 100, 200, and 400 μmol/L, their lead hyperaccumulation potential was established. I. Raskin et al. [48] have established that plants accumulating 1.0 g/kg of dry weight in tissues can be considered plant hyperaccumulators of Pb [48]. Castor plants were accumulated on a hydroponic medium—from 10.54 to 24.61 g Pb/kg [49]. The use of metal chelating agents, such as EDTA, can increase the translocation of lead to the aerial organs.

In castor oil plants Ricinus communis L., which were grown on soil contaminated with lubricating oils (1–6% oil/soil), content of heavy metals, like Mn, Ni, and Pb were greatest in leaves and cadmium - in plant roots [50]. Application of 5 mM/kg of EDTA increased in the proportion of phytoavailable Pb, Zn, and Cd. The absorption of heavy metals increased in mustard white (*Sinapis alba*), radish (*Raphanus sativus oleiformis*), and amaranth (*Amaranthus* spp.). In mustard concentration of Pb was 479.71 mg/kg; Zn - 524.68 mg/kg; and Cd - 7.93 mg/kg, and phytoextraction potentials were 1.32 kg/ha, 1.44 kg/ha, and 0.022 kg/ha for Pb, Zn and Cd, respectively [51].

In a comparative experiment with *Helianthus annuus, Nicotiana tabacum*, and *Vetiveria zizanioides* grown in a hydroponic medium containing Pb $(NO_3)_2$ at concentrations of 0.25 and 2.5 mmol/L with or without chelating agents (EDTA or DTPA), it was found that the presence of metal chelating agents increased phytoextraction of lead. Most of the lead was accumulated in the leaves of plants. It was found that sunflower plants accumulate more lead than other species. Lead at a concentration of 2.5 mmol/L led to a strong increase in its concentration in plant tissues compared to the concentration in the growing medium. The bioconcentration factor was higher in sunflower plants than in the other two species of *N. tabacum* and *V. zizanioides*. In sunflower plants, the bioconcentration factor was 2.4 and 1.9 times more than in *Nicotiana tabacum* and *Vetiveria zizanioides*, respectively. The largest amount of lead was found in the roots, stems, and leaves of *H. annuus* grown at 2.5 mmol/L EDTA [52].

Sunflower showed good results in soil contaminated with arsenic. Pentavalent arsenate ($AsO4^-$) is very resistant in soils and is present in well-aerated soils. As a result, arsenic contamination of agricultural soils is a big problem. It is known that arsenates and phosphates ($PO4^-$) are chemically similar and therefore compete for joint sites in the soil. Therefore, in order to reduce the binding of arsenic to soil particles and to improve the phytoextraction of it from the soil, it is advisable to add ammonia. Addition of phosphate increases the content of arsenate in the soil solution by replacing arsenate at specific anion exchange sites of the soil, which will increase the bioavailability of arsenic for plant roots. Phosphate fertilizers increase As accumulation in plants by stimulating the phosphate-absorbing mechanism. It was found that sunflower (*Helianthus annuus* L.) can be a candidate for phytoextraction of arsenic when phosphorus is added as a mobilizing agent [53].

Sunflower plants in hydroponic conditions accumulated great amounts of nickel (Ni) and lead (Pb) in the shoots and roots. Accumulation of Ni and Pb (55.82 and 72.28 mg/kg) was increased in the presence of EDTA. It was shown that in sunflower concentration and total accumulation of Pb was more than Ni [54]. It was revealed that sunflower *Helianthus annuus* accumulated most amount of the lead compared to other plant species, like *Brassica juncea* (L.), *Brassica nigra* (L.), *Raphanus sativus* L., and *Ipomea triloba* L. [55]. The study of phytoextraction potential of plants such as *Helianthus annuus*, *Echinochloa crus-galli*, *Abutilon avicennae*, and *Aeschynomene indica* grown on soils polluted with cadmium (Cd), lead (Pb), and 2,4,6-trinitrotoluene (TNT) has shown that the concentration of lead was the highest in *A. avicennae* and *H. annuus*. The removal of cadmium was also high in these plants. The highest values for cadmium were found in plants *E. crus-galli* (50.1%) and *H. annuus* (41.3%) [56]. In experiment with using other chelating agent, like DTPA (3 mmol) for rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*) in soil contaminated by lead and zinc (234.6 mg/kg and 1364.4 mg/kg, respectively), concentration of these metals in sunflower plants was higher as compared to rapeseed [57].

The results of experiments with two sunflower species *Tithonia diversifolia* and *Helianthus annuus* showed that these plants have accumulated great amounts of lead and zinc in the leaves, stems, and roots. The concentrations of Pb in the leaves were 87.3 mg/kg, 71.3 mg/kg, and 71.5 mg/kg and in the stems 79.3, 77.8, and 60.7 mg/kg at 4 weeks, 6 weeks, and 8 weeks after planting, respectively. In roots, it was 99.4 mg/

kg, 97.4 mg/g, and 77.7 mg/kg at 4 weeks, 6 weeks, and 8 weeks AP, respectively. The same pattern was observed in *Helianthus annuus*. The studied plants have accumulated Zn in great amount in aboveground parts compared to roots. The translocation coefficient factor and bioconcentration factor of Pb and Zn with these plant species were greater than 1. But translocation factor of Zn was more than Pb. The authors concluded that the two species of sunflower *Tithonia diversifolia* and *Helianthus annuus* can be used in phytoremediation successfully [58]. Other researchers also state that the study of accumulation of heavy metals in *Helianthus annuus* showed that sunflower plants have accumulated great amounts of heavy metals [59, 60].

In other experiments with sunflower plants, heavy metals are accumulated mainly in the roots with little translocation of heavy metals from the roots to shoots [61]. Vermicompost amendments have increased uptake of Pb, Zn, and Cd by sunflower plants [62]. Patel et al. have determined that phytoextraction of copper by sunflower plants was higher than lead. Application of metal chelating agents like EDTA, a decrease in pH, and the addition of ammonium sulfate in the growth medium increased the uptake of metals by plants. Application of EDTA increased the heavy metal uptake by plants to a greater extent than the using of ammonium sulfate and the decreasing in pH [63]. The main reason of the application of *Helianthus annuus* L. in phytoremediation is that sunflower plants grow fast, accumulate great biomass, and are able to uptake heavy metals in large amounts [64–68].

### 13.2.1 Metal Accumulation Ability of Sunflower and the Mixture of Lawn Grasses

The main reason to develop phytoremediation technology using energy-rich crops is that energy valuable cultures accumulate a large biomass of aboveground organs and are able to accumulate large amounts of heavy metals in their parts. Metal chelating agents will enhance the phytoextraction of metals that have low bioavailability and will also increase the translocation of metals to the aerial organs [69, 70]. Another possibility for phytoremediation is the use of wild grass species for phytoremediation. It was investigated the metal accumulation capacity of grass species of *Poa pratensis*, *Lolium perenne*, and *Festuca rubra* [71]. The studied grass species had translocation factor <1 and bioconcentration factor for roots >1. *P. pratensis* had lower phytostabilization potential than the other grasses but had a higher translocation factor and lower tolerance to cadmium. *L. perenne* has shown more tolerance to Cd and accumulation of Cd in largest amount. The authors concluded that *L. perenne* would be useful for phytostabilization of soils characterized by a relatively small pollution by cadmium. Other researchers found that the grasses which accumulate great biomass of well-developed root system are tolerant to heavy metals. Wild grass species have shown a high ability to accumulate heavy metals in shoots and roots. These peculiarities of grass species justify their use in phytoremediation [72–74].

In our previous studies, it was found that wild grass species *Thlaspi arvense*, *Agropyron repens*, *Setaria viridis*, *Dactylis glomerata*, and *Phleum pratense* have accumulated heavy metals in large quantities in the roots. In general, all the studied species were relatively resistant to the action of heavy metals and accumulated them to varying degrees mainly in the roots of plants, with the exception of *T. arvense*. From this it can be concluded that these species can be used for phytoremediation of soils contaminated with metals such as Zn, Pb, Cu, and Cd, in particular for phytostabilization. For phytoremediation of soils contaminated by Zn, almost all these species can be used - *T. arvense*, *A. repens*, *S. viridis*, and *D. glomerata*, and for Pb-contaminated soils - *A. repens*, *S. viridis*, *T. arvense*, and *Ph. pratense*. For the cleaning of soils from Cu and Cd, the use of *T. arvense* and *A. repens* was recommended [27]. Thus, sunflower plants and wild grass species have high metal accumulation capacity. The aim of our research was to study heavy metal accumulation capacity of sunflower plants (*Helianthus annuus* L.) and wild grass species and their phytoextraction potential.

### *13.2.2 Material and Methods*

Seeds of the mixture of lawn grasses *Poa pratensis*, *Festuca rubra*, and *Arrhenatherum elatius* (1:1:1) and sunflower (*Helianthus annuus* L.) were sown on the pots (1 $m^2$) on the territory of the metallurgic factory “KazZinc” in Ust-Kamenogorsk City in East Kazakhstan. On separate plots with an area of 1 $m^2$, 10 seeds of sunflower plants and 100 seeds of the mixture of lawn grasses were sown. After 3 months the plants were removed for analysis. Before planting and after removing of plants, the soil samples were taken for analysis. The content of heavy metals in plant parts (in aboveground organs and roots) was determined. The content of trace metals in shoots and roots was determined as described next. Plant samples (0.5 g) were digested in a mixture of 5 mL of 50% $HNO_3$ and 0.5 mL HCl at 95 ± 5 °C according to standards for operation procedures [75]. Samples were transferred to digestion block (section) at temperature 90 ± 5 °C, closed by glass, and heated without bringing to a boil for 10–15 min. Then they were cooled and added 5 mL of concentrated $HNO_3$, moved in digestion block with 90 ± 5 °C, closed by glass, and heated without bringing to a boil for 30 min before the disappearance of brown fumes. Then the samples were cooled and added 2 mL of water and 3 mL of $H_2O_2$, continued heating up until the volume has been reduced to about 5 mL, removed from digestion blocks, allowed to cool, filtered, and added with deionized water up to a final volume to 50 mL. Samples were analyzed using the appropriate SOP [76].

The concentrations of metals in plants and soils were measured by atomic absorption spectrophotometry using an installed Winlab A Analyst 300 (Perkin Elmer, Germany) [76] with an installed and aligned HCL/EDL lamp. HCL lamps were stabilized/aligned for 25 min and EDL lamps - for 45 min; an operating pressure for acetylene was ~0.7 kgf/$cm^2$ and for compressed air - 2.8–3.0 kgf/$cm^2$. Following calibration, samples were analyzed.

After removing of plants, the plant part biomass was measured. Plants were dismembered on the aboveground part and roots. To determine the dry biomass, the plants were placed in a thermostat and dried at temperature 105 °C to constant weight, cooled to room temperature, and weighed.

Assessment criteria for the accumulation capacity of plant bioconcentration factor (BCF) and shoot/root ratio were used. BCA was determined according to the following formula:

$$\text{BCF} = \text{Cplant}/\text{Csoil},$$

where Cplant and Csoil are concentrations of heavy metals in plant parts and soil, accordingly [77].

Shoot/root ratio was calculated according to the following formula:

Shoot/root ratio = concentration of metal in the aboveground organs/concentration of metal in the roots.

Determination of the content of metal in plant parts in percent (%) was carried out by the following way: % (g/100 g) = metal concentration (g/kg) × 100 g/1000 g. As assessment criteria of the level of cleaning of soil, it was used the value of the removal of heavy metals by plant parts and the residual amount of metals in the soil after the experiment (mg/kg). The residual amount of metals in the soil after experiment was calculated by the following formula:

Content of heavy metals in the soil after removing of plants (% to metal concentration before planting) = (concentration of metal after removal of plants (mg/kg)/concentration of metal in the soil before planting) × 100%

The removal of metals by plant parts from the area 1 $m^2$ was calculated by the following formula:

The removal of heavy metals by plants ($g/m^2$) = concentration of heavy metals (g/kg) × yield ($g/m^2$)/1000 g. The concentration value in mg/kg was previously converted into g/kg.

## *13.2.3 Results and Discussion*

### 13.2.3.1 The Study of the Metal Accumulation Ability of Sunflower Plants (*Helianthus annuus* L.) and a Mixture of Lawn Grasses

Sunflower plants (*Helianthus annuus* L.) and a mixture of lawn grasses—*Poa pratensis*, *Festuca rubra*, and *Arrhenatherum elatius*—were planted in the territory of the lead-zinc metallurgical factory in Ust-Kamenogorsk City. Sunflower plants and a mixture of lawn grasses were planted separately in areas of 1 $m^2$ in three replicates (plots of 1 $m^2$ under sunflower and lawn grasses). On the plots 1 $m^2$, 10 sunflower plants were planted, and on a site with lawn grasses, 100 plants were sown. Determination of the concentration of heavy metals, such as cadmium (Cd), copper (Cu), lead (Pb), and

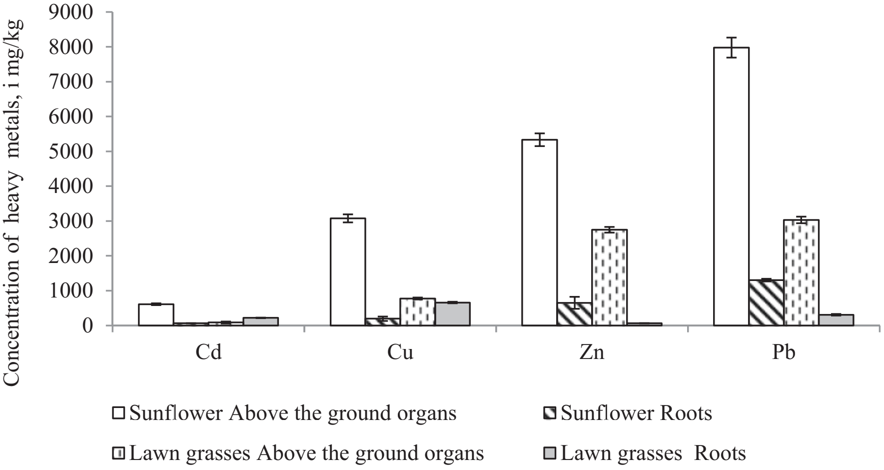


**Fig. 13.1** Content of heavy metals in plants parts of sunflower and lawn grasses

zinc (Zn), showed that the content of these metals in the aerial organs of sunflower changed in the following order (mg/kg), Pb (7978.0) > Zn (5333.0) > Cu (3076.0) > Cd (612.0), and in roots Pb (1304.0) > Zn (652.0) > Cu (195.0) > Cd (65.0). The study of metal accumulating capacity of sunflower plants and a mixture of lawn grasses showed that zinc in the aboveground organs and roots of both sunflowers and in a mixture of lawn grasses is accumulated in greatest amount (Fig. 13.1). Cadmium is found in the lowest concentration in the organs of these plants.

According to the content in the aboveground organs of lawn grasses, heavy metals were arranged in the following order, Pb (3031.36) > Zn (2748.70) > Cu (775.87) > Cd (87.10), and in the roots Pb (661.0) > Zn (309.0) > Cu (220.0) > Cd (66.0). The concentration of heavy metals such as lead, zinc, copper, and cadmium in the aerial organs of sunflower was higher than in the aboveground organs of lawn grasses by 2.63, 1.94, 3.96, and 7.03 times, respectively. The highest excess in the aboveground organs is observed in cadmium and the lowest in zinc. In the roots of sunflower, the concentration of lead and zinc was also higher compared to lawn grasses. Concentration of lead in the roots of sunflower was 1.97 times higher and zinc 2.11 times more compared to the roots of lawn grasses. Concentration of copper, on the contrary, was slightly higher in the roots of lawn grasses than in sunflower (by 1.12 times), and the concentration of cadmium in the roots of sunflower and lawn grasses was almost the same (65.0 mg/kg and 66.0 mg/kg, respectively). In the roots, the highest excess was observed for zinc.

The content of heavy metals in the aerial organs of sunflower and lawn grasses was much higher, compared with the roots, probably due to strong atmospheric pollution.

The ratio of the content of metals in the aerial organs to their content in the roots is of great importance for cleaning the soil with the help of plants. The higher this value, the higher the potential ability of plants to clean the soil from metals. This index varies in sunflower in the following order: Cu (15.8.0) > Cd (9.4) > Zn

**Table 13.1** Heavy metals content in plant samples in percent

| Plant parts | Cu g/100 g | Pb g/100 g | Cd g/100 g | Zn g/100 g |
|---|---|---|---|---|
| Sunflower plants | | | | |
| Aboveground organs | 0.31 | 0.8 | 0.061 | 0.53 |
| Roots | 0.02 | 0.13 | 0.007 | 0.07 |
| Lawn grasses | | | | |
| Aboveground organs | 0.08 | 0.3 | 0.009 | 0.27 |
| Roots | 0.022 | 0.07 | 0.007 | 0.03 |

(8.2) > Pb (6.1). For lawn grasses, the shoot/root ratio decreased in the following order: Zn (8.9) > Pb (4.6) > Cu (3.5) > Cd (1.3). For lawn grasses, unlike sunflower, the shoot/root ratio was higher for zinc and lead compared to copper and cadmium. A study of the content of heavy metals in plant organs showed that zinc ions were accumulated most in the aerial organs and roots of sunflower and a mixture of lawn grasses (Fig. 13.1). Cadmium is found in the lowest concentration in plant organs.

The percentage of heavy metals in plant organs is an integral indicator in the selection of plants for phytoremediation. In all studied plants, this indicator was below than 1 (Table 13.1).

The percentage of lead was highest in the aerial organs and roots of sunflower (0.8% and 0.13%, respectively) compared with other metals, and the lowest percentage of cadmium was 0.06% and 0.01% in the aerial organs and roots, respectively. Lawn grasses had the same pattern. The percentage of lead in the aerial organs was 0.3% and in the roots 0.07%; cadmium was 0.01% and 0.007% in the aerial organs and roots, respectively. The percentage of heavy metals in sunflower organs varied in the following order (%): in the aerial organs, Pb (0.8) > Zn (0.53) > Cu (0.31) > Cd (0.061), and in the roots Pb (0.13) > Zn (0.07) > Cu (0.02) > Cd (0.007).

In lawn grasses, the percentage of heavy metals decreased in the following order: aboveground organs (%), Pb (0.3) > Zn (0.27) > Cu (0.08) > Cd (0.009), and in the roots Pb (0.07) > Zn (0.03) > Cu (0.022) > Cd (0.007).

When comparing the content of metals in the aerial organs and in roots of the studied plants, it was found that the percentage of Pb content in roots of sunflower plants was 6.2 times less than in the aboveground parts. Concerning zinc, its content in the aboveground organs of sunflower exceeded that in roots by 7.57 times. The content of Cd in the aerial organs of sunflower exceeded its content in the roots by 8.7 times. The highest excess was observed for copper—its content in the aerial organs in percent was more than the percentage in the roots by 15.5 times. Lawn grasses had the same pattern. The percentage of metals in the aboveground organs exceeded its percentage in the roots—Pb, 4.29 times; Zn, 9 times; Cu, 3.63 times; and Cd, 1.29 times. If we compare the percentage of metals in the organs of sunflower and lawn grasses among themselves, we can see that in the aboveground organs of sunflower, the percentage of lead was 2.67 times more; zinc percentage content, 1.96 times more; copper, 3.88 times more; and cadmium, 6.78 times more than in lawn grasses.

In sunflower roots, the percentage of lead was 1.86 times more than that of lawn grass, and zinc content in 2.33 times greater, while the percentage of copper and cadmium in the roots of both plant species was the same—0.007% and 0.007%, respectively. Thus, the concentration of metals in the aerial plant organs was higher than in the roots. The coefficient of translocation of metals from the roots to the aboveground organs exceeded than 1. Obviously, this indicator is not a natural phenomenon and is the result of strong atmospheric pollution. Characteristically, the shoot/root ratio of cadmium and copper in sunflower was higher than that of zinc and lead, and in contrast to cadmium and copper, the shoot/root ratio of lead and zinc was greatest. The percentage of lead was greatest in the aerial organs and roots of the studied plants, and the smallest percentage of cadmium was also found.

#### 13.2.3.2 Determination of Bioaccumulation Coefficient of Lawn Species and Sunflower Plants

Another important point is the ratio of the content of metals in the aerial plant organs to the content in the soil. As a rule, this value is great in plant hyperaccumulators [44]. To estimate the degree of bioaccumulation of heavy metals by the organs of the studied plants, the bioconcentration factor (bioaccumulation coefficient) of metals for the aerial organs and roots of sunflower and lawn grass plants was calculated (Table 13.2). Plants at the end of the experiment were collected from contaminated sites to determine the content of heavy metals in their organs. Soil samples from these sites were also taken for analysis. Using these values, the bioconcentration factor of the metals studied for sunflower and lawn grasses was determined.

In sunflower, the bioconcentration factor of Pb for aerial organs and roots was greatest and BCF of Zn the smallest. For the aboveground organs, the bioconcentration factor of all the metals studied was >1. Probably, the reason for this is strong atmospheric pollution. The bioconcentration factor in sunflower roots was >1 only for Pb (4.2).

The bioconcentration factor of heavy metals in the aerial organs compared to the roots of sunflower was higher for cadmium, copper, and zinc, while for lead, it was

**Table 13.2** BCF heavy metals for sunflower and lawn grasses

| Metals | Cu, mg/kg | Pb, mg/kg | Cd, mg/kg | Zn, mg/kg |
|---|---|---|---|---|
| Sunflower | | | | |
| Soils under sunflower | 870.0 ± 29.3 | 1900.0 ± 62.2 | 210.0 ± 7.6 | 2510.0 ± 97.8 |
| Aboveground organs | 3076.0 ± 102.3 | 7978.0 ± 25.16 | 612.0 ± 22.3 | 5333.0 ± 18.8 |
| Roots | 195.0 ± 6.1 | 1304.0 ± 4.156 | 65.0 ± 2.1 | 652.0 ± 18.3 |
| BCF for aboveground organs | 3.54 ± 0.093 | 4.19 ± 0.14 | 2.9 ± 0.09 | 2.12 ± 0.07 |
| BCF for roots | 0.22 ± 0.07 | 4.2 ± 0.09 | 0.31 ± 0.01 | 0.26 ± 0.008 |
| Lawn grasses | | | | |
| Soils under sunflower | 980.0 ± 31.10 | 2210.0 ± 26.6 | 260 ± 5 | 2470 ± 27 |
| Aboveground organs | 775.87 ± 22.3 | 3031.36 ± 1.2 | 87.10 ± 2.5 | 2748.70 ± 9.3 |
| Roots | 220.0 ± 9.20 | 661.0 ± 21.3 | 66.0 ± 2.6 | 309.0 ± 9.2 |
| BCF for aboveground organs | 0.79 ± 0.025 | 1.37 ± 0.05 | 0.34 ± 0.015 | 1.11 ± 0.05 |
| BCF for roots | 0.22 ± 0.008 | 0.3 ± 0.013 | 0.25 ± 0.01 | 0.13 ± 0.004 |

approximately the same. The bioconcentration factor of sunflower plants decreases in the following order: for aerial organs, Pb (4.19) > Cu (3.54) > Cd (2.9) > Zn (2.12), and for the roots Pb (4.2) > Cd (0.31) > Zn (0.26) > Cu (0.22). In lawn grasses, as in sunflower, the lead bioconcentration factor for the aboveground organs and roots was the largest (1.37 and 0.3, respectively). In the aerial organs, the lowest value of the bioconcentration factor was observed for cadmium (0.34) and in the roots for zinc (0.13). In lawn grasses, the bioconcentration factor of lead and zinc in the aerial organs was above 1, and in the roots, this index was <1 for all metals studied. The bioconcentration factor of lawn grasses decreased in the following order: for aboveground organs, Pb (1.37) > Zn (1.11) > Cu (0.79) > Cd (0.34), and for the roots Pb (0.3) > Cd (0.25) > Cu (0.22) > Zn (0.13). In lawn grasses, the bioconcentration factor of all the heavy metals studied in the aerial organs was higher in comparison with the roots. Probably, this is a consequence of strong atmospheric pollution on the territory of the plant.

#### 13.2.3.3 Determination of the Removal of Heavy Metals from the Soil by the Organs of Sunflower and Lawn Grass Plants

The question of whether the degree of metal accumulation is more important for the phytoremediation process or the accumulation of a significant aboveground mass is controversial for many researchers. Chaney et al. believe that the ability to hyperaccumulate metals and exhibit hyper-resistance to high metal concentrations is the most important plant properties for phytoremediation than the ability to accumulate large biomass [2]. But when comparing the hyperaccumulation ability of plants, it is also considered expedient to take into account not only the concentration of metal in plants (metal content per unit of plant weight) but also the amount of metal extracted from a given area. So, if one species strongly suppresses the accumulation of biomass of the aerial organs and the other to a lesser degree, the concentration of metal in the aerial organs of the latter may be lower than in the first due to the dilution effect. The absolute value of the metal content in plants in terms of a certain area will give a more correct picture for the estimation of the hyperaccumulation activity of plants in a comparative analysis [43]. Taking into account the concentration of heavy metals in plant organs and the yield of dry biomass from 1 $m^2$, the removal of heavy metals by plant organs was determined, and the degree of soil purification by the investigated plants was estimated.

For the use of plants for the purification of soils from heavy metals, the necessary index, which should be taken into account in phytoremediation, is the absolute value of the biomass of the aerial organs and plant roots from a certain area. It was determined the accumulation of biomass of the aboveground organs and roots of sunflower plants and mixture of lawn grasses grown on the area 1 $m^2$ around the lead-zinc metallurgical plant in Ust-Kamenogorsk City. It was calculated the value of biomass (aboveground organs and roots) per one plant for sunflower and mixture of lawn grasses and biomass of all plants, collected from the plots with an area of 1 $m^2$. Sunflower plants accumulated significant biomass in comparison with lawn

**Table 13.3** Accumulation of biomass by aerial organs and roots of sunflower and a mixture of lawn grasses

| Sunflower | | |
|---|---|---|
| Plant parts | Dry weight per one plant, mg | mg per 1 $m^2$<br>10 plants |
| Aboveground organs | 829.0 ± 31.9 | 8290.0 ± 262.6 |
| Roots | 862.0 ± 25.2 | 8620.0 ± 301.21 |
| Mixture of lawn grasses | | |
| Plant parts | Dry weight per one plant, mg | mg per 1 $m^2$<br>100 plants |
| Aboveground organs | 297.0 ± 7.1 | 29,700.0 ± 934.1 |
| Roots | 21.0 ± 8.3 | 2100.0 ± 71.5 |

grasses (Table 13.3). The dry biomass of the aboveground organs of sunflower per plant exceeded the biomass of grass lawns by 2.79 times and biomass of sunflower roots by 41 times.

Biomass, collected from an area of 1 $m^2$, was significant. The biomass of aerial organs from 100 plants of lawn grasses per 1 $m^2$ exceeded than that of sunflower by 3.58 times. But the root biomass of 100 lawn grass plants from an area of 1 $m^2$ was less by 4.1 times than root biomass of 10 sunflower plants from the plot with the same area. Before planting plants and at the end of the experiment, the content of heavy metals in the soil was determined. According to the initial content in the soil under sunflower plants, the heavy metals were arranged in the following order (mg/kg): Pb (11,035.0) > Zn (5181.0) > Cu (3258.0) > Cd (303.0) (Table 13.4).

According to the initial content in the soil under lawn grasses, heavy metals are arranged in the following order (mg/kg): Pb (9410.0) > Zn (4871.0) > Cu (3420.0) > Cd (280.0).

Taking into account the biomass of the aboveground organs and roots of sunflower and lawn grasses from the area of 1 $m^2$, the removal of heavy metals by sunflower and lawn grass organs was calculated. According to the removal of heavy metals from 1 $m^2$ by the aboveground organs of sunflower (ten plants), metals are arranged in the following order (mg), Pb (66.138) > Zn (44.211) > Cu (25.5) > Cd (5.074), and by roots Pb (11.24) > Zn (5.62) > Cu (1.68) > Cd (0.56) (Fig. 13.2). According to the removal of heavy metals by the aerial organs of lawn grasses (100 plants), the metals are arranged in the following order (mg), Pb (90.03) > Zn (81.636) > Cu (23.043) > Cd (2.59), and by roots Pb (1.388) > Zn (0.65) > Cu (0.462) > Cd (0.139).

The removal of copper by the aerial organs of sunflower little more than that of lawn grasses (25.5 mg Cu and 23.043 mg Cu in the aerial parts of sunflower and grasses, respectively). The removal of cadmium by the aboveground organs of sunflower was higher than that of lawn plants by 1.96 times although total biomass of the aboveground organs of sunflower from the area of 1 $m^2$ was lower as compared to lawn grasses. It was a consequence of a large accumulation of these metals by aerial organs of sunflower, and the concentration of Cu in the aerial organs of sunflower was about four times greater than that of lawn grasses

**Table 13.4** The content of heavy metals in soil sown with sunflower and lawn grasses

| Soil | Cu, mg/kg | % | Pb, mg/kg | % | Cd, mg/kg | % | Zn, mg/kg | % |
|---|---|---|---|---|---|---|---|---|
| The content of heavy metals in the soil under sunflower before the experiment | 3258.0 ± 170.0 | 100 | 11,035.0 ± 419.0 | 100 | 303.0 ± 9.6 | 100 | 5181.0 ± 218.2 | 100 |
| The content of heavy metals in the soil under sunflower after the experiment | 870.0 ± 25.2 | 27 | 1900.0 ± 72.4 | 17 | 210.0 ± 7.60 | 69 | 2510.0 ± 8.9 | 48 |
| The content of heavy metals in the soil under lawn grasses before to the experiment | 3420.0 ± 12.0 | 100 | 9410.0 ± 361.3 | 100 | 280.0 ± 8.5 | 100 | 4871.0 ± 181.4 | 100 |
| The content of heavy metals in the soil under lawn grass after the experiment | 980.0 ± 42.0 | 29 | 2210.0 ± 69.1 | 24 | 260.0 ± 8.2 | 93 | 247.0 ± 8.170 | 51 |

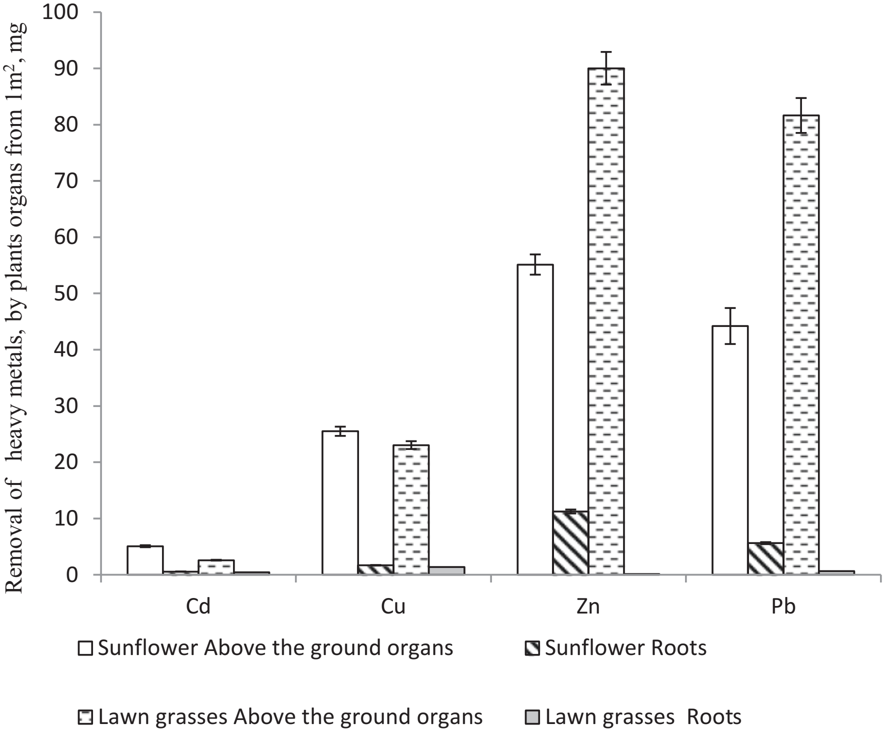


**Fig. 13.2** The removal of heavy metals by plant organs

and concentration of Cd also more in sunflower aboveground organs than that in lawn grasses (1.9 times).

The removal of lead and zinc by the aboveground organs of sunflower was lower than that of the aboveground organs of lawn grasses: Pb, 1.63 times, and Zn, 1.85 times. The concentration of lead in the aerial organs of sunflower was 2.63 times and that of zinc was 1.94 times higher than in lawn grasses.

The removal by roots of all heavy metals studied was higher in sunflower plants. The removal of lead, zinc, copper, and cadmium by the roots of sunflower was higher than in lawn grasses by 8, 8.6, 3.65, and 4 times, respectively. The degree of soil purification was determined from the difference in the concentration of heavy metals in the soil before planting and at the end of the experiment. It was established that the greatest degree of soil purification from the studied metals was found in sunflower plants.

For the residual content of heavy metals in the soil under sunflower plants, the metals were arranged in the following order (%), Cd (69) > Zn (48) > Cu (27) > Pb (17), and, in the soil under a mixture of lawn grasses (%), Cd (93) > Zn (51) > Cu (29) > Pb (24) (Table 13.4). According to this indicator, it is possible to judge the degree of soil purification by the investigated plants. The higher the percentage of the metal content (residual amount) in the soil, the lower the degree of purification. From the obtained data, it follows that the degree of soil purification by plants from lead was the greatest and cadmium the lowest. The degree of soil purification by sunflower plants was higher for all metals tested than for lawn grasses.

Thus, in all the investigated plants, the bioconcentration factor of lead for the aerial organs and roots was the highest. For the aboveground organs, the bioconcentration factor of all the metals studied was above unity. The bioconcentration factor of all investigated heavy metals in the aerial organs of plants was higher in comparison with the roots. Probably, this is a consequence of strong atmospheric pollution on the territory of the plant. The removal of lead and zinc by the aboveground organs of sunflower was lower than that of the aboveground organs of lawn grasses. The removal of copper by the aerial organs of both plant species was approximately the same, and the removal of cadmium by the aboveground organs of sunflower was higher than that of lawn plants. Biomass of aerial organs from 1 $m^2$ in lawn grasses is 3.58 times more than in sunflower. And the biomass of roots, on the contrary, exceeds in sunflower, in comparison with lawn grasses—by 4.1 times. The degree of soil purification by plants from lead was the highest and from cadmium the lowest. The degree of soil purification by sunflower plants was higher for all metals tested than by lawn grasses.

Thus, it can be concluded that the plants of the sunflower can be successfully used for phytoremediation of soils contaminated with heavy metals.

## 13.3 Conclusion

Thus, bioremediation of contaminated soils is the most promising and less expensive way of cleaning the environment from contamination. To date, phytoremediation is recognized throughout the world as the most cost-effective and environmentally friendly technology. For Kazakhstan, the use of species widespread on the territory of the Republic for phytoremediation is appropriate, since the introduction of European species of plant hyperaccumulators will require additional costs. Therefore, the search for plant hyperaccumulators in the territory of Kazakhstan is the paramount task of investigators working in this direction. Oli seed plants which accumulate high biomass and are able to accumulate large amounts of heavy metals and wild grass species can be successfully used for phytoremediation.

**Acknowledgements** The author expresses great gratitude to the employees of the Laboratory of Plant Physiology and Biochemistry of The Institute of Plant Biology and Biotechnology B. A. Sarsenbayev and B. N. Usenbekov for their participation in research of study of metal accumulation capacity of sunflower plants and conducting the related field work.

## References

1. Suleev DK, Sagitov SI, Sagitov PI, Zhumagulov KK (2004) Ecology and nature management. The concept of a transition to sustainable development. Nauka, Almaty 391 p
2. Chaney RL, Malik M, Li YM et al (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
3. Chaney RL, Li YM, Scott JA (1998) Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. CRC Press, Boca Raton, FL 37 p
4. The concept of ecological safety of the Republic of Kazakhstan (1996) Noosphere 1:135–146
5. Fry SC, Miller JG, Panwill JC (2002) A proposed role for copper ions in cell wall loosening. Plant and Soil 247:57–67
6. Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L., cv Azad). J Exp Bot 52(358):1101–1109
7. Zornoza P, Vazquez S, Esteban E, Fernandez-Pascual M (2002) Cadmiumstressinnodulated-whitelupin: strategiesto avoid toxicity. Plant Physiol Biochem 40:1003–1009
8. Reichman SM (2002) The responses of plants to metal toxicity: a review focusing on copper, manganese and zinc. The Australian Minerals & Energy Environment Foundation, Melbourne, VIC, p 54
9. Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MNV (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35(4):985–999
10. Kumar A, Prasad MNV, Achary VMM, Panda BB (2013) Elucidation of lead-induced oxidative stress in Talinum triangulare roots by analysis of antioxidant responses and DNA damage at cellular level. Environ Sci Pollut Res 20(7):4551–4561
11. Ebbs S, Lau J, Ahner B (2002) Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Planta 214:635–640
12. Krotz RM, Evangelou BP, Wagner GJ (1989) Relationships between cadmium, zinc, Cd-peptide and organic acid in tobacco suspension cells. Plant Physiol 91:780–787
13. Rauser WE (1995) Phytochelatins and related peptides. Plant Physiol 109:1141–1149
14. Shkolnik NY, Alekseeva-Popova IV (1983) Rastenia v extremalnih usloviah mineralnogo pitania. Nauka, Leningrad 176 p, In Russian
15. Chaney R, Brown S, Li Y-M, Angle S, Horner F, Green C (1995) Potential use of hyperaccumulator plant species to decontaminate metal polluted soils. Min Environ Manag 3(3):9–11
16. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
17. Prasad MNV (2003) Prakticheskoe ispolzovanie rastenii dlya vosstanovklenia ecosystem, zagryaznennih metallami. Fiziol Rastenii 50(3):764–780
18. Duffus JH (2002) “Heavy metals” – a meaningless term? (IUPAC Technical Report). Pure Appl Chem 74(5):793–807
19. Bandman AL, Volkova NV, Grehova TD (1998) Vrednie himicheskie veshesnva. Himia, Leningrad 592 p, In Russian
20. Chernavskaya NM (1989) Physiologia rastitelnih organizmova I rol metallov. Nauka, Moskva 156 p, In Russian
21. Harvey LJ, McArdle HJ (2008) Biomarkers of copper status: a brief update. Br J Nutr 99(S3):10–13 PubMed: 18598583
22. Stern BR (2010) Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. Toxicol Environ Health A 73(2):114–127
23. Tchounwou PB, Ishaque A, Schneider J (2001) Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Mol Cell Biochem 222:21–28 PubMed: 11678604

24. Tchounwou PB, Yedjou CG, Foxx D, Ishaque A, Shen E (2004) Lead-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2). Mol Cell Biochem 255:161–170 PubMed: 14971657
25. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82(8):493–512 PubMed: 18496671
26. Patlolla A, Barnes C, Yedjou C, Velma V, Tchounwou PB (2009) Oxidative stress, DNA damage and antioxidant enzyme activity induced by hexavalent chromium in Sprague Dawley rats. Environ Toxicol 24(1):66–73 PubMed: 18508361
27. Atabayeva S (2016) Heavy metals accumulation ability of wild grass species from industrial area of Kazakhstan. In: Ansari AA et al (eds) Phytoremediation: management of environmental contaminants. Section 4. Phytoremediation applications for metal contaminated soils using terrestrial plants. Springer, New York, NY, pp 157–200
28. Gimeno-Garcia E, Andreu V, Boluda R (1996) Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environ Pollut 92:19–22
29. Andreu V, Boluda R (1995) Application of contamination indexes on different farming soils. Bull Environ Pollut 104:271–282
30. Lee JS, Chon HT, Kim KW (1988) Migration and dispersion of trace elements in the rock-soil-soil plant system in areas underlain by black shales and states of the Okchon Zone, Korea. J Geochem Explor 65:61–78
31. Jackson AP, Alloway BJ (1992) The transfer of cadmium from agricultural soils to the human food chain. In: Adriano DC (ed) Biochemistry of trace metals. Lewis Publishers, Boca Raton, FL, pp 109–158
32. FAO/WHO. Joint Committee on Food Additives and Contaminants. Position paper on cadmium (prepared by France). 27th Session. The Hague, The Netherlands. 20–24 March, 1995. 32 p
33. Tasekeev M (2004) Bioremediatsia toksichnih promishlennih othodov. Promishlennost Kazakhstana 5(26):59–63
34. Kvesitadze GI, Hatisashvili GA, Sadunishvili TA, Evstigneeva ZG (2005) Metabolism antropogennih toksikantov v visshih rasteniah. Nauka, Moskva 197 p, In Russian
35. Brown SL, Chaney RL, Angle JS, Baker AJ (1995) Zinc and cadmium uptake of *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Soc Am J 59:125–133
36. Prasad MN (2003) Prakticheskoe ispolzovanie rasenii dlia vosstanovlenia ekosyste, zagryaznennih tiazhelimi metallami. Fiziol Rastenii 50(5):764–780 In Russian
37. Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. J Environ Biol 28(2):367–376
38. Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134:335–342
39. Li YM, Chaney R, Brewer E, Roserberg R, Angle JS, Baker A, Reeves R, Nelkin J (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant and Soil 249:107–115
40. Broadly MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702
41. Palmergen MG, Clemens S, Williams LE, Kramer U, Borg S, Schjorring JK, Sanders D (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473
42. Brooks RR (1998) Plants that hyperaccumulate heavy metals, vol 53. CAB International, Wallingford
43. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. AB International, Wallingford, pp 261–287
44. Lasat MM, Fuhrman M, Ebbs SD (2003) Phytoextraction of radiocesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. J Environ Qual 27:165–169
45. Baker AJ, McGrath SP, Reeves RD (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal polluted soils. In: Contaminated soil and water. Lewis Publishers, Boca-Raton, FL, pp 85–107

46. Kos B, Grčman H, Leštan D (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil Environ 49:548–553
47. Lesage E, Meers E, Vervaeke P, Lamsal S, Hopgood M, Tack FM (2005) Enhanced phytoextraction: II. Effect of EDTA and citric acid on heavy metal uptake by Helianthus annuus from a calcareous soil. Int J Phytoremediation 7(2):143–152
48. Raskin I, PBA K, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
49. Romeiro S, Lagôa AMM, Furlani PR, De Abreu CA, De Abreu MF, Erismann NM (2006) Lead uptake and tolerance of *Ricinus communis* L. Braz J Plant Physiol 18(4):18–25
50. Vwioko DE, Anoliefo G, Fashemi SD (2006) Castor oil grown in soil contaminated with spent lubricating oil. J Appl Sci Environ Manag 10(3):127–134
51. Marchiol L, Assoaris 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(1):21–27
52. Boonyapookana B, Parkpian P, Techapinyawat S, De Laune RD, Jugsujinda A (2005) Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). J Environ Sci Health A Toxic Hazard Subst Environ Eng 40(1):117–137
53. Gulz PA, Gupta SK, Schulin R (2003) P-enhanced phytoextraction of arsenic from contaminated soil using sunflower. Proceedings of the 7th International Conference on the Biogeochemistry of Trace Elements (7th ICOBTE), 15–19 June, 2003, Uppsala, Sweden. Book of Abstracts. – Vol I-II. P. 148–149
54. Mukhtar S, Bhatti HN, Khalid M, Haq MA, Shahzad SM (2010) Potential of sunflower (helianthus annuus L.) for phytoremediation of nickel (Ni) and lead (Pb) contaminated water. Pak J Bot 42(6):4017–4026
55. Begonia GB (1997) Comparative lead uptake and responses of some plants grown on lead contaminated soils. Department of Biology, Jackson State University, Jackson, MS 39217. http://www.msstate.edu/org/MAS/ejour3.html
56. Lee I, Baek K, Kim H, Kim S, Kim J, Kwon Y, Chang Y, Bae B (2007) Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J Environ Sci Health A Tox Hazard Subst Environ Eng 42(13):2039–2045
57. Solhi M, Hajabbasi MA, Shareatmadari H (2005) Heavy metals extraction potential of sunflower (*Helianthus annuus*) and canola (*Brassica napus*), Caspian. J Env Sci 31:35–42
58. Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA, Adesodun JK (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(1–4):195–201
59. Madejon P, Murillo JM, Maranon T, Cabrera F, Soriano MA (2003) Trace element and nutrient accumulation in sunflower plants two years after the Aznacolla mine spill. Sci Total Environ 37:239–257
60. Marchiol L, Fellet G, Perosa D, Zerbi G (2007) Removal of trace, metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted with zinc by industrial wastes: a field experience. Plant Physiol Biochem 45:379–387
61. Lin J, Jiang W, Lin D (2003) Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (*Helianthus annuus* L.). Bioresour Technol 86:151–155
62. Angelova V, Perifanova-Nemska MN, Uzunova GP, Ivanov KI, Lee HQ (2016) Potential of sunflower (Helianthus annuus L.) for phytoremediation for soils contaminated with heavy metals. Int J Environ Ecol Eng 10(9):576–583
63. Patel SJ, Bhattacharya P, Banu S, Bai L, Namratha R (2015) Phytoremediation of copper and lead by using sunflower. Indian mustard and water hyacinth plants. Int J Sci Res 4(5):113–115 ISSN (Online): 2319-7064
64. Zavoda T, Cutright J, Szpak I, Fallon E (2001) Uptake, selectivity and inhibition of hydroponic treatment of contaminants. J Environ Eng 127:502–508

65. Prasad MNV (2007) Sunflower (*Helianthus annuus* L.) – a potential crop for environmental industry. Helia 30:167–174
66. 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 Eur J Agric Environ Sci 4(1):93–103
67. Herrero EM, Lopez-Gonzalvez A, Ruiz MA, Lucas-Garcia JA, Barbas C (2003) Uptake and distribution of zinc, cadmium, lead and copper in *Brassica napus* var *oleifera* and *Helianthus annuus* grown in contaminated soils. Int J Phytoremediation 5:153
68. Rivelli AR, De Maria S, Puschenreiter M, Gherbin P (2012) Accumulation of cadmium, zinc and copper by *Helianthus annuus* L. Impact on plant growth and uptake of nutritional elements. Int J Phytoremediation 14:320–334
69. Chen H, Cutright TJ (2002) The interactive effects of chelator, fertilizer and rhizobacteria for enhancing phytoremediation of heavy metal contaminated soil. J Soil Sediment 2:203–210
70. Martins CDC, Liduino VS, Oliveira FJS, Sérvulo EFC (2014) Phytoremediation of soil multi-contaminated with hydrocarbons and heavy metals using sunflowers. Int J Eng Technol 14(5):144305–147171
71. Gołda S, Korzeniowska J (2016) Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. Environ Protect Nat Resour 27(1 (67)):8–14. https://doi.org/10.1515/oszn-2016-0003
72. Aibubu N, Liu Y, Zeng G, Wang X, Chen B, Song H, Xu L (2010) Cadmium accumulation in *Vetiveria zizanioides* and its effects on growth, physiological and biochemical characters. Bioresour Technol 101:6297–6303
73. Xu P, Wang Z (2013) Physiological mechanism of hypertolerance of cadmium in Kentucky bluegrass and tall fescue: chemical forms and tissue distribution. Environ Exp Bot 96:35–34
74. Abaga NOZ, Dousset S, Mbengue S, Munier-Lamy C (2014) Is vetiver grass of interest for the remediation of Cu and Cd to protect marketing gardens in Burkina Faso. Chemosphere 113:2–47
75. LMN/SOP-06 (2006) Digestion of hard samples for heavy metal determination in the flame, 10 p
76. LMN/SOP-08 (FLAA) (2001) Work process of Analyst 300 Perkin Elmer (in the flame), 14 p
77. Agoramoorthy G, Chen F-A, Hsu MJ (2008) Threat of heavy metal pollution in halophytic and mangrove plants of Tami Nadu, India. Environ Pollut 155:320–326

# Chapter 14
# Woody Species in Phytoremediation Applications for Contaminated Soils

**Elena Masarovičová and Katarína Kráľová**

## 14.1 Introduction

Trees held special significance for humans from ancient times providing them fuel, building materials, ornamental objects, oracles, or weaponry (in details see [1]). Since then woody plants found many more applications in plenty of other practical fields. Besides the most important production function woody plants possess also further, non-production functions. These plants play a significant role in reducing erosion and moderating the climate. The last mentioned function of the woody plants is extraordinarily important from the aspect of global atmosphere warming. Trees not only remove carbon dioxide from the atmosphere and store large amount of carbon in their tissues but also release large part of oxygen into the atmosphere. Some of them belong also to the important metalliferous plant species. Moreover, enormous leaf biomass is after decomposition the source of mineral nutrition in the soil and can also serve as a filter against various pollutants (hygienic function of the forest ecosystems). Finally, trees and forests provide a **habitat** for many species of plants and animals (e.g., [2]).

New biotechnological approach showed that woody species have also significant application in many phytoremediation technologies [3, 4]. For instance, the poplars not only remediate the contaminated soils but also work effectively with contaminated wastewater, landfill leachate, and tannery waste out flows [3]. Species such poplar, willow, black locust, ash, or alder for their convenient biological features can be successfully used to clean up substrates contaminated by both inorganic and

---

E. Masarovičová (✉)
Faculty of Natural Sciences, Department of Soil Science, Comenius University in Bratislava, Bratislava, Slovak Republic
e-mail: elena.masarovicova@uniba.sk

K. Kráľová
Faculty of Natural Sciences, Institute of Chemistry, Comenius University in Bratislava, Bratislava, Slovak Republic

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_14

organic pollutants. These plants have perennial character, long life span, high transpiration rate, quick regeneration of removed aboveground parts, and easy vegetative reproduction (cf. [3]). Moreover, some of them are fast-growing trees that have an extensive and massive root system penetrating deeply into the soil and ensuring efficient uptake of water containing the pollutants from the substrate. Mainly poplar and willow have been shown to be excellent species for phytoremediation purposes because they can be cultivated at high rates of growth and thus produce a large biomass. Leaves of this biomass have not only large transpiration potential but they also can uptake large amount of contaminated water. The use of plants producing large biomass for metal extraction from soil was proposed as an alternative to hyperaccumulators because high biomass production establishes to compensate moderate heavy metal concentrations in their shoots (in detail see [5]). Phytoremediation covers a wide range of pollutants like inorganic chemicals including heavy metals, metalloids, many organic substances (including persistent organic pollutants), and radioactive elements. Specific application of phytoremediation is realized in the region with large area of salinization of soils when these sites acquire better quality. Further research is also needed in the field of genetic engineering to improve the phytoremediation abilities of transgenic plants and to understand the mechanisms and effectiveness of phytoremediation techniques in order to make these technologies more effective, time-saving, and economically feasible [6].

Phytoremediation has gained much popularity over the last 20 years and has been considered as an acceptable technique in many countries due to its cost-effectiveness compared to traditional practices. Although the concept of phytoremediation is few decades old and has been applied on a wide range of pollutants, its sustainability is still questioned at various scientific forums. Therefore, it is the need of the hour to remediate our valuable resources with due considerations for future generations [7].

## 14.2 Phytoremediation: Useful Tool to Remediate Contaminated Environment

**Phytoremediation** refers to the biotechnologies that use living plants to clean up habitat—soil, water, and air contaminated with hazardous chemicals. Phytoremediation is a complex technology which comprises several techniques with respect to the specificity in physiological, morpho-anatomical, biochemical, and molecular responses of plants to excessive concentrations of different contaminants [4]. It is a cost-effective and plant-based approach of remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment and to metabolize different molecules in their tissues. As mentioned above, phytoremediation covers a wide range of pollutants like inorganic chemicals including heavy metals, metalloids, many organic substances (including persistent organic pollutants), and radioactive elements (e.g., [7]). However, toxic heavy metals and organic pollutants are the major targets for these processes. To optimize and improve phytoremediation, knowledge of the physiological processes and cleanup mechanisms together with biological and engineering strategies rapidly began to emerge in recent years.

It should be stressed that the development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a "green" sustainable process [3].

### *14.2.1 Classification*

Processes utilized in phytoremediation procedures can be shortly classified as follows:

**Phytoextraction** (decrease of soil metal concentrations by cultivating plants with a high capacity for metal accumulation in shoots), **rhizofiltration** (cleaning contaminated surface waters or wastewaters by adsorption or precipitation of metals onto roots or absorption by roots or other submerged organs of metal-tolerant aquatic plants), **phytostabilization** (immobilization of contaminant metals in soils or sediments by root uptake, adsorption onto roots, or precipitation in the rhizosphere), **phytodegradation** (elimination of organic pollutants by decomposition through internal or secreted plant enzymes or products), **rhizodegradation** (decomposition of organic pollutants by means of rhizosphere microorganisms), **phytovolatilization** (organic pollutants or certain metals (e.g., Hg or Se) absorbed by plants are released into the atmosphere by transpiration, either in their original form or after metabolic modification), and **hydraulic control** (uptake of large amounts of water that prevent the spread of contaminated wastewater into adjacent uncontaminated areas) (in detail see [3, 4, 8, 9]).

We would like to mention that for the last 20 years **phytoextraction (or phytoaccumulation)** has been growing rapidly in worldwide popularity. In general, this process has been tried more often for extracting heavy metals than for organic substances.

Strategies of the plants grown on the metal-containing soil (classified as accumulators and excluders) were based on the ratio between leaf/root metal concentrations [10]. This conception was later improved suggesting the following two groups of plants: metal excluders and metal non-excluders (indicators, hyperaccumulators) [11]. Conditions for abovementioned classification of plant strategies described by Ma et al. [12] are defined by two further characteristics: bioconcentration or bioaccumulation factor (BCF or BAF) and translocation factor (TF). Both factors have to be considered for evaluation whether a particular plant is a metal hyperaccumulator. The term BCF, defined as the ratio of metal concentrations in plant dry mass ($\mu g\ g^{-1}$ d.m.) to those in soils ($\mu g\ g^{-1}$ soil), has been used to determine the effectiveness of plants in removing metals from soils [13]. In the case of aquatic plants or plants cultivated in hydroponics BCF expresses the ratio of metal concentration in plant dry mass (μg $g^{-1}$ d.m.) to that in external solution (μg $cm^{-3}$). The plants growing on contaminated soils absorb contaminants through the roots and store them in root tissues and/or transport them into the stems and/or leaves. Living plants may continue to absorb contaminant until it is harvested. After harvest, a lower level of the contaminant can remain in the soil, so the growth/harvest cycle must usually be repeated to achieve a significant cleanup. After this process, the cleaned soil can support formation of some other vegetation. The main advantage of phytoextraction is environmental friendliness. It should be mentioned that traditional methods that are used for cleaning up toxic metal-contaminated soil disrupt soil structure and reduce soil productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to soil quality. Moreover, this approach is less expensive than any other cleanup process.

The further remarkable phytoremediation procedure is **phytotransformation** that represents a change in chemical structure without complete breakdown of the compound. After uptake of the xenobiotics, plant enzymes (such are peroxidases, phenoloxidases, esterases, or nitroreductases) increase the polarity of these substances by adding functional groups such as hydroxyl groups. During the further phase known as conjugation, plant biomolecules (e.g., glucose, amino acids) are added to the polarized xenobiotic to further increase of the polarity. Both abovementioned processes serve to increase the polarity and reduce the toxicity of the compounds as well as allow for easy transport of the xenobiotics along aqueous channels. However, in the final stage of phytotransformation, a sequestration of the xenobiotics occurs within the plant. The xenobiotics polymerize in a lignin-like manner and develop a complex structure that is sequestered in the plant tissues. This process ensures that xenobiotic is safely stored and thus does not affect the functioning of the plant.

**Rhizodegradation** (also known as **phytostimulation**) is degradation of contaminants (mainly different organic compounds) through the use of microbial activity that is enhanced by the presence of plant roots in the rhizosphere. This presents soil surrounding of root system that is directly influenced by root secretions named exudates. Within plant–microbe interactions, roots release these exudates, i.e., various natural substances such as sugars, alcohols, acids, or specific enzymes that stimulate microbiological activity in the soil. Microorganisms (e.g., yeast, fungi, bacteria) consume these contaminants as their source of energy and nutrition. In this process of biodegradation, certain microorganisms are capable of breaking down hazardous substances into nontoxic and harmless products (in detail see [14]). In process of rhizodegradation plant-supplied substrates stimulate microbial communities in plant root zones to cause contaminant dissipation. In spite of great interest and study, aspects of rhizodegradation have remained inadequately understood, much the same way as many rhizosphere phenomena, thereby delaying its routine implementation for cleaning up specific soil contaminants [15]. It should be mentioned that it is possible to develop transgenic plants with improved plant–microbe interactions. However, rhizodegradation as well as abovementioned application of genetically transformed plants is so comprehensive topic that should need special attention within extra chapter.

### 14.2.2 Application

Phytoremediation may be applied wherever the habitat (soil, water, air) has become polluted or is suffering ongoing chronic pollution. Examples, where this approach has been successfully used, include the restoration of abandoned metal mine workings and sites where some organic pollutants (e.g., polychlorinated biphenyls) have been dumped during manufacture and mitigation of ongoing coal mine discharges reducing the negative impact of contaminants in soils, water, and air. Over the past years, this biotechnology has become increasingly popular and has been employed at sites with soils contaminated mainly with lead, uranium, and arsenic. While it has the advantage that environmental concerns may be treated in situ, one major disadvantage of phytoremediation is that it requires a long-term commitment because the process is dependent on a plant's ability to grow and thrive in an environment that is not suitable for normal plant growth. Specific application of phytoremediation is in the region with large area of salinization of soils when these sites acquire better quality (process of land improvement and land evaluation, as well) (e.g., [16]).

Further research is needed in the field of genetic engineering to improve the phytoremediation abilities of transgenic plants and to understand the mechanisms and effectiveness of phytoremediation techniques in order to make these technologies more effective, time-saving, and economically feasible [6].

### 14.2.3 Advantages and Limitations

It could be shortly concluded that the cost of phytoremediation (both in situ and ex situ) is lower than that of traditional processes; it is potentially the least harmful procedure by reason that it uses naturally occurring organisms (plants) and preserves the environment in a more natural state. Moreover, plants that are exploited can be easily monitored, and valuable metals can be reused by companies specializing in "phytomining," as well. The metals accumulated in wood mass could be after separation reused in other sectors of industry (e.g., glassmaking). On the other hand, wood biomass is exercised as a biofuel.

However, phytoremediation is limited to the surface area and depth occupied by the root system. Survival of the plants is affected by the both properties and toxicity of the soil. It is not possible to completely prevent the leaching of contaminants into the groundwater or transfer of contaminants (especially metals) accumulated in plants into the food chain.

Phytoremediation has many advantageous features that make it an appropriate and successful technology, giving practitioners a valuable option for remediation. Its major advantage is the low cost to be 50–80% lower than estimated for some alternative applications of phytoremediation [3].

## 14.3 Fundamental Approaches in Experimental Design of Woody Plants Under Contaminated Conditions

In general, there are three fundamental approaches in experimental design for the woody plants cultivated under contaminated conditions: hydroponics, pots or boxes, and field experiments.

In the first two procedures, the plants are cultivated in growth chamber or cabinet with regulation of environmental factors (mainly irradiance, air temperature and humidity, day/night period, mineral nutrition). The most difficult (and thus also scarce) are field experiments that are usually realized at different dumping places, sites after ecological and harmful events, etc. However, the most important is the fact to have plants that are in good physiological fitness. Cultivation of woody plants (cuttings) in hydroponics, pots, and boxes on contaminated substrate enables rapid selection of woody species showing potential to accumulate/hyperaccumulate toxic metals or to eliminate organic contaminants from the substrate.

Correct experimental design with woody species is necessary to prepare young individuals from the cuttings that have good developed shoots as well as roots. Thus, in our earlier paper [17], we studied growth parameters (including rooting and root growth) of six fast-growing trees, *Salix viminalis* L., *S. alba* L., clone 21, *S. purpurea* L., and *S. cinerea* L., and two poplar species—*Populus x euramericana* cv. Gigant and *Populus x euramericana* cv. Robusta cultivated under two different conditions. Stem cuttings approx. 18 cm long from last year shoots were cut in March before the beginning of growing season. The cuttings were grown hydroponically in growth cabinet under the controlled conditions: Since effect of both different cultivation conditions and the cadmium was observed, first part of cuttings (variant A) was directly rooted and grown in control, 100 μmol $dm^{-3}$ $Ca(NO_3)_2$, and in Cd concentration, 10 μmol $dm^{-3}$ $Cd(NO_3)_2$, combined with 100 μmol $dm^{-3}$ $Ca(NO_3)_2$ treatment for 21 days. Second part of cuttings (variant B) was firstly rooted in Knop nutrient solution for 10 days. Then the plants were transferred into 100 μmol $dm^{-3}$ $Ca(NO_3)_2$, and after 3 days half of them was placed into 10 μmol $dm^{-3}$ $Cd(NO_3)_2$ for 7 days. In the variant B, the total time of hydroponic cultivation was the same as in the variant A. The solutions were changed every 3 days to prevent depletion of metals, nutrients, and oxygen. Twenty-one-day-old plants were washed in distilled water and used for experimental evaluation. It was found that the roots responded to Cd treatment more sensitively than the shoots. Cd treatment suppressed rooting and root growth (length and biomass production) as well as its development in all tested species. Root system of *S. cinerea*, *S. alba*, and *Populus x euramericana* cv. Robusta was more tolerant to Cd stress than the root system of the other studied species. Shoot growth parameters of *Salix* species were significantly reduced unlike *Populus* species, which were not affected by Cd treatment. Using similar methodical approach, Lunáčková et al. [18] tested abovementioned woody species to find potentially suitable clones able to tolerate and accumulate high Cd concentration in their organs without significant inhibition or damage of physiological processes, production characteristics, and primary metabolite content. The authors found that from all tested species grown at 10 µM $Cd(NO_3)_2$, *S. viminalis* and *Populus x euramericana* cv. Gigant were the most sensitive ones with the lowest accumulation of Cd into their shoots. The highest Cd accumulation in the shoots was found in *S. purpurea*, *S. cinerea*, *S. alba*, and *Populus x euramericana* cv. Robusta which were considered as more Cd-tolerant species. Relatively higher Cd accumulation in the leaves together with better root growth was established in *S. alba*.

After that Lunáčková et al. [19] compared some physiological, production, and structural characteristics of *S. alba* L. and *Populus x euramericana* cv. Robusta under two variants of cultivation: rooting in Knop nutrient solution prior to Cd treatment and direct cultivation in Cd. Some production parameters of *S. alba* roots (root cumulative length, number, and biomass production) and some physiological characteristics of *S. alba* leaves (assimilation pigment content, net photosynthetic rate, starch content, specific leaf mass) were positively influenced by pre-growing in Knop solution. Cd enhanced values of specific leaf mass in both species and caused xeromorphic character of leaves—increased stomata density but reduced stomata sizes. Assimilation pigment and starch contents, net photosynthetic rate, and specific leaf mass were positively influenced by indirect treatment. Indirect treatment lowered root Cd uptake in willow, Cd accumulation in cuttings of both species, and Cd accumulation in poplar shoot. Remarkable finding was that roots and shoots of *P. euroamericana* cv. Robusta rooted in Knop nutrient solution were more sensitive to toxic effect of Cd than plants exposed directly to Cd treatment. Pre-growing in Knop nutrient solution lowered root uptake of Cd in *S. alba*, accumulation of Cd in cuttings of both species, and translocation, and accumulation of Cd into the shoots in *P. euroamericana* cv. Robusta. Structural changes induced by Cd indicated better adaptation of roots grown during the whole experimental period in Cd than of roots formed in Knop solution and then transferred into Cd solution. The analyses of Cd content in roots, cuttings, and shoots showed that Cd ions were accumulated mainly in the roots.

Investigating physiological processes related to the uptake and accumulation of heavy metals by woody plants is of great practical importance for a better understanding of phytoextraction and enlarges the possibilities of the exploitation of trees for the remediation of polluted sites. According to this, the determination of reliable physiological/biochemical indicators for plants' successful survival and remediation potential in unfavorable ecological conditions is of crucial importance for distinguishing genotypes with high adaptive potential in contaminated environments. According to Pajevič et al. [4], the plants' selection criteria for high phytoextraction capacities are photosynthetic and transpiration potential, produced enzymes involved in detoxification and their activity, and biomass production, which is related to growth and survival rate, root system, and other criteria which affect the adaptive ability to tolerate different contaminants. In this sense, the aim of breeding programs is to produce genotypes (cultivars, clones) characterized by superior growth and resistance to high levels of pollutants which have to be extracted from the soil to the aboveground plant parts.

It could be concluded that there is still much fundamental and applied research needed to underpin phytoremediation technology, but this could be undertaken in conjunction with actual remediation scheme, which would achieve the dual purpose of treating contaminated sites and providing demonstration sites to show the application of phytoremediation [3].

## 14.4 Phytoaccumulation of the Most Important Metal Contaminants by Fast-Growing Woody Plants

### *14.4.1* **Salix** ***Species***

The results of a meta-analysis concerning heavy metal uptake by plant parts of willow species showed that all parts of willow species accumulated significantly more Cd, Pb, and Zn in contaminated soils than in uncontaminated soils. However, based on different metal accumulations among plant parts, willow species were found to be proven to be successful accumulators of Cd (twigs and leaves), Pb (roots and twigs), and Zn (twigs), and lower soil pH enhanced Cd accumulation rate in stems, which notably increased also with exposure time [20].

Among nine *Salix* taxa grown on soil containing high concentrations of Cu, Pb, and Zn, the highest metal contents were estimated in *S. x smithiana* roots (116 ± 8.76 mg $kg^{-1}$ d.w. (Cu), 87.84 ± 7.30 mg $kg^{-1}$ d.w. (Pb), and 203.42 ± 14.62 mg $kg^{-1}$ d.w. (Zn), respectively). In this experimental area, acidification of the rhizosphere and a higher concentration of acids, mainly oxalic, malic, malonic, acetic, and citric acids, were observed, while the levels of oxalic, malic, acetic, and citric acids increased in the roots of *Salix* taxa, and in the leaves formic and succinic acids were also detected. The highest concentration of acids in the rhizosphere and roots (73.48 ± 6.77 μM 100 $g^{-1}$ d.w. and 49.79 ± 2.65 μM 100 $g^{-1}$ d.w., respectively) was estimated for *S. x smithiana*, and their higher content in leaves was found for *S. alba* and *S. viminalis* "PR" (78.12 ± 3.95 μM 100 $g^{-1}$ d.w. and 71.12 ± 3.75 μM 100 $g^{-1}$ d.w., respectively) [21].

On the other hand, exogenously applied citric acid alleviated Cd toxicity through its chelating properties and increased activity of antioxidant enzymes that resulted in better encompassment of oxidative stress [22].

Based on physiological and biochemical responses of *Salix integra* Thunb to combined stress of Cu and flooding, this *Salix* species could be considered as highly tolerant [23]. Comparative study focused on the response of *Quercus* spp. and *Salix* spp. seedlings cultivated in pots containing 50% and 100% Pb/Zn mine tailings showed low metal TFs for all tested species, whereby TF values related to Cd (1.03–1.45) and Zn (1.42–2.18) estimated for *S. integra* notably exceeded those determined for the other species, and this species would be suitable for phytoremediation. On the other hand, as convenable candidate for phytostabilization purposes *Q. virginiana* showing high tolerance and low TF could be considered [24].

Mleczek et al. [25] tested 145 *Salix* taxa cultivated in an area affected by industrial activity, whereby the highest Zn, Cu, and Pb content in shoots was estimated in *S. eriocephala* 7 (58.99 ± 4.30 mg kg$^{-1}$ d.w.), *S. purpurea x viminalis* 8 (33.38 ± 2.91 mg kg$^{-1}$ d.w.), and *S. fragilis* 1 (24.64 ± 1.97 mg kg$^{-1}$ d.w.), while in washed leaves it was observed in *S. alba x triandra* (122.87 ± 12.33 mg kg$^{-1}$ d.w.), *S. purpurea* 26 (106.02 ± 11.12 mg kg$^{-1}$ d.w.), and *S. purpurea* 45 (55.06 ± 5.75 mg kg$^{-1}$ d.w.), respectively. However, as the most suitable for phytoremediation purposes, *S. acutifolia* was evaluated showing not only effective accumulation of all three metals but also producing shoots with optimal length and diameter.

Young plants of *Salix schwerinii* x *Salix viminalis* E. L. Wolf cultivated in pot experiment on soils contaminated with Cr, Zn, Cu, Ni, and total petroleum hydrocarbons were able to accumulate considerable amounts of abovementioned metals with mean concentrations in plant organs 17.05–250.45 mg Cr kg$^{-1}$, 142.32–1616.59 mg Zn kg$^{-1}$, 12.11–223.74 mg Cu kg$^{-1}$, and 10.11–75.90 mg Ni kg$^{-1}$. Moreover, a moderate reduction in total petroleum hydrocarbons in the contaminated soil was also observed [26].

Based on the transcriptome sequencing and expression analysis of Cd transport and detoxification-related genes in Cd-accumulating *Salix integra* metal tolerance protein (MTP1), ABCB25, NRAMP5, and ZIP1 may be involved in the Cd transport and detoxification in leaves, while NRAMP2, ZIPB, and NRAMP5 may be related to Cd transport in roots [27].

Proteomic approach used by Zemleduch-Barylska and Lorenc-Plucinska [28] to identify changes of leaf and fine roots proteins in *S. viminalis* grown on Cr-rich tannery waste from an active landfill compared to control soil conditions showed changes in metabolism with increases in energy production processes, their greater efficiency being estimated for leaves rather than root development, and this willow species was found to be not suitable for remediation of Cr-contaminated soil-containing tannery waste.

In two willow species cultivated on Technosols from a former gold mining site characterized with polymetallic contamination including As, Sb, and Pb, these metals were accumulated mainly in the rhizosphere of plants; however *S. purpurea* which accumulated more efficiently As in the plant's upper parts did not translocate Pb and Sb to its shoots, unlike to *S. viminalis* which was able to transfer these two metals in aboveground part [29].

In the field experiment investigating the ability of willow and poplar clones for remediation of soils moderately contaminated with Cd, Mn, and Zn using short rotation coppice plantations, the *Salix* clones were found to remove higher Cd, Mn, and Zn amounts compared to the *Populus* clones; the overall removal of metals was higher in older wood classes due to higher biomass yield, although young shoots showed usually higher metal content connected with lower wood/bark ratios as well as higher metal concentrations in bark suggesting benefits of longer rotations [30].

Field evaluation of eight willows under short rotation coppice cultivated on agricultural soil contaminated with Cd (6.5 ± 0.8 mg kg$^{-1}$) and Zn (377 ± 69 mg kg$^{-1}$) showed promising removal potentials of 72 g Cd $ha^{-1}$ $year^{-1}$ and 2.0 kg Zn $ha^{-1}$ $year^{-1}$, and removal of both metals can be increased by 40% if leaves are harvested, as well [31].

Investigation of combined effects of elevated $CO_2$ and Cd-contaminated soil on Cd accumulation of *Populus x euramericana* and *Salix jiangsuensis* showed that the increase of plant growth and total Cd uptake at elevated $CO_2$ was greater in highly than in low Cd-contaminated soil suggesting that stimulated plant growth by increasing leaf photosynthesis was reflected in improved phytoremediation efficiency [32].

Greger and Landberg [33] reported that 4 years of willow cultivation reduced Cd concentration in the soil by up to 27% and in grains of the post-cultivated wheat by up to 33%, and already 1 year of willow cultivation resulted in notable decrease of Cd in post grown wheat grains.

*S. babylonica* Linn. and *S. jiangsuensis* J172 showing tolerance indices of 91.15% and 84.26% in which the uptaken Pb from the soil reached 140.20 mg and 149.49 mg, respectively, at soil $Pb^{2+}$ concentration 800 mg $kg^{-1}$ were evaluated as species with greater potential for restoration of medium soil Pb contamination [34].

*S. viminalis* and *Ailanthus altissima* planted in soil contaminated with $Cr^{6+}$ and irrigated with water containing $Cr^{6+}$ (10 mg $dm^{-3}$) were able to reduce the Cr soil content after 360 days of cultivation from 70 (initial) to 32 mg Cr $kg^{-1}$ and 41 mg Cr $kg^{-1}$ dry soil and accumulated in the roots 2029 mg Cr $kg^{-1}$ d.w. and 358 mg Cr $kg^{-1}$ d.w., respectively, whereby the majority of Cr in all plant tissues was in the form of $Cr^{3+}$, *Ailanthus* exhibiting the lowest reduction of $Cr^{6+}$ in the roots. Based on root growth rate inhibition, *Salix* was found to be the more tolerant species to Cr toxicity and showed also higher Cr translocation from roots to leaves [35].

Quantitative expression analyses of 20 genes in leaves of *Salix caprea* grown on metal-polluted and unpolluted sites showed that some metallothioneins and cell wall-modifying genes were induced irrespective of the genotype's origin and metal uptake capacity, while a cysteine biosynthesis gene was expressed constitutively higher in the metallicolous genotype, and largest group of genes was only induced in the metallicolous genotype [36].

Tognetti et al. [37] reviewed the findings related to phytotechnology in shaping the multifunctional tree with special emphasis on *Salicacae* spp., particularly with regard to tree responses to environmental pollution, and suggested that the main challenge in the future will be maximizing the yield of multipurpose tree plantations while preserving or restoring ecosystem services of close-to-nature willow–poplar stands (e.g., riparian forests).

Hrynkiewicz and Baum [38] compared *Salix dasyclados* with usually stronger natural mycorrhizal colonization with *S. viminalis* showing lower natural mycorrhizal colonization with respect to the efficiency of phytoextraction of Cd, Pb, Cu and Zn from contaminated soil. The concentrations of heavy metals in the biomass of *S. dasyclados* were in general higher than in *S. viminalis* irrespective of inoculation with the ectomycorrhizal fungus. Inoculation with *Amanita muscaria* caused notable reduction of Cu concentration in the trunks of both *Salix* taxa; however it did not affected the levels of other heavy metals in the biomass. Higher perceptiveness of willow clones for mycorrhizal inoculum correlated with an elevated total extraction of heavy metals from contaminated soils, and as the main benefit of mycorrhizal formation of willows in phytoremediation of contaminated soils improved biomass production with relatively unchangeable metal concentrations was found.

It was mentioned that in the topsoil of 14 commercial willow short rotation coppice fields in Sweden after 10–20 years, 12% lower Cd levels were estimated compared to that observed in the reference fields [39].

At higher Cu concentration in soil, the biomass of *Salix jiangsuensis* CL "J-172" and *Salix babylonica* Linn showed a notable decrease and increased metal levels in the roots of both species, while at flooding beside increased Cu accumulation in the roots, decreased Cu accumulation in the stems and leaves was observed [40].

In both As-tolerant (*Salix viminalis x Salix miyabeana*) and an As-sensitive willow (*S. eriocephala*), addition of $PO_4^{3-}$ ions caused enhanced As accumulation in the tolerant genotype to a greater extent than in As-sensitive one, and increased expression of PHT1 transporters as a result of exposure to As in an As-sensitive genotype contributed to rapid toxicity; however the differences in detoxification capacity between genotypes are not connected with upregulation of γ-glutamylcysteine synthetase or phytochelatin synthase [41].

In a pot experiment investigating effects of endo- and ectomycorrhizal fungi on *S. alba* L. and *Populus nigra* L., it was found that poplar was colonized predominantly by *Glomus intraradices* and willow by *Hebeloma mesophaeum*, whereby *H. mesophaeum* increased willow height and biomass, while *G. intraradices* decreased poplar height; however *H. mesophaeum* caused considerable reduction of Cd and Fe accumulation in both willow and poplar shoots, respectively [42].

Gomes et al. [43] following cultivation of 1-year-old plantlets of *S. humboldtiana* Willd for 30 days in pots containing 0 or 15–30% (v/v) of contaminated soil with 18,600 mg Zn, 140 mg Cd, 450 mg Cu, and 410 mg Pb per kg soil estimated high levels of metals in leaves of willow ranging from 362 to 878 mg Zn $kg^{-1}$ d.w. and from 19 to 35 mg Cd $kg^{-1}$ d.w., whereby plants showed phytotoxicity symptoms caused by high Zn and Cd levels.

In leaves of willows grown on soil from the surroundings of a uranium mine treated with 5 mmol citric acid per 1 kg of soil in five doses of agent applied during a 5-week period, approx. 3.7 times more radium ($^{226}$Ra) was estimated compared to the control what was reflected also in notable increase of TF (1.26 ± 0.26 compared to 0.34 ± 0.17, respectively), while for uranium ($^{235}$U) this TF increase was lower (0.082 ± 0.210 compared to 0.040 ± 530, respectively). Addition of citric acid caused an enhanced uptake of essential elements Fe, Mn, and Cu that compete with uranium for complexing agent, and U uptake was significantly lower than that of Mn or Fe [44].

Zhivotovsky et al. [45] observed differences in uptake and translocation of Pb in willow cultivated in pot and field experiments, respectively. While in the pot experiment the plants were tolerant to soil Pb concentration of 21.36 g $kg^{-1}$ during 6 months and at application of ethylenediaminetetraacetic acid (EDTA), >1000 mg Pb $kg^{-1}$ was translocated into aboveground tissues, in the field experiments carried out in EDTA-treated soil, only 200 mg Pb $kg^{-1}$ was estimated in the aboveground tissues of willows after 4.5 months.

In the field trial lasting 45 days ethyl lactate-EDTA composite system was found to enhance Cd uptake in Cd-contaminated soil by autochthonous willow (*Salix x aureo-pendula* CL "J1011") to greater extent as application of EDTA alone what was reflected in 29% decrease of Cd soil concentration and 146.5% increase of Cd concentration in willow leaves compared to 20% reduction of Cd soil concentration and 56.7% increase of Cd concentration in leaves observed with alone EDTA treatment [46].

Investigation of Cd, Cu, Zn, and Pb uptakes by *Salix viminalis* grown on strongly polluted calcareous soils containing 18 mg Cd $kg^{-1}$, 1400 mg Cu $kg^{-1}$, 500 mg Pb $kg^{-1}$, and 3300 mg Zn $kg^{-1}$, as well as on moderately polluted soils with 2.5 mg Cd $kg^{-1}$ and 400 mg Zn $kg^{-1}$, respectively, showed that these were 2–10 times higher than uptakes by plants cultivated in growth chamber. Although the willow grown on strongly polluted soil, the metal accumulation in leaves of reached ≥80 mg Cd $kg^{-1}$ and ≥3000 mg Zn $kg^{-1}$; however, due to the fact that growth of willows was adversely affected, they were not suitable for phytoremediation purposes. On the other hand, at moderately polluted soils, they were able to extract 0.13% of total Cd and 0.29% of the total Zn per year, while Cu and Pb remained strongly fixed in calcareous soils [47]. Inoculation with the fungal strain *Hebeloma crustuliniforme* in combination with *Micrococcus luteus* resulted in the increase of total Cd and Zn accumulated in the shoot biomass of *Salix viminalis x caprea* up to 53% and in combination with *Sphingomonas* sp. up to 62%, respectively. The bacteria also improved plant growth, and the mycorrhizal dependency of willows colonized with the ectomycorrhizal fungus *H. crustuliniforme* [48].

Among six willow species (*S. babylonica*, *S. caprea*, *S. dasyclados*, *S. matsudana x alba*, *S. purpurea*, and *S. smithiana*) cultivated in soil contaminated with Cd and Zn, the highest Cd concentrations in leaves, 440 mg $kg^{-1}$, were found in two clones of S. smithiana (soil metal content: 32.7 mg Cd $kg^{-1}$ and 1760 mg Zn $kg^{-1}$) and 70 mg $kg^{-1}$ (soil metal content: 4.34 mg Cd $kg^{-1}$ and 220 mg Zn $kg^{-1}$), respectively, and the BCFs of the best performing clone were 15.9 for Cd and 3.93 for Zn on the less contaminated soil [49].

## *14.4.2* **Populus** *Species*

Among *Populus deltoides* (clone B-81) and *Populus x euramericana* (clone Pannonia) plants cultivated in soil moderately contaminated with Cd (8.14 mg $kg^{-1}$ soil) under semi-controlled conditions for 6 weeks, higher adverse effect on biomass production and morphological characteristics was estimated for *P. x euramericana*, but high tolerance to Cd was observed in both clones, the tolerance index being higher in *P. deltoides* than in *P. x euramericana* (82.2 vs. 66.5, respectively). Total Cd content accumulated in roots, shoots, and leaves was 58.0%, 18.2%, and 23.8% in *P. x euramericana* compared to 46.7%, 39.9%, and 13.4% estimated in *P.*

*deltoides* suggesting that *P. deltoides* could be used for both phytoremediation and phytostabilization purposes [50].

Ariani et al. [51] compared changes in epigenetic modifications in *Populus canadensis* I-214 clone under excess Zn and found that genes with a H3K4me3 modification (indicating trimethylation of lysine 4 on the histone H3 protein subunit) were high expressed, while genes with a H3K27me3 modification (indicating trimethylation of lysine 27 on the histone H3 protein subunit) on the 5′ untranslated region were low-expressed, whereby H3K4me3 modifications in roots were enriched in genes involved in carbon catabolism, in nitrogen metabolism, and in regulation of subcellular vesicular trafficking, and H3K27me3 modifications showed enrichment mainly in genes involved in photosynthetic processes. Short-term defense system in poplar plants exposed to Cd could be stimulated by additional nitrogen through glutathione synthetase and phytochelatin synthase synthetic pathways resulting in alleviation of the toxic symptoms in plants [52].

Rome et al. [53] investigated expression of specific genes involved in Cd uptake, translocation, vacuolar compartmentalization, and recycling in *Populus alba* Villafranca clone and observed that Cd exposure led to initially increased expression of the poplar homologues of IRT1, NRAMP, and OPT3, which was indeed reduced after longer-term Cd exposure. Cd exposure resulted in higher leaf and stem concentrations of some essential metals (Fe, Ca, Cu, and Mg) compared to control plants after 1 day and 1 week of experiment; however following 1 month Cd treatment, lower concentration of Mn, Fe, Cu, Co, and Mg in poplar roots was detected.

Exposure to Cd (10 mg Cd $kg^{-1}$ dry substrate) promoted the colonization by *Glomus intraradices* in male but not in female *P. deltoides* plants. Inoculation with *G. intraradices* alleviated the phytotoxic effects of Cd in females which were more sensitive to Cd stress than male plants by stimulating antioxidant enzymes resulting in reduced levels of reactive oxygen species and restricted Cd transfer to the shoots, while in male plants enhanced Cd accumulation was observed, and in both sexes more Cd was sequestered in the root systems [54]. Progress and prospect of research in transgenic poplar related also to phytoremediation were summarized by Ding et al. [55].

Treated wastewater representing a potential source of nutrients for reforestation with poplar trees could be used as an alternative strategy for recycling wastewater. Irrigation of 1-year-old *P. alba* cv. MA-104 with treated wastewater for 90 days resulted in increased biomass production by 36% and enhanced Cd and Pb accumulation capacity [56].

Kubatova et al. [57] studied the removal of Cd, Pb, and Zn from metal-contaminated soil by fast-growing clones of *S. schwerinii x S. viminalis*, *Salix x smithiana*, *Populus maximowiczii x P. nigra*, and *P. nigra* using short rotation coppice plantations. Application of sewage sludge resulted in higher shoot yield in the second harvest and reduced shoot metal concentration compared to control, whereby considerably higher removal of abovementioned elements in the shoots in the second harvest (except the clone of *P. nigra*) was connected with improved biomass yield. The best removal of Cd (up to 0.94%) and Zn (up to 0.34%) of the total soil element content was obtained with *Salix x smithiana*, and Pb was less available to plants than Cd and Zn, respectively.

Pb uptake was found to increase drought tolerance of wild-type and transgenic poplar (*Populus tremula x P. alba*) overexpressing gsh 1 *gene* encoding γ-glutamylcysteine synthetase. Higher Pb accumulation in the roots of transgenic plants was connected with effective chelation by glutathione (GSH), while the presence of Pb reduced GSH content in leaves of both plant types and transgenic plants exhibited higher adaptability when exposed simultaneously to mild drought and Pb. However, although enhanced Pb accumulation in the roots due to water stress was estimated, rewatering led to Pb leakage out of the roots suggesting that the transgenic plants cannot be considered as a good candidate for phytoremediation of Pb [58].

In general, the enrichment ability of heavy metals (HM) by polar was found to decrease in following order: Zn > Cu > Cd > Pb > Ni > Cr, whereby the highest HM enrichment capability showed the leaves. The total transfer ability of HM decreased as follows: Cu > Cd > Zn > Pb > Ni > Cr [59].

Exogenous abscisic acid (ABA) alleviated Zn uptake and accumulation in *Populus x canescens* seedlings grown in sand culture and exposed to excess Zn for 7 days, likely by modulating the transcript levels of key genes involved in Zn uptake and detoxification.

In poplar roots excess Zn resulted in enhanced transcript levels of several genes involved in Zn uptake and detoxification (e.g., yellow stripe-like family protein 2 and plant Cd resistance protein 2), which were repressed by exogenous ABA application [60].

As shown by an experiment in Hoagland nutrient solution, higher $Cd^{2+}$ uptake rates and elevated transcript levels of several genes involved in $Cd^{2+}$ transport and detoxification were estimated in transgenic poplar *P. tremula x P. alba* compared with wild-type plants. Improved Cd accumulation in the aerial parts of transgenic plants was connected with the overexpression of bacterial γ-glutamylcysteine synthetase in the cytosol of *P. tremula x P. alba* resulting in higher GSH concentrations in leaves. Moreover, in transgenic plants lower $O_2^-$ and $H_2O_2$ concentrations but higher concentrations of total thiols, GSH, and oxidized GSH in roots and/or leaves and stimulated foliar GSH reductase activity were estimated compared to wild plants. Thus, the higher tolerance of transgenic polar to $Cd^{2+}$ could be connected with GSH-mediated induction of the transcription of genes involved in $Cd^{2+}$ transport and detoxification [61].

The BCF estimated for *P. alba* L. var. *pyramidalis* Bunge grown on Cd-contaminated calcareous soils decreased from 2.37 to 0.25 with increasing soil Cd concentration and with stand age under field conditions, whereby Cd concentrations in individual plant organs decreased as follows: leaves > stems > roots [62].

Addition of chelating agents EDTA and ethylene glycol-(2-aminoethylether)-*N*,*N*,*N′*,*N′*-tetraacetic acid (EGTA) resulted in notable increase of the Cd concentration and amount in leaves of *P. alba* L. var. *pyramidalis* Bunge plants cultivated in two severely contaminated calcareous arable soils originating from a mining and smelting region using pot experiment, and due to stimulated translocation of metal from roots to leaves, >70% of Cd amount in poplar shoots distributed in leaves. Although high tolerance of *P. pyramidalis* to Cd was observed, its use for phytostabilization could be accompanied with collections of leaf fall to avoid the recycling of leaf-bound Cd [63].

Polyaspartate, a biodegradable chelatant, was found to improve the phytostabilization of Cu and Zn in *P. alba* L. (clone AL35) grown on agricultural soil highly contaminated with these two metals, and in the case of Cu it enhanced also the phytoextraction capacity [64].

Arbuscular mycorrhizal fungi (AMF) forming symbiotic associations with the plant roots improve nutrient uptake and enhance phytoextraction of HMs. Cuttings of the *P. alba* clone, AL35, characterized with high accumulation ability related to Cd and Zn and high foliar polyamine (PA) levels which were pre-inoculated with *Glomus mosseae* or *G. intraradices* and then transferred to pots containing HM-polluted soil showed comparable growth to that of controls grown on unpolluted soil but higher accumulated Cu and Zn amounts as well as an overall upregulation of metallothionein and PA biosynthetic genes, together with increased PA levels. A number of genes identified using a genome-wide transcriptomic analysis mostly belonged to stress-related functional categories of defense and secondary metabolism that were differentially regulated in mycorrhizal vs. non-mycorrhizal plants, and based on the results of proteomic analysis, it was found that depending on sampling time, changes in protein profiles were differentially affected by AMF and/or HMs [65].

A review paper related to the ecological role, applications, and scientific perspectives of *Populus* spp. in the twenty-first century was presented by Stobrawa [66].

The study performed with *Populus nigra* “Italica” plants treated with a combination of different concentrations of Cd (10, 25, 50 mg $kg^{-1}$ soil) and Pb (400, 800, 1200 mg $kg^{-1}$ soil) confirmed their suitability for phytoextraction processes of Cd in moderately contaminated soils as well as for phytostabilization in heavily contaminated soil, while for Pb remediation only phytostabilization process could be considered [67].

Pottier et al. [68] investigated genotypic variations in the dynamics of metal concentrations in poplar leaves in a field study and found that the leaf metal concentrations of individual genotypes showed notable differences, and Zn and Cd accumulation continued until leaf abscission in all genotypes, while Mg was remobilized during senescence, and also a positive correlation between natural resistance-associated macrophage protein 1 (NRAMP1) expression levels and BCF related to Zn was estimated.

The cuttings of the poplar clones AL22 (*P. alba* L.) and N12 (*P. nigra* L.) showing tolerance to Cu and Zn cultivated in iron-rich soil collected from an urban–industrial area accumulated Cd and Zn predominantly, while Cu, Fe, and Pb were mainly accumulated in roots. While N12 clone accumulated ≈10 times higher Cd concentrations than AL22 clone, accumulated Cu in AL22 exceeded that in N12 and enhanced Fe availability resulted in reduced uptake and accumulation of metals [69].

In *P. alba* L. var. *pyramidalis* Bunge plants grown on calcareous soils contaminated with multiple metals, the greatest accumulation of Cd and Zn was estimated in the leaves, while Cu and Pb were allocated mainly in the roots. The highest tissue concentrations of individual metals estimated in the pot experiment, namely, 40.76 mg $kg^{-1}$ for Cd, 8.21 mg $kg^{-1}$ for Cu, 41.62 mg $kg^{-1}$ for Pb, and 696 mg $kg^{-1}$ for Zn, were all noted in the multi-metal-contaminated soil, and the foliar concentrations of Cu and Pb were at most 8 mg $kg^{-1}$ and 5 mg $kg^{-1}$, respectively. Despite high Cd and Zn amounts accumulated in the leaves, phytoextraction using *P. alba* L. var. *pyramidalis* may take at least 24 years and 16 years for Cd and Zn, respectively [70].

Investigation of Cd, Cr, and Ni uptake from soil contaminated by these metals by different organs of *P. alba* in a pot experiment showed that the highest accumulation of all studied metals was found in the leaves; the concentrations of Cd and Cr were found to be higher in the fallen leaves compared to the green leaves; Cd and Ni were not transported from the leaves to the roots and stems, or vice versa, until the fall season, while the transport of Cr from the roots and stems to the leaves was observed at treatments with 240 mg Cr $kg^{-1}$ and 480 mg Cr $kg^{-1}$, respectively [71].

In greenhouse pot bioremediation experiments performed for 5 months using *Populus deltoides* LH05-17 grown with 300 mg $kg^{-1}$ As amendment in soil inoculated with rhizobacterium strain D14, 54% As in the soil was removed, while at the uninoculated treatments, it was only 43%, and also an increase of As concentrations in roots, stems, and leaves by 229%, 113%, and 291%, respectively, was observed, whereby approximately 45% As was translocated from roots to the aboveground tissues [72].

## 14.5 Phytoaccumulation of Metal Contaminants by Some Other Common Woody Species

Ten years after application of silico-aluminous (CFA1) and sulfo-calcic (CFA2) coal fly ashes (CFAs) to agricultural soil contaminated with high concentrations of Cd, Pb, and Zn, the effect of CFAs on *Robinia pseudoacacia*, *Alnus glutinosa*, *Acer pseudoplatanus*, and *S. alba* was investigated, and it was found that CFA amendments caused a decrease of the $CaCl_2$ -extractable fraction of Cd and Zn from the soil, alleviated oxidative stress in tree leaves, but they did not notably affect the concentrations of metal trace element in leaves. Due to application of CFA2, *A. glutinosa* and *R. pseudoacacia* showed considerable increase in the percentages of C16:0, C18:0, and C18:2 fatty acids, while that of C18:3 decreased and increased activities of superoxide dismutase (SOD) and peroxidase (POD) in leaves was detected. While application of CFA2 adversely affected cell membranes fluidity in leaves of these two species reflected in a decrease of C18:3 fatty acid content, it showed opposite effect on *A. pseudoplatanus*. On the other hand, reduction of lipid and DNA oxidation following application of CFA1 was associated with the absence or low induction of anti-oxidative processes [73]. In a previous similar experiment, Pourrut et al. [74] estimated that *A. glutinosa*, *A. pseudoplatanus*, and *R. pseudoacacia* could be considered as Cd, Zn, and Pb excluder due to accumulation of low metal concentrations in their leaves and young twigs, whereby application of CFA1 and CFA2 amendments to metal-contaminated soil strongly decreased metal translocation to aboveground parts of these trees, CFA2 being more efficient. Based on BCF values, the decrease of metal concentrations in the tree species was as follows: Zn > Cd > Pb in *A. glutinosa* leaves and twigs, Zn ≈ Cd > Pb in *R. pseudoacacia* twigs, and Cd > Zn > Pb for *A. pseudoplatanus*.

The comparison of metal accumulation and translocation in *Acer rubrum* and *P. tremuloides* growing in Northern Ontario showed that leaf tissues of trembling aspen accumulated more Ni and Zn than roots, while in red maple the concentration of metals in individual plant parts was low with regard to the corresponding bioavailable metal levels in soil. In *P. tremuloides* the estimated TFs for Fe (0.52–3.26), Mg (3.39–5.47), Ni (2.6–16.4), and Zn (1.41–4.1) were significantly higher than those determined for *A. rubrum* (Fe, 0.08–0.17; Mg, 2.62–4.13; Ni, 0.26–0.81; Zn, 0.71–0.90) suggesting that *P. tremuloides* could be considered as an accumulator for Ni and Zn, while *A. rubrum* is an excluder for Zn, and in soil contaminated with Ni, it uses the avoidance strategy [75].

Study of germination and early seedlings growth of *Ailanthus altissima* (Mill.) Swingle and *Acer negundo* L. exposed to 20, 50, and 90 μM Cd or Pb showed that both species were tolerant to presence of these metals; however, *A. altissima* showed higher tolerance to Cd and Pb treatment [76].

Evaluation of foliar, wood, and bark metal concentrations 2 and 12 years after afforestation of metal-polluted calcareous upland dredged sediment landfills with an oxidized topsoil showed that metal concentrations in bark and wood of maple, ash, and oak were generally lower than concentrations in the leaves of the same species, while normal foliar metal concentrations in ash, maple, oak, black alder, and small-leaved lime had normal foliar metal concentrations, Cd concentrations in maple being somewhat higher than in oak, ash, lime, and alder. On the other hand, very high Cd and Zn concentrations in hybrid poplar were estimated [77].

From five tree species planted on a mound constructed of dredged sediment slightly polluted with heavy metals, normal concentrations of Cd, Cu, Pb, and Zn were found in foliage of *Acer pseudoplatanus* L., *Alnus glutinosa* L. Gaertn., *F. excelsior* L., and *R. pseudoacacia* L., and these tree species were considered to be suitable for phytostabilization under the given conditions, while in leaves of *Populus alba* L., 8.0 mg Cd kg$^{-1}$ and 465 mg kg$^{-1}$ Zn were detected suggesting that autumn litter fall of poplar can represent a risk of Cd and Zn input into the ecosystem [78].

Compared to non-mycorrhized white birch plants (*Betula pubescens* Ehr.) grown on metal-polluted industrial soil, the mycorrhized ones showed after 60 days of cultivation higher biomass and lower metal accumulation connected with reduced hydrogen peroxide content and diminished activities of the antioxidant enzymes, suggesting that the symbiotic fungus could act as a barrier to the entrance of metals into the host plants [79]. On the other hand, plants of mycorrhized *Betula celtiberica* Rothm. & Vasc. grown in vitro in the presence of 10 mg Cd kg$^{-1}$ had accumulated more Cd in their shoots than the non-mycorrhized ones (526.03 ± 6.03 mg

$kg^{-1}$ d.w. and 95 ± 5335 mg $kg^{-1}$ d.w., respectively) and showed higher shoot/root ratio (0.344 vs. 0.239) suggesting improved Cd transport to the shoot. Moreover, the mycorrhized plants were characterized also with better development [80].

Zloch et al. [81] analyzed siderophore biosynthesis by siderophore-producing bacterial strains isolated from the roots and rhizosphere of *B. pendula* L. and *A. glutinosa* L. growing at two heavy metal-contaminated sites in Southern Poland in the presence of increasing concentrations of $Cd^{2+}$ (0.5–3 mM) under Fe deficiency. They observed higher abundance of siderophore-producing bacterial strains in the rhizosphere (47%) compared with root endophytes (18%), whereby the most effective siderophore synthesis was exhibited by the strains belonging to the genus *Streptomyces* which in the presence of $Cd^{2+}$ ions secreted siderophores such as hydroxamates, catecholates, and phenolates, and the addition of $Cd^{2+}$ resulted in higher siderophore (mainly ferrioxamine B) synthesis.

*B. pendula* was reported to be able accumulate high amounts of Zn in its leaves, in both the controlled (41–180 mg $kg^{-1}$) and in the contaminated environments (245–482 mg $kg^{-1}$), and "cleansing" of the soil was reflected in considerably lower Zn levels in the leaves of *Solidago virgaurea* grown near the trees than at 7 m away. While BCF of Zn in the leaves of *B. pendula* was 2.66 in the waste heap and 7.29 in the control area, the corresponding BCFs for *S. virgaurea* plants growing at 0.5 m distance of waste heap were 0.32 and 0.25 for control area, respectively; BCFs for *S. virgaurea* plants growing at 7.0 m distance of waste heap were 0.81 and 0.97 for control area. Zn content in the leaves of *B. pendula* was approx. 7–10 times higher than that of *S. virgaurea* leaves (corresponding to "normal" Zn contents), and according to the researchers, this species could be included in the group of hyperaccumulators of Zn [82].

White birch (*Betula papyrifera*) grown in Northern Ontario was reported to be a Ni and Zn accumulator with a translocation factor of 6.4 and 81, respectively, and an indicator of Cu and Pb [83].

As high concentrations of Pb and Zn as well as a high pH characteristic for soil at abandoned lead and zinc mine in Galicia (NW Spain) have adverse effect on revegetation, this limitation could be overcome by the use of organic amendments contributing to fixing of heavy metals or by implanting spontaneous vegetation adapted to these environmental conditions, e.g., species such as common broom (*Cytisus scoparius*) or white birch (*Betula celtiberica*) [84].

Investigation of bioaccumulation of As and Cd in birch and lime from the Bor region (East Serbia) which is known for Cu production showed highest As and Cd levels in plant material collected from the site in close proximity to the pollution source characterized with the greatest metal concentrations in soil and the lowest soil pH. The mean values of BCFs for roots and washed leaves and corresponding TFs estimated for As with lime were 0.030, 0.030, and 1.01, and with birch they were 0.013, 0.12, and 0.98, respectively, while for Cd the observed values were 0.10, 0.06, and 0.67 for lime and 0.09, 0.08, and 096 for birch. The estimated values of BCF < 1 and mobility ratio <1 indicated low rate of uptake and accumulation of As and Cd in both woody species, whereby better potential to express linear correlation between concentrations in plant parts and soil was observed with birch, and it was suggested that the trees could be used for phytostabilization purposes and as a vegetation protective barrier from considerable atmospheric pollution [85].

Screening of metal accumulation by several woody species grown on contaminated sites in the north part of France showed that highest Zn and Cd accumulation in leaves (950 mg Zn kg$^{-1}$ d.w. and 44 mg Cd kg$^{-1}$ d.w., respectively) was observed in *P. tremula x P. tremuloides*, whereby for Zn content positive correlation with Cd content was estimated, both in leaves and stems, and generally high BCF values for poplar and willow compared to other tested species suggested their good potential to be used in phytoremediation. On the other hand, Mn was accumulated in oak and birch species to higher extent than in other woody species [86].

At valorization of heavy metal-contaminated tree biomass, the pyrolysis temperature is imperative, because at temperatures generally used for the co-combustion or fast pyrolysis of biomass, some of the heavy metals can volatilize [87, 88].

Baltrenaite and Butkus [89] simulated transport of Zn, Pb, Cu, Ni, and Mn from the soil amended with industrial sewage sludge to seedlings of coniferous and leafy trees by adapting a generalized model of contaminant uptake by plants created by Hung and Muckay [90] and found that the results of modeling showed only low differences compared to those of measurements (about 6% in leaves, 5% in the stem, and 8% in roots).

Among *A. glutinosa*, *B. pendula*, and *Sambucus nigra* grown in $^{226}$Ra contaminated soil, the highest accumulation was estimated in birch, reaching 0.41 Bq g$^{-1}$ d.w. in the leaves at the end of the vegetation period and the lowest one in alder. Consequently, for the potential use of these trees for phytoremediation, removal of fallen leaves in the end of vegetation period is necessary [91].

*Salix caprea* L., *B. pendula* Roth. and a shrub, *Rubus idaeus* L., grown on soils of uranium-bearing dumps (Sudety Mts. SW Poland) containing a high proportion of polymetallic minerals accumulated high concentrations of HMs, whereby highest levels of Cd were accumulated in leaves of *S. caprea* (0.83–12.13 mg kg$^{-1}$; mean 4.11 ± 3.01 mg kg$^{-1}$), while in *R. idaeus* leaves, the lowest concentrations of heavy metals (except Mn reaching 182–949 mg kg$^{-1}$ with a mean of 449 ± 214 mg kg$^{-1}$) were detected [92].

Controlled laboratory studies using *Betula occidentalis* Hook. and *Carex microptera* Mack. cultivated on Pb-contaminated soil (3000 mg Pb kg$^{-1}$) and tailings (13,000 mg Pb kg$^{-1}$) collected from an abandoned mining site in Utah showed that lead was excluded by birch ($\leq$300 mg kg$^{-1}$ d.w.) that resulted in higher Pb concentration in rooting zone of investigated substrates, while in *C. microptera*, Pb accumulation in the aboveground tissue reached $\geq$1000 mg kg$^{-1}$ d.w. The obtained results suggested that metal-resistant plants can promote soil Pb stabilization; however this ability is affected by both soil characteristics determining solubility and mobility of Pb and the Pb concentration in soil [93].

Investigation of vanadium uptake and translocation in *Rhus copallinum*, *Betula populifolia*, and *P. deltoides* grown at a brownfield site in New Jersey, USA, showed that vanadium concentration in the plants decreased in a sequence of root > leaf > stem, and its translocation efficiency showed a decrease with increasing metal concentration in the soil, suggesting that vanadium absorption by the plant roots could be inhibited by its excessive level in the soil [94].

*Betula schmidtii* showing the shoot to root Cd concentration ratios over 1.26 following treatment with Cd was recommended for phytoremediation purposes by Oh et al. [95]. Seedlings of *B. schmidtii* cultivated in pots and irrigated with water containing 800 ppm and 1500 ppm of $Pb(NO_3)_2$, respectively, showed tolerance to Pb and were found to accumulate 602.0 mg Pb $kg^{-1}$ d.w. in shoots [96].

Roots of *R. pseudoacacia* L. seedlings inoculated with an isolate (BGC XJ01A) of arbuscular mycorrhizal fungus *Funneliformis mossea* cultivated in soil containing Pb (0, 90, 900, and 3000 mg Pb $kg^{-1}$ soil) showed considerably lower metal retention in roots in the inoculated seedlings, whereby higher proportion of Pb in the cell wall and soluble fractions and reduced proportion of Pb in the organelle fraction of roots, stems, and leaves were observed, the largest proportion of Pb being segregated in the cell wall fraction. Inoculation resulted in elevated content of pectate- and protein-integrated Pb and Pb phosphate and lower water-soluble Pb in individual plant organs and in improved plant biomass, height, and photosynthesis of black locust seedlings [97].

Elevated temperature caused increased microbial activity in rhizosphere soil of *R. pseudoacacia* seedlings exposed to Cd and Pb and resulted in notable enhancement of the removal rate of both metals in rhizosphere soils, being greater for Cd than for Pb [98].

Jia et al. [99] reported that elevated $CO_2$ affected secondary metabolite contents in *R. pseudoacacia* seedlings grown in Cd- and Pb-contaminated soils, whereby combine exposure to both metals resulted in greater increase in secondary metabolites (with the exception of condensed tannins in leaves and total alkaloids in stems) compared to treatment with individual metals, and saponins in leaves and alkaloids in stems were found to show stronger changes than other secondary metabolites. Changes in the production of secondary metabolites in *R. pseudoacacia* seedlings exposed to HMs by elevated $CO_2$ could be connected with modulated plant protection and defense mechanisms. Because under elevated $CO_2$ increased Cd and Pb uptake was estimated, this woody species could be a good candidate for the phytoremediation of heavy metal-contaminated soils under global environmental scenarios.

Pb concentrations in the leaves of *Cupressus sempervirens*, Arizona cypress and bioda thuja, grown around the battery production plant in Gaziantep, Turkey, reached 5768 mg $kg^{-1}$, 5340 mg $kg^{-1}$, and 2200 mg $kg^{-1}$, respectively, what represents 2000 times higher values compared to those determined for plants grown in uncontaminated regions, while Cd levels estimated in *Cupressus arizona* and bioda thuja grown around the battery plant and textile industry were up to 180 mg $kg^{-1}$ and 270 mg $kg^{-1}$, respectively [100]. Phytoextraction study focused on the ability of five woody species to extract Cu, Zn, or Cd from a polluted soil to their aboveground tissues showed that *Salix* and *Betula* transferred Zn and Cd to leaves and twigs, and *Alnus*, *Fraxinus*, and *Sorbus* excluded these metals from their aboveground tissues, whereby transfer of Cu to shoots was not observed [101].

Pinheiro et al. [102] exposed plants of *Fraxinus angustifolia* to metal-contaminated soils from the Cunha Baixa uranium mine (Mangualde, Portugal) for a period of about 3 months and found that the trees did not hyperaccumulate metals but extracted Sr at a higher rate and they were able to resist and adapt to the adverse conditions of contamination that could be utilized for phytoremediation and specifically phytostabilization purposes; however changes in the bacterial root communities compared to trees cultivated in uncontaminated soils were observed.

Low-methylesterified homogalacturonan, pectin epitope (JIM5-P), was reported as the cell wall polymer that may determine the capacity of cell wall for Pb binding and sequestration [103]. Hybrid aspen responded to Pb treatment by cell wall thickenings rich in such low-methylesterified pectins enabling binding of metal ions resulting in large accumulated Pb amounts that could be considered as a defense strategy of plants to cope with Pb [104].

Langer et al. [105] investigated the ectomycorrhizal impact on Zn accumulation of *P. tremula* L. grown in metalliferous soil with increasing levels of Zn concentration and found that due to barrier properties in the mycorrhizal treatments, the mycorrhizal inoculation of *P. tremula* could be advantageous in revegetation and phytostabilization of metal-polluted sites.

In hybrid aspen (*Populus tremula x tremuloides*) cultivated in field conditions in soil contaminated with several metals (Cd, Cr, Cu, Ni, V, Zn), strong correlation between Cd and Zn concentrations was estimated with maximal foliar concentrations of 35 mg Cd kg$^{-1}$ d.w. and 2400 mg Zn kg$^{-1}$ d.w., respectively, whereby the levels of foliar metallothionein 2b (MT2b) correlated with Cd and Zn concentrations suggesting that MT2b expression is one of the responses of *P. tremula x tremuloides* to chronic metal exposure [106].

For the amelioration of environmental problems at local (e.g., phytoremediation) and global (e.g., increased greenhouse effect) scales, short rotation forestry plantations employing high-density plantations of fast-growing tree species with a rotation period of fewer than 10–12 years can be used [107]. Evaluation of the potential of short rotation coppice remediation of $^{137}$Cs- and $^{90}$Sr-contaminated sites using *Salix caprea* and *P. tremula* showed that *S. caprea* accumulated greater levels of $^{137}$Cs than *P. tremula*, while accumulation of $^{90}$Sr in both species was comparable. Accumulation of $^{137}$Cs was greatest in the roots, while that of $^{90}$Sr in the leaves indicated higher TF for $^{90}$Sr compared to $^{137}$Cs. Based on the TF values, the researchers estimated remediation times for *S. caprea* as 92 years and 56 years for $^{137}$Cs and $^{90}$Sr, respectively. Short rotation coppice remediation of $^{137}$Cs- and $^{90}$Sr-contaminated soil using willows is favorable because of higher biomass, and the use of soil amendments improving bioavailability of $^{137}$Cs could enhance the phytoremediation effectiveness [108].

He et al. [109] using transcriptomic and physiological analyses studied Cd accumulation and detoxification in Cd-treated *Populus x canescens* (a hybrid between *P. alba* and *P. tremula*) plants cultivated in sand. Cd was situated in the phloem of the bark, and subcellular Cd compartmentalization was observed primarily in vacuoles of phloem cells. Due to Cd exposure, approximately 48% of the differentially regulated transcripts formed a coregulation network, and 43 hub genes were found to play crucial role not only in cross talk among distinct biological processes but also in coordinating the transcriptomic regulation in the bark of *Populus x canescens*. Cd accumulation was accompanied with decreased total N, P, and Ca and increased S content in the bark and induction of oxidative stress and antioxidants.

Sterile line of poplar *P. alba x P. tremula* var. *glandulosa* transformed with a heavy metal-resistance gene, ScYCF1 (yeast cadmium factor 1), which encodes a transporter that sequesters toxic metal(loid)s into the vacuoles of budding yeast, was found to establish an extensive root system in mine tailing soil and accumulated increased amounts of Cd, Zn, and Pb in the root suggesting that YCF1-expressing poplar could be used for phytostabilization and phytoattenuation in highly contaminated regions [110].

Durand et al. [111] analyzed proteomic changes in leaves and cambial zone of *P. tremula x P. alba* (717-1B4 genotype) after 61 days of exposure to Cd (360 mg kg$^{-1}$ soil d.w.) and observed changes in the display of 120 spots for leaf tissue and 153 spots for the cambial zone. Reduced photosynthesis caused serious reorganization of carbon and carbohydrate metabolisms in both tissues, and stress of cambial cells was connected with actually present Cd inside the tissue as well as by a deprivation of photosynthates due to leaf stress.

Analysis of serpentinophytes from northeast of Portugal for trace metal accumulation showed that *Ulmus procera* accumulated in the twigs 173.4 mg Cr kg$^{-1}$ d.w., and Mn accumulation in tissues of *Castanea sativa*, *Quercus pyrenaica*, *Fraxinus vulgaris*, and *Quercus ilex* was estimated as 125 mg kg$^{-1}$ d.w., 110 mg kg$^{-1}$ d.w., 109 mg kg$^{-1}$ d.w., and 108 mg kg$^{-1}$ d.w., respectively [112].

Parraga-Aguado et al. [113] assessed the metal(loid)s availability and uptake by *Pinus halepensis* Miller in a Mediterranean forest impacted by abandoned tailings containing high concentrations of Pb and As and found that due to higher root systems providing a better soil retention as well as relative low metal accumulation, this species could be applied in the phytostabilization of tailings.

## 14.6 Phytoaccumulation of Metal Contaminants by Some Remarkable (Non-exotic, Exotic, and Invasive) Woody Species

Alahabadi et al. [114] performed a comparative study on capability of different tree species in accumulating heavy metals from soil and ambient air and found that based on notable metal accumulating ability, the species suitable for phytoextraction are *Pinus eldarica* and *Morus alba* for Zn, *Fraxinus excelsior* and *Morus alba* for Cu, *P. eldarica* and *Ailanthus glutinosa* for Pb, and *P. eldarica* and *Nigral morus* for Cd. The maximum BCF values of Zn (0.647), Pb (0.227), and Cd (0.647) for leaves were found in *P. eldarica*; however this species did not show the maximum capability to accumulate multi-heavy metals. Using comparison of comprehensive bioconcentration index (CBCI) reflecting the capability of woody species in accumulating multi-HMs from soil, the highest CBCI values in leaves were observed in *P. eldarica* (6.72), *Populus deltoides* (6.17), and *Morus alba* (5.97).

Abbasi et al. [115] investigated the growth and nutrient uptake in 1-year-old potted seedlings of three forest species native to Iran, *Acer cappadocicum, Fraxinus excelsior*, and *Platycladus orientalis* exposed to Pb (100–500 mg $kg^{-1}$) in soil for 6 months, and observed a gradual decrease in dry weight of leaf and shoot of plants with increasing Pb concentration, minor decline of P, and unaffected uptake of N and K. P. *orientalis* was not only more tolerant to toxic metal treatment than the two broad-leaf species, but this conifer showed also highest values of tolerance index (TI; the ratio of dry weights of the seedlings grown in contaminated soil and control), TF, and BCF.

Based on the evaluation of $^{137}$Cs concentrations in new leaves of deciduous trees *Chengiopanax sciadophylloides* and *Acer crataegifolium* 2 years after the Fukushima Daiichi nuclear power plant accident, the concentration ratios (CR) reflecting $^{137}$Cs concentration in plant (Bq $kg^{-1}$ d.w.) per $^{137}$Cs concentration in soil (Bq $kg^{-1}$ d.w.) suggested that these species are accumulators of $^{137}$Cs and could be used for phytoremediation. For example, the CR values estimated for *C. sciadophylloides* ranged between 0.49 and 0.60 in 2011 and increased to 2.3–11 in 2012, while for *A. crataegifolium* in 2012, CR values ranging from 3.9 to 11 were observed [116].

Screening for native metal accumulator woody plants in Zanjan, Iran, showed that among eight tested trees grown on heavy metal-contaminated soil, *Cupressus sempervirens* var. *arizonica* was found to be the best accumulator of Pb (1346.680 mg $kg^{-1}$ d.w.), *Thuja orientalis* the best phytoextractor for Fe (308.652 mg $kg^{-1}$ d.w.), and *P. nigra* the best accumulator for Mn, Zn, and Cd (47.125 mg $kg^{-1}$ d.w., 291.750 mg $kg^{-1}$ d.w., and 4.166 mg $kg^{-1}$ d.w., respectively) when compared to metal accumulation ability of rest-tested trees *Ulmus pumila*, *F. excelsior*, *R. pseudoacacia*, *Acer hyracanum*, and *Salix alba* [117].

Liu et al. [118] studied heavy metal accumulation by native plants grown in a Mn mining area of Guangxi, South China, at soil characterized by low pH and low N and P levels in which Mn and Cd concentrations exceeded the pollution threshold, whereby *Camellia oleifera* was able to accumulate high concentrations of these metals in the aerial parts (5608.3 mg Mn $kg^{-1}$ and 1.15 mg Cd $kg^{-1}$, respectively) that could be utilized in soil phytoremediation. Pb/Zn concentrations in the six species cultivated in soil substrate obtained from Pb/Zn mine tailing containing 1220 mg Pb $kg^{-1}$ and 2134 mg Zn $kg^{-1}$, respectively, and an average pH value of 7.3 were generally in the order of *Alnus nepalensis* > *Pinus yunnanensis* > *Betula alnoides* > *Eucalyptus globulus* > *Acacia dealbata* > *Cupressus duclouxiana*, showing notable higher metal levels in root tissue than in leaf and stems. In general, Pb concentrations in the seedlings decreased in the order of root > shoot > leaf (except for *P. yunnanensis*), while that of Zn decreased as follows: root > leaf > shoot (except for *B. alnoides*). As appropriate species which could be used in afforestation of mine tailing areas with high levels of Pb/Zn, *Alnus nepalensis* accumulating 4.70 mg Pb and 6.92 mg Zn per seedling and *B. alnoides* with 1.85 mg Pb and 3.74 mg Zn per seedling could be considered [119].

Ozen and Yaman [120] determined histidine and Pb in leaves of six woody species grown on industrial areas in Turkey, including Gaziantep and Bursa cities. The following concentrations of histidine/Pb (in mg $kg^{-1}$) were estimated: 2–9/4–378 for *Morus*, 6 and 13/1–122 for *R. pseudoacacia*, 2 and 10/1–14 for *Populus nigra*, 3 and 10/1.6–224 for *Thuja* (family *Cupressaceae*), 1 and 11/1.5–57 for *Cupressus arizonica*, and 4/1.8 for *Cedrus libani*. Thus, the leaves of *Morus*, *Thuja*, and *R. pseudoacacia* could be recommended for biomonitoring or as hyperaccumulators for Pb. *Cupressus sempervirens* was reported to be a suitable species for phytoaccumulation of Pb [117].

Lu et al. [121] tested *Salix matsudana*, *R. pseudoacacia*, *Picea crassifolia*, and *Lycium barbarum* grown on soils containing 107–3045 mg $kg^{-1}$ Ni, 116–2580 mg $kg^{-1}$ Cu, 7.1–22.7 mg $kg^{-1}$ Cd, 115–897 mg $kg^{-1}$ Cr, and 23.2–144.3 mg $kg^{-1}$ Co for phytoremediation purposes, and because the values of BCF and TF were found to be less than 1 for all five metals, these species could be considered as suitable for the phytostabilization of metal-polluted sites. On the other hand, *Caragana korshinskii* showed BCF for Cd > 1.

Evaluation of heavy metal accumulation in woody plants grown in a Pb/Zn mine area at Northwest China showed that *Ailanthus altissima* (Mill.) Swingle, *Cotinus coggygria* Scop., *Populus simonii*, *P. purdomii*, and *R. pseudoacacia* could be considered as suitable candidates for phytoextraction and/or phytostabilization purposes, whereby association with arbuscular mycorrhizal fungi improved the efficiency of phytoremediation. While *P. purdomii* Rehd. accumulated 432.08 mg Zn $kg^{-1}$ and 140.85 mg Cu $kg^{-1}$ in leaves, in roots of *R. pseudoacacia* and *P. simonii*, 712.37 mg Pb $kg^{-1}$ and 3.86 mg Cd $kg^{-1}$ were estimated suggesting that under metal stress both translocation of higher extent of essential metals into the aerial parts and higher retention of toxic metals in roots can protect the aboveground parts from damage [122].

Results of the analysis of heavy metal enrichment ability of woody plants at ancient copper mine site in Tonglushan of Hubei Province showed that *Platanus x acerifolia* (Ait.) Willd and *Broussonetia papyrifera* (Linn.) L' Hert. ex Vent. are suitable for planting in Pb-contaminated area, while *Viburnum odoratissimum* var. *awabuki*, *Firmiana platanifolia*, *R. pseudoacacia*, and *Melia azedarach* Linn. could be used in Cd-polluted area. On the other hand, for decontamination of Cu–Cd–Pb complex pollution area, *V. odoratissimum* var. *awabuki*, *M. azedarach*, *Ligustrum lucidum*, *F. platanifolia*, *Osmanthus fragrans* (Thunb.), and *R. pseudoacacia* are suitable. Strong enrichment ability to Cu, Cd, and Pb which was reflected in averages of complex enrichment coefficient on these metals exceeding 1 was estimated for *V. odoratissimum* var. *awabuki*, *M. azedarach*, *L. lucidum*, *F. platanifolia*, and *O. fragrans* [123].

Among woody plants grown on soils surrounding the old flotation tailings pond of the copper mine containing high levels of Cu (1585.6 mg $kg^{-1}$), Fe (29 462.5 mg $kg^{-1}$), and As (171.7 mg $kg^{-1}$), respectively, the estimated BCF and TF values for *Prunus persica* were 1.20 and 3.95 for Cu, 1.5 and 6.0 for Zn, and 1.96 and 5.44 for Pb, and for *Juglans regia* L., they were 8.76 and 17.75 for Zn, and *R.*

*pseudoacacia* L. was selected as the most suitable species for phytostabilization of Zn [124].

The evaluation of the uptake ability of Pb and Cd by 1-year-old leaves and shoots of *Platanus orientalis*, *R. pseudoacacia*, and *Fraxinus rotundifolia* grown in Karaj city, the western Tehran (Iran), showed that shoots of *F. rotundifolia* and *R. pseudoacacia* accumulated considerably higher Cd amount than *P. orientalis*, while no significant differences among the trees were estimated in leaf Cd and Pb content. On the other hand, notably higher Pb levels in shoots of *R. pseudoacacia* were estimated compared to the other species. The total metal accumulation capacity can be evaluated using metal accumulation index (MAI); the highest MAI value of 2.21 was observed for *R. pseudoacacia* leaves, while *F. rotundifolia* had the highest MAI value of 2.4 for shoots [125].

The addition of 1040 mg $kg^{-1}$ $Mg^{2+}$ was able to improve the growth of *Torreya grandis* seedlings which was adversely affected due to cultivation in soils treated with 1400 mg $kg^{-1}$ $Pb^{2+}$, increased root growth and oxidative activity, protected the root ultrastructure, and resulted in five times higher concentrations of $Pb^{2+}$ in the roots and four times higher translocation of $Mg^{2+}$ to the shoots than in plants without $Mg^{2+}$ application [126].

Among investigated woody species *Albizia amara*, *Casuarina equisetifolia*, *Tectona grandis*, and *Leucaena luecocephala* tested for Cr phytoaccumulation, the highest increase in Cr content showed *A. amara* when treated with 20 mM citric acid addition, whereby $Cr^{6+}$ showed more adverse effect on plant growth than $Cr^{3+}$, and roots accumulated >10-fold more Cr than the shoots [127].

*Eucalyptus camaldulensis* could be regarded as a suitable species for phytostabilization of Cd-contaminated soil and application of organic fertilizers (cow manure, pig manure, and organic fertilizer) to soils resulted in lower Cd accumulation in plants compared to plants without fertilizer treatment, whereby the TF values < 1 and a BCF values for the root >1 indicated that this species can stabilize Cd in the roots. Lower Cd concentrations in plants were connected with dilution effect caused by improved plant growth rates at fertilizer application which was higher than the ability of plants to accumulate Cd [128].

Eucalyptus camaldulensis cultivated on soils containing 1069 mg $kg^{-1}$ of As and 4086 mg $kg^{-1}$ of Pb was found to be tolerant to this soil contamination; it showed low BCFs for soil contaminants and accumulated in leaves low amounts of As and Pb, whereby the accumulation of toxic metals in flower buds was even lower than in leaves suggesting its suitability for phytostabilization of metal-contaminated soils. On the other hand, *Salix purpurea* accumulated in leaves about 15 times higher Cd concentration and 6 times higher Zn concentration than *E. camaldulensis* [129].

AM spores from native plant species collected from the mining site which were multiplied and inoculated onto *E. camaldulensis* were found to improve its growth as well as its tolerance to high soil Cu content [130].

Seedlings of *Acacia aulacocarpa* and *Eucalyptus urophylla* inoculated with ectomycorrhizal fungus *Pisolithus* 23-01 which were cultivated in Cu mine tailing soil showed improved plant growth and Cu tolerance suggesting that they can be selected for rehabilitation of Cu mine tailings in the Philippines [131].

An overrepresentation of enzymes involved in photosynthesis was estimated in *E. camaldulensis* grown in HM-contaminated areas, and the activation of these energetic pathways increased by soil microorganism (AMs/PGPRs) additives, and trees exposed to HMs exhibited an overrepresentation of antioxidant enzymes, chaperones, and proteins involved in GSH metabolism [132].

*E. urophylla* and *E. citriodora* seedlings cultivated in soil containing high levels of arsenate were characterized with notably reduced root and shoot dry mass and the height of both species, adverse effects being more pronounced in *E. urophylla* plants. As was accumulated mainly in roots, and increasing As concentration reduced TF and BCF, whereby *E. citriodora* plants showed higher tolerance to As than *E. urophylla* and translocated this element to the shoot [133]. As suitable eucalypt species for As phytoextraction of As-polluted soils, *E. grandis* accumulating As in the root system and stem was recommended [134].

Sanchez-Palacios et al. [135] reported that arsenic in the tissue of *Eucalyptus cladocalyx* was found in form of As(III) coordinated to phytochelatins (PCs), mainly as As(III)-PC3, and As complexation was detected in the root endodermis while in vascular bundles was undetectable suggesting that these complexes significantly affected As tolerance. *E. cladocalyx*, as an ideal candidate for the long-term phytostabilization of As-polluted areas land and mine tailings, was recommended also by King et al. [136].

*Eucalyptus globulus* trees grown on metal-polluted soil can remove much more Cd and Hg than those from uncontaminated soils that is connected with the fact that *E. globulus* can produce more biomass and consequently it can uptake more heavy metals than non-metalliferous plants per year [137].

Mughini et al. [138] tested 13 hybrid *Eucalyptus* clones (*E. camaldulensis x E. viminalis*; *E. camaldulesis x E. grandis*; *E. camaldulensis x E. globulus* subsp. *bicostata*) for the As and HM uptake from soil in a Mediterranean environment and estimated considerably higher levels of As, Cu, Pb, and Zn in leaves than in stems and branches suggesting *Eucalyptus* species as a suitable short rotation woody crops for phytoremediation of HM-contaminated soils.

*E. camaldulensis* grown spontaneously in the area of the former zinc smelter "Pertusola Sud" (Crotone, Italy) on soil considerably contaminated by metals (Cd, Cu, Ge, Hg, In, Pb, Tl, and Zn) and metalloids (As and Sb) showed high Sb accumulation in the leaves, and estimated values of BCF and TF related to this metal were 1.11 and 1.71, respectively [139].

Meeinkuirt et al. [140] investigated the effect of applied fertilizer on growth and Pb accumulation of six tree species (*Leucaena leucocephala*, *Acacia mangium*, *Peltophorum pterocarpum*, *Pterocarpus macrocarpus*, *Lagerstroemia floribunda*, *E. camaldulensis*). In pot experiment following application of cow manure, the highest root Pb accumulation (>10,000 mg $kg^{-1}$) as well as the highest Pb uptake per plant was observed in *L. floribunda* and *P. macrocarpus*, while in field experiment *E. camaldulensis* and *A. mangium* treated with Osmocote fertilizer exhibited the highest Pb uptake (600–800 μg $plant^{-1}$).

After 1 year of phytoremediation of tannery sludge dumps of common effluent treatment plant containing high concentrations of HMs in mg $kg^{-1}$ d.w. [1667 (Fe), 628 (Cr), 592 (Zn), 427 (Pb), 354 (Cu), 210 (Mn), 125 (Cd), and 76 (Ni), respectively] by woody plant species *Terminalia arjuna*, *Prosopis juliflora*, *Populus alba*, *Eucalyptus tereticornis*, and *Dendrocalamus strictus*, high BCFs for different metals were detected showing a decrease in the order Fe > Cr > Mn > Ni > Cd > Pb > Zn > Cu, and the removal of toxic metals decreased as follows: Cr (70.22%) > Ni (59.21%) > Cd (58.4%) > Fe (49.75%) > Mn (30.95%) > Zn (22.80%) > Cu (20.46%) > Pb (14.05%) [141].

The saprophytic fungi *Trametes versicolor* and *Coriolopsis rigida* were found to increase the shoot dry weight and the tolerance of *E. globulus* to Zn when these plants were colonized with arbuscular mycorrhizal fungi *Glomus deserticola* and also increased Zn uptake by plants. It can be stated that in the presence of Zn, *G. deserticola* played a filtering/sequestering role resulting in improved Zn uptake and accumulation, while saprophytic fungi affected the colonization and metabolic activity of the AMF, and in this way they had impact on the tolerance and the accumulation of Zn in *E. globulus* [142]. On the other hand, inoculation with *G. deserticola* and saprobe fungus *Coriolopsis rigida* caused considerable enhancement of Cu uptake by *E. globulus* at 2000 mg Cu kg$^{-1}$ sand:soil (1:1; v/v) up to levels reached by hyperaccumulative plants [143].

Evaluation of different agroforestry tree species for their suitability in the phytoremediation of seleniferous soils performed by Dhillon et al. [144] showed that effective removal of Se takes place through the stem portion of different trees where it constitutes 30–50% of total Se, whereby in one growing season, *Dalbergia sissoo* aged 24 years, 10-year-old *Populus deltoides*, and 10-year-old *Eucalyptus* hybrid were found to remove 2385 g Se per hectare, 1845 g Se per hectare, and 1407 g Se per hectare, respectively, and under an agroforestry farming system of poplar/mentha/wheat, the removal increased to 4207 g Se per hectare resulting in 43–65% Se reduction for the soil surface layer and from 13% to 20% for the whole soil profile.

Mycorrhizal fungi *Glomus mosseae* and *G. deserticola* were found to increase the shoot dry weight of *E. globulus* treated with 1500 mg kg$^{-1}$ Al compared to control, and this effect was increased by co-inoculation with saprobe fungus *Trichoderma koningii* and was accompanied with the highest Al accumulation in the shoot suggesting that such treatment contributes to the increase in resistance of *E. globulus* to Al. The formation of stable Al complexes with short-chain organic acids could represent an internal Al detoxification mechanism reflected in Al tolerance of eucalypt plants [145].

Among three woody species, *Cassia siamea*, *Azadirachta indica* (neem), and *Holoptelia integrifolia* grown on the manganese mine tailing dump, *H. integrifolia* was able to accumulate high Mn amounts under stress [146].

The tolerance to Pb pollution of six tree species exposed to $Pb(NO_3)_2$ and $Pb(CH_3COO)_2$ was as follows, *Amaorpha fruticosa* L. > *Platycladus orientalis* L. > *Koelreuteria paniculata* Laxm. > *R. psedoacacia* L. > *Pinus tabuliformis* Carr. > *Hippophae rhamnoides* L., suggesting that Pb-tolerant species *A. fruticosa* L. and *P. orientalis* L. could be used for phytoremediation purposes, while

Pb-sensitive *H. rhamnoides* L. could be used as an indicative plant to diagnose the toxicity of Pb contamination on soil quality [147].

In 6-month-old seedlings of *Pinus densiflora* and *Quercus variabilis* separately inoculated with three species of ectomycorrhizal fungi (*Pisolithus* sp., *Cenococcum geophilum*, *Laccaria laccata*) and transplanted to the copper tailing significantly enhanced growth and nutrient uptake 6 months after planting was observed, whereby *P. densiflora* plants accumulated higher amounts of Cu and Zn in roots compared to non-inoculated seedlings; however, inoculation was found to inhibit the HM accumulation in shoots [148].

Wild and selected genotypes of *Pinus pinaster* inoculated with ectomycorrhizal fungi, *Suillus bovinus* and *Rhizopogon roseolus*, and exposed to soil contaminated at 15 and 30 mg Cd kg$^{-1}$ responded differently to Cd exposure depending on the mycorrhizal association. While the non-inoculated wild genotype accumulated 1.7-fold more Cd in the shoots at 30 mg Cd kg$^{-1}$ than the selected genotype, Cd concentration in the roots of the selected genotype showed a decrease at inoculation with *R. roseolus*, in contrast to wild genotype for which opposite trend was observed [149].

Inoculating the native plant *Alnus firma* with *Bacillus thuringiensis* GDB-1 isolated from roots of *Pinus sylvestris* showing the capacity to remove heavy metals from mine tailing promoted the growth of *A. firma* seedlings, likely due to production of indole acetic acid, siderophores, 1-aminocyclopropane-1-carboxylic acid deaminase, and P solubilization. It increased HM concentrations in roots by 154% (Ni), 135% (Cd), 120% (Zn), 117% (Pb), 114% (Cu), and 113% (As), and in shoots by 175% (Ni), 160% (Cd), 137% (Zn), 137% (Pb), 161.1% (Cu), and 110.1% (As), and because of improved metal phytoaccumulation efficiency, this species is suitable for phytoremediation of soil-containing HM-contaminated mine tailings [150].

Screening of 12 woody species focused on their ability to remediate HMs showed that for the removal of Cu, Cr, and Ni, szechwan juniper and poplar were the most suitable, while removal effects of Zn were better by *Prunus persica*, *Osmnthus fragrans*, and *Sophora japonica*. *Magnolia grandiflora* and *Sophora japonica* were classified as plant species showing better removal of Hg, while *Cedrus deodara* showed good removal effects of Cd and Cu [151].

## 14.7 Rhizoremediation of Organic Contaminants by Woody Species

### *14.7.1 Polycyclic Aromatic Hydrocarbons*

The rhizoremediation of organic contaminants is connected predominantly with the ability of certain plants to increase microbial xenobiotic degradation rates [152, 153], whereby microbial stimulation in soil is accomplished through root exudation [154, 155].

For efficient hydrocarbon rhizoremediation applications, *Salix* spp. are particularly suitable because they can improve degradation of organic pollutants in soil, being tolerant to high pollutant concentrations; they possess extensive root systems [156–158] and could be cultivated in many climatic zones [159].

Jia et al. [160] performed a pot experiment focused on the study of phenanthrene (Phe) and pyrene (Py) dissipation in spiked sediments using *Avicennia marina* (Forsk.) Vierh. and estimated notably enhanced dissipation of both polycyclic aromatic hydrocarbons (PAH) in the rhizosphere compared with non-rhizosphere sediments, whereby dissipation was stimulated considerably over the contribution of direct plant uptake and accumulation of both PAH. In the rhizosphere sediments, under 50 mg kg$^{-1}$ PAH contamination, the Phe and Py residual were considerably lower (8.1 mg kg$^{-1}$ and 13.2 mg kg$^{-1}$, respectively) after 120 days than in non-rhizosphere sediments (12.4 mg kg$^{-1}$ for Phe and 16.6 mg kg$^{-1}$ for Py). The estimated BCFs related to roots were 0.42–0.60 for Phe and 0.66–0.81 for Py, respectively, suggesting that *A. marina* was not a hyperaccumulator plant for PAHs. With increasing PAH concentrations, notable increase of antioxidant and detoxification enzyme activities in roots and leaves were observed suggesting that *A. marina* had defense mechanism capacity against abiotic stress.

Page et al. [161] reported that *Salix purpurea* stimulated the expression of specific bacterial xenobiotic degradation genes in a soil contaminated with hydrocarbons. Considerably higher abundance of transcripts encoding for alkane 1-monooxygenases, cytochrome P450 monooxygenases, laccase/polyphenol oxidases, and biphenyl 2,3-dioxygenase small subunits were estimated in the vicinity of the plant's roots than in bulk soil indicating that *S. purpurea*-microbe systems could target the organic contaminants such as $C_4$–$C_{16}$ alkanes, fluoranthene (Fla), anthracene, benzo(a)pyrene, biphenyl, and polychlorinated biphenyls (PCBs), and it was found that members of the bacterial orders *Actinomycetales*, *Rhodospirillales*, *Burkholderiales*, *Alteromonadales*, *Solirubrobacterales*, *Caulobacterales*, and *Rhizobiales* are active participants in the exposed partnerships.

Because pine (*Pinus elliottii*) needle litter-derived dissolved organic matter was found to reduce the strong affinities of both Phe and Fla to soil and significantly inhibited PAHs sorption and supported PAHs desorption, it contributed to higher leaching of PAH in soil [162].

Wang et al. [163] reported that the concentrations of the low-molecular-weight organic acids (LMWOAs) from root exudates and the dehydrogenase activity in the rhizosphere sediments of three mangrove plant species *Kandelia obovata* Sheue, *Bruguiera gymnorrhiza* (L.) Poir, and *Avicennia marina* (Forsk.) Vierh. cultivated on sediment contaminated with PAHs (fluorene, Phe, anthracene, Fla, Py, chrysene, benzo[a]pyrene, and benzo[k]fluorene) changed species specifically with the levels of PAH contamination. In the tested plant species, the highest concentration among LMWOAs showed citric acid, followed by succinic acid, whereby succinic acid was positively related to the removal of all the PAHs except chrysene, and positive correlations were estimated also between the removed extent of Fla, Py, chrysene, benzo[a]pyrene, and benzo[k]fluorene, and all LMWOAs, except citric acid. LMWOAs enhanced dehydrogenase activity that contributed to more effective PAH removal. The removal of all PAH, but particularly of Py, chrysene, benzo[k]fluorene, and benzo[a]pyrene, was notably higher with *K. obovata* and *B. gymnorrhiza* compared to *A. marina* and at high PAH contamination level as the most efficient from the aspect of PAH removal was found to be *B. gymnorrhiza*.

While in unamended soil, the plants generally increase the accessibility of PAHs, both active carbon and biochar decreased the bioaccessibility of PAHs in soil, and willow grown in active carbon- and biochar-amended soil accumulated less Phe than in the control soil [164].

The investigation of the degradation of PAH in an aged creosote-contaminated soil in the presence of *Salix viminalis* in a greenhouse experiment showed that 100% of Phe and 80% of Py were degraded compared to 68% and 63% without plants, and *S. viminalis* also significantly increased microbial populations [165].

*Betula pendula* and *Morus rubra* cultivated either separately or together with perennial ryegrass (*Lolium perenne*) in a greenhouse experiment in long-term PAH-contaminated soil were tested with respect to their effect on enhanced biodegradation of 15 selected PAHs, and it was found that after 1 year of cultivation, the overall content of PAHs declined to 50% (for Fla and Py it represented even 27% and 21.6%) of the initial PAH amount and almost entirely undegraded remained only benzo[ghi]perylene and indeno[1,2,3-cd]pyrene [166]. *B. pendula* showing a rhizosphere effect reflected in augmented extradiol dioxygenase diversity in rhizosphere caused more efficient Py degradation [167].

In greenhouse experiment, the rate of degradation or biotransformation of PAHs in soils contaminated with petroleum-derived hydrocarbons using planted woody species decreased as follows, *Salix nigra* Marshall > *Populus deltoides x P. nigra* DN 34 > *Fraxinus pennsylvanica* Marshall > unplanted soil, whereby ash and hybrid poplar were able notably decrease acenaphthene, anthracene, Fla, naphthalene, and Phe in soil compared to the unplanted soil control [168].

Willows *S. rubens* and *S. triandra* cultivated in soils contaminated with petroleum-derived hydrocarbons were able to reduce the total hydrocarbons concentration by 98.56% and 98.65%, respectively; the content of PAHs content was remarkably reduced as well, and Py, chrysene, benzo[k]fluoranthene, and benzo[a]pyrene concentrations decreased to undetectable levels. Although the contaminants affected the development of *S. rubens* grown on PAH-contaminated soil more than that of *S. triandra*, it could be recommended for phytoremediation of hydrocarbon contaminated soils because it is a native species [157].

### *14.7.2 Polychlorinated Biphenyls*

The 209 PCB congeners belong to persistent toxic pollutants acting as environmental estrogens and consequently showing adverse effects on both animals and humans [169, 170].

A little more than 1 year after planting poplar clone (Monviso) caused a general decrease in contaminant occurrence and an increase in microbial activity in the area chronically polluted by PCB and heavy metals close to the city of Taranto in Southern Italy and improved soil quality what was reflected in notable decrease in PCB congeners and reduced levels in all heavy metals. With the exception of congener PCB 28 (2,4,4′-trichlorobiphenyl) a negligible transportation of PCB 52 (2,2′,5,5′-tetrachlorobiphenyl), PCB 101 (2,2′,4,5,5′-pentachlorobiphenyl), PCB 118 (2,3′,4,4′,5-pentachlorobiphenyl), PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl), PCB 138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), and PCB 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl) into poplar tissue was observed 420 days after the poplar planting, the highest BCF related to roots was estimated for PCB118 [171]. Although highly hydrophobic PCBs are not expected to enter the transpiration stream, plant root exudates can form more hydrophilic complex with PCBs enabling their transportation into plants [172].

The whole poplar (*Populus deltoides x nigra*, DN34) plants were found to facilitate the degradation of PCBs and had positive effect on the cleaning of the commercial garden soil and was able for complete removal of at least 19 of the 29 potential PCB congeners (two congeners were completely removed after 96 days). For example, PCB 52 declined by 22.3% in the planted system, while in the unplanted reactors, its decrease was only 0.1% [173]. In whole poplar plants, PCB can be transformed into hydroxylated metabolites and subsequently, sulfate conjugates (e.g., [174–177]); moreover, enantioselective uptake and translocation of PCB 95 (2,2′,3,5′,6-pentachlorobiphenyl) in whole poplar plants were reported [178]. For example, *Populus deltoides x nigra* DN34 plants metabolized 4-chlorobiphenyl (PCB 3) to monohydroxy metabolites 2′-hydroxy-4-chlorobiphenyl, 3′-hydroxy-4-chlorobiphenyl, and 4′-hydroxy-4-chlorobiphenyl which were identified in hydroponic solution and in different parts of poplar plant [174]. It was reported that poplars can enantioselectively biotransform at least one chiral OH-PCB, namely, 5-hydroxy-2,2′,3,5′,6-pentachlorobiphenyl (5-OH-PCB 95). The 5-OH-PCB 95 was found to be notably enantioselectively biotransformed inside poplar tissues while in the hydroponic solution remained nearly its racemic mixtures [179]. In the roots of whole poplar plants exposed to racemic PCB 95 for 30 days as the major metabolite, 4′-hydroxy-2,2′,3,5′,6-pentachlorobiphenyl (4′-OH-PCB 95) was observed, and biotransformation of chiral PCBs to OH-PCBs by plants could be considered as an important source of enantiomerically enriched OH-PCBs in the environment [180].

Poplar plants were found to metabolize PCB 3 (4-chlorobiphenyl) during 25 day exposures into three sulfate metabolites, including 2′-PCB 3 sulfate, 3′-PCB 3 sulfate, and 4′-PCB 3 sulfate; however, their concentrations in the roots were much lower than those of hydroxylated PCB 3 [177].

*Paulownia tomentosa* and *Cytisus scoparius* were found to be less effective in reduction of PAHs and PCBs in the polluted soil than *Populus alba*; however *C. scoparius* which was least effective plant in soil decontamination showed the highest efficiency in soil metabolic stimulation [181].

Planted microcosms with fully developed roots and rhizosphere of poplar cultivated in soil containing PCB congeners PCB 52, PCB 77 (3,3′,4,4′-tetrachlorobiphenyl), and PCB 153 exhibited greater reductive dechlorination of PCBs compared to the unplanted reactors. For example, PCB 153 and PCB 52 showed a 55% and 63% diminution in corresponding soil PCBs after 32 weeks in the single congener exposure experiment in the poplar planted system. Transformation products observed in the planted systems were similar to those in the unplanted systems [182].

Slater et al. [183] tested the ability of *Salix alaxensis* and *Picea glauca* white spruce, tree species native to Alaska, to promote microbial biodegradation of PCBs via the release of phytochemicals upon fine root death in an experiment based on addition of crushed fine roots, biphenyl as PCB analogue, or salicylate, a secondary metabolite of willow, to microcosms containing PCB-spiked soil and 180 days after treatment found significantly greater PCB loss, including PCB 77, PCB 105, and PBC 169 (3,3′,4,4′,5,5′-hexachlorobiphenyl) in soil treated with willow root crushates compared to untreated soils showing similar extent of degraded PCB as well as degraded congeners to biphenyl-treated microcosms. Considerably lower soil toxicity was estimated for soil treated with willow root crushates for 180 days (log $EC_{50}$: 2.0 ± 0.2) than for untreated soil (log $EC_{50}$: 1.5 ± 0.1) and biphenyl-treated soil (log $EC_{50}$: 0.86 ± 0.04), while toxicity of willow-treated soil was comparable with that of uncontaminated soil (log $EC_{50}$: 1.8 ± 0.2). On the other hand, the PCB loss was not enhanced by *P. glauca* or salicylate suggesting that *S. alaxensis* could be used for rhizoremediation by altering microbial community structure, enhancing the loss of some PCB congeners, and reducing the toxicity of the soil environment.

Investigation of the structural and functional changes occurring in a PCB-contaminated soil ecosystem after planting *S. viminalis* showed that during the development of the root system, the rhizospheric bacterial community which evolved from the native soil community was changed, *Proteobacteria* dominated both in rhizosphere and soil showing enrichment in the rhizosphere, and higher abundance of *Betaproteobacteria* in the native and in the planted PCB-polluted soil was estimated as well [152].

Among five mature trees growing naturally in a PCB-contaminated site approx. 2.7–56.7-fold, higher numbers of PCB degraders were estimated in the root zones of *Pinus nigra* and *Salix caprea* than in the root zones of *Fraxinus excelsior*, *B. pendula*, and *R. pseudoacacia* or non-root-containing soil in certain seasons and at certain soil depths, whereby the majority of culturable PCB degraders associated with plants were identified as members of the genus *Rhodococcus*. At a depth of 0–20 cm in *P. nigra* rhizosphere, notably higher number of PCB degraders was estimated than in *R. pseudoacacia*, while the number of PCB metabolizers in willow rhizosphere at a depth of 40–60 cm exceeded that of *F. excelsior* and *R. pseudoacacia* rhizosphere at depth of 20–40 cm [184].

### *14.7.3 BTEX and TCE*

**BTX** referring to mixtures of **b**enzene, **t**oluene, and the three **x**ylenene isomers, all of which are aromatic hydrocarbons and **TCE** (**tri**chloro**e**thylene), a *chlorinated hydrocarbon* commonly used as an industrial solvent belong to *volatile organic contaminants*.

A number of isolates of endophytic bacteria isolated from the root, stem, and leaf of two cultivars of poplar tree growing on a site contaminated with BTEX showed the ability to degrade these contaminants or to grow in the presence of TCE, suggesting potential of poplar trees to be used in effective phytoremediation [185]. As low-cost remediation technique to restore areas contaminated with chlorinated solvents and BTEX compounds, phytoremediation has been proposed also by Collins et al. [186], and the use of transgenic plants could be considered as one of possible ways to more effective phytoremediation.

In willows which were grown in glass cylinders filled with compost above water-saturated quartz sand in the presence of TCE, >98% of TCE was lost via evapotranspiration in 1 month after adding TCE, and almost 1% of TCE was metabolized in the shoots with trichloroacetic acid and dichloroacetic acid as dominant metabolites [187]. The capacity of *B. pendula* to phytoremediate TCE was found to be comparable to *Populus* spp. and *Eucalyptus sideroxylon rosea*, i.e., tree species applied in field-scale TCE phytoremediation [188].

### *14.7.4 Petroleum/Diesel*

Total petroleum hydrocarbons (TPH) is a term used for any mixture of volatile and extractable hydrocarbons that are found in crude oil, for example, hexane, benzene, toluene, xylenes, naphthalene, and fluorene.

Hybrid poplar trees grown at former oil tank farm sites were able to decrease the concentration of benzene, toluene, ethylbenzene, xylene, and gasoline range organics by about 81%, 90%, 67%, 78%, and 82%, respectively, in the lower soil horizons, and their roots dewatered soils and allowed penetration of oxygen deep into the soil profile [189].

Colonization of hybrid poplar roots (*P. deltoides x* [*P. laurifolia x P. nigra*] cv. Walker) by ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker & Couch increased total fine root production in diesel-contaminated soil (5000 mg diesel fuel kg soil$^{-1}$) to 56.58 g m$^{-2}$ compared to 22.59 g m$^{-2}$ in the uncolonized diesel-contaminated treatment, and notably greater concentrations of TPH were found to be sequestered in hybrid poplar root/fungal-sheath complexes from the colonized treatment compared to the roots of the uncolonized treatment [190].

*Tectona grandis* and *Gmelina arborea* were reported as tree species suitable for phytoremediation of crude oil-contaminated soils, mainly at low pollutant concentrations [191], while tropical woody plants, kiawe (*Prosopis pallida*), milo

(*Thespesia populnea*), and kou (*Cordia subcordata*) showed potential for use in phytoremediation of petroleum hydrocarbons in coastal tropical soils [192].

Jones et al. [193] investigated the interaction between *Prosopis pallida*, *Thespesia populnea*, *Cordia subcordata* (trees) and *Scaevola sericea*, *Myoporum sandwicense*, and *Nerium oleander* (shrubs) and microorganisms during petroleum hydrocarbon bioremediation in Pacific Islands coastal soils and found that in the presence of roots in a contaminated zone of soil, the hydrocarbon degraders which prefer the contaminant will use the contaminant as a carbon source resulting in efficacious removal of hydrocarbons, while for the hydrocarbon degraders considering the root exudates and the contaminant as equally attractive, the degradation of hydrocarbons will be less successful. Although no correlation was estimated between the reduction of the levels of TPH in the diesel in soil planted with three abovementioned trees and the levels of hydrocarbon-degrading microorganisms in the rhizosphere of these tree species, qualitative differences in root exudates of plants could result in qualitative or nutritional changes among the populations of hydrocarbon-degrading microorganisms.

An outdoor pot experiment, in which 16 black poplar clones were cultivated in soil contaminated with diesel fuel, showed that the diesel contamination had a more pronounced effect on rhizosphere communities than the plant variety, and based on the presence of genes encoding alkane hydroxylase, the ability to utilize alkanes, and abundance in the rhizosphere, among 14 diesel degrading strains isolated from the rhizosphere, three promising plant/microbe combinations were identified for phytoremediation purposes [194].

In a pot experiment, the leaf wilting of *Robinia pseudoacacia* cultivated in petroleum-contaminated soil was not observable even when petroleum concentration was 40,000 mg $kg^{-1}$ suggesting the potential of this species to remediate petroleum-contaminated soils [195].

Pot experiments using contaminated sandy soil showed that phytoremediation of low-level TPH contamination was found to be most efficient if fertilization with N-P-K water-soluble fertilizer, or fertilizer with molasses amendments was used in combination with plant species; however, the same level of remediation was reached by the addition of grasses and/or willow combinations without amendment or by fertilization of sandy soil [196].

In a pot study, willow native to Alaska was found to accelerate the degradation of diesel range organics, and together with fertilizer, it increased aromatic degradation by shifting microbial community structure and the identity of active naphthalene degraders [197].

The potential of *Dracaena reflexa* and *Podocarpus polystachyus* to remove hydrocarbons from 2.5% to 1% diesel fuel-contaminated soil amended individually with 5% organic wastes (tea leaf, soy cake, and potato skin) was tested for a period of 270 days, and it was found that soy cake amendment caused a 90% and 99% oil loss in soil contaminated with 2.5% and 1% oil exceeding that obtained with *D. reflexa* in unamended soil; for *P. polystachyus* this loss reached only 84% and 91%, respectively. The tree plants did not accumulate hydrocarbon from the soil; however, high number of hydrocarbon-utilizing bacteria in the rhizosphere indicated that oil degradation occurred by rhizodegradation [198].

### *14.7.5 Explosives*

2,4,6-Trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) are the most powerful and commonly used explosives representing persistent pollutants contaminating soil and groundwater, and for their removal plants could be used. Metabolism of TNT in plants occurs by reduction as well as by oxidation, and the distribution of the transformation products, conjugates, and bound residues appears to be consistent with the green liver model concept, and it can be assumed that plants metabolize RDX also according to the green liver concept [199].

Biodegradation of nitro-substituted explosives TNT, RDX, and HMX by phytosymbiotic *Methylobacterium* sp. (strain BJ001) associated with poplar tissues (*Populus deltoides x nigra* DN34) was described by Van Aken et al. [200]. Metabolites detected from RDX transformation included a mononitroso RDX derivative and a polar compound tentatively identified as methylenedinitramine.

Transgenic aspen (*Populus tremula x tremuloides* var. Etropole) expressing bacterial nitroreductase gene, pnrA, was found to be highly interesting for phytoremediation applications on TNT-contaminated soil and underground aquifers. Because lower expression of the transgene in plant roots compared to shoots was observed, by improvement of the *pnrA* expression in aspen roots, the remediation capacity could be enhanced [201].

Using 4-year-old hybrid willow (*Salix* spec., clone EW-20) and *Picea abies* cultivated in sand or ammunition plant soil and exposed to a single pulse application with water solved $^{14}$C-TNT (reaching an initial concentration of 5.2 mg TNT per kg dry soil), 60 days after treatment, it was found that TNT was readily transformed in tree tissue (about 80% of $^{14}$C was non-extractably bound in roots, stems, wood, and leaves, or needles), and in tree root extracts no TNT and none of the known metabolites, mono-amino-dinitrotoluenes, diaminonitrotoluenes, and trinitrobenzene and no dinitrotoluenes were present. Consequently, it could be concluded that both short rotation *Salicaceae* trees and conifer forests possess a dendroremediation potential for TNT-polluted soils [202].

## 14.8 Phytoremediation of Saline Soils by Woody Species

Soil salinity, especially in arid and semiarid regions, represents serious problem because of significant yield losses, and therefore attention is focused on the removing of soil salinity using various amendments or salt-tolerant plant species, and salinity tolerance is an important adaptive trait for land reclamation [203]. Because due to salinity, more than half a billion hectares of land are not being properly used for crop production worldwide, phytoremediation used to improve the deteriorated soils could markedly contribute to sustainable agricultural productivity [16].

Mirck and Zalesny [204] in a mini-review focused their interest on the key salt tolerance studies and classified the salt tolerance levels of poplars and willows which were found to range from sensitive to moderately tolerant.

Phytoremediation of sodic soils is based on the ability of plant roots to increase the dissolution rate of calcite, thereby resulting in enhanced levels of $Ca^{2+}$ in soil solution to effectively replace $Na^+$ from the cation exchange complex, and it is especially effective when used on moderately saline–sodic and sodic soils [205].

Halophytes, plants which can survive, grow, and reproduce in soils containing high salt concentration and also resist to heavy metal toxicity, could be successfully used for phytoremediation in heavy metal-contaminated saline soils [206].

Because plants can adapt to saline conditions by different mechanisms, Walter [207] classified them into tree types: (1) salt excluding, having a root system possessing ultrafiltration mechanism (e.g., *Rhizophora mucronata*, *Ceriops candolleana*, *Bruguiera gymnorrhiza*, *Kandelia candel*), (2) salt excreting which regulate internal salt levels through foliar glands (e.g., *Avicennia officinalis*, *Avicennia alba*, *Avicennia marina*, *Aegiceros corniculatum*), and (3) salt accumulating which are able to accumulate high salt concentrations in their cells and tissues and can control salt toxicity by developing succulence (e.g., *Sonneratia acida*, *Sonneratia alba*, *Limnitzera racemosa*, *Excoecaria agallocha*, *Salvadora persica Suaeda nudiflora*).

Biosaline agriculture utilizes salt-tolerant plants for the rehabilitation of saline ecosystem [208]. Thus, the challenges for biosaline agriculture in the twenty-first century represent extensive using of salt-contaminated resources to cope food requirements utilizing the potential of salt-tolerant plants for landscape reintegration and soil rehabilitation [209].

According to Ashraf et al. [210], among salt-tolerant woody species applicable to restore productivity of saline ecosystem belong *Suaeda fruticosa*, *Kochia indica*, *Atriplex amnicola*, and *Sesbania aculeata* (shrubs) as well as *Acacia ampliceps*, *Prosopis chilensis*, *Acacia nilotica*, *Eucalyptus striaticalyx*, *Prosopis cineraria*, *Casuarina glauca*, *Prosopis tamarogo*, and *Leucaena leucocephala*. *Eucalyptus* tree which can be utilized for timber and fuel wood and its leaves contain essential oils was selected as especially suitable tree species for saline and water-logged environments because its use in rehabilitation of saline ecosystems could be economical and profitable.

Over a summer period from investigated three eucalypt species, the mean daily water use of *Eucalyptus cladocalyx* was about sixfold greater than that of *E. melliodora* and fourfold greater than that of *E. polybractea*. *E. cladocalyx* showed also higher tolerance to extreme temperature, and based on the results of sap flow, monitoring this species could be used to reduce salinity of ground water [211].

Due to high evapotranspiration, high growth rates and abilities to accumulate salts and specific ions tree could be considered as a suitable candidate for wastewater reuse.

High levels of salts in artificial wastewaters containing also Se and B which was used for treatment of poplar hardwood cuttings had adverse effect on plant growth causing leaf damage and shedding, and salinity also affected Se and B accumulation patterns in poplar leaves. The Cl levels in the leaves and stems predominantly determined the salt tolerance of poplar species, although this was affected also by growth and vigor characteristics of plants and by the allometric relationships between leaves and stems influencing the sinks in which ions could accumulate before reaching toxic levels. Poplars were reported to be more salt tolerant than avocado trees, however notably less salt tolerant than eucalypt [212].

In contrast to *Salix discolor* and *S. eriocephala* which were adversely affected by salinity, the total aboveground dry mass of *Salix interior* showed a slight increase in medium salinity treatment and further raised in high salinity treatment, its basal diameter showed an increase with increasing salinity, and *S. interior* exhibited also greater stimulation of maximum rate of carboxylation and electron transport compared to both other abovementioned willow cultivars. The saline tolerance of *S. interior* could be connected with the natural selection in the arid regions of the Southwest USA, where *S. interior* is believed to have its evolutionary origins [213]. *S. interior* showed greater water-use efficiency than *S. discolor* and *S. eriocephala*, and under control conditions its leaf $Na^+$ concentration was 13-fold higher of that of *S. discolor* and *S. eriocephala*. However, while in *S. interior* the saline treatment caused 17% increase of the leaf $Na^+$ concentration, in both other tested willows, leaf $Na^+$ concentration at medium saline treatment was sixfold higher than in the control [214].

Plants of *Populus euphratica* Oliv. and *Populus tomentosa* Carr which were watered with 100 mM NaCl for 8 days and then 200 mM NaCl for 12 days were found to exhibit soil NaCl concentrations of 60 mM and 95 mM, respectively. *P. tomentosa* showing leaf necrosis at salinity treatment was found to be more sensitive to salinity than *P. euphratica* which was not damaged. *Populus euphratica* displayed notably lower rates of net root uptake and transport of salt ions ($Na^+$ and $Cl^-$) to the shoots under salinity, showed lower unit transpiration rates and lower salt concentrations in the xylem at salinity treatment resulting in its greater capacity for salt exclusion. Moreover, it was estimated that *P. euphratica* more effectively restricted radial salt transport in roots by blocking apoplasmic salt transport and sequestering more $Cl^-$ in cortical vacuoles, and in the presence of high external $Na^+$ concentrations, it maintained higher $K^+$ uptake and transport than *P. tomentosa* [215].

Among 1-year-old seedlings of *Populus euphratica* Cliv. and 1-year-old rooted cuttings of *P. euramericana* cv. Italica and *P. popularis* exposed to increasing soil NaCl concentration, *P. euphratica* was found to be the most tolerant species. Among both other sensitive poplar genotypes, Italica exhibited greater capacity to accumulate salt in cortical vacuoles compared with *P. popularis*. Limited ion loading into the xylem during radial transport in *P. euphratica* at high salinity contributed to the restriction of subsequent axial transport [216].

*Salix miyabeana*, *S. purpurea*, and *S. sachalinensis x S. miyabeana* grown in organically amended Solvay waste and irrigated with storm water containing 163–

8125 mg $Cl^-$ $dm^{-3}$ exhibited altered stomatal conductance after 4.5 weeks, while height and leaf length was adversely affected after 6 and 7 weeks, *S. sachalinensis x S. miyabeana* being indeed the most sensitive species; however, it showed the greatest evapotranspiration values during the 10-week trial, and storm water with relatively high $Cl^-$ ion concentrations (up to 1625 mg $Cl^-$ $dm^{-3}$) did not have short-term effects on biomass accumulation and evapotranspiration [217].

The degree of reduction in evapotranspiration of *S. viminalis* plants cultivated in a pot-based experiment in the presence of different chloride concentrations (0–422 mmol $dm^{-3}$) was directly related to $Cl^-$ concentration and, in the short term, evaporative demand, whereby $Cl^-$ concentration >70 mmol $dm^{-3}$ prevented the sustainable growth and development of plants [218].

After 7 years of growing of three poplar clones in highly saline clay containing B and Se as well as in non-saline sandy loam soils, lower microbial biomass and the amount and proportion of AMF community were estimated in saline soil, and also the amounts of phospholipid fatty acids (PLFAs) of Gram-positive bacteria and actinomycetes were notably lower in plants cultivated in saline soil compared to non-saline soil in contrast to saprophytic fungal, Gram-negative bacterial and eukaryotic PLFAs which were comparable in non-saline soil and saline soil, respectively. The researchers stated that salinity and B affected microbial biomass and AMF, but the investigated poplar clones were able to recycle the necessary amount of nutrients to promote and preserve saprophytic fungal and bacterial communities from the effects caused by poor soil quality [219].

The Pb uptake of 6-week-old *Eucalyptus camaldulensis* plants exposed to both salinity stress (200 mM NaCl) and Pb (20 mg $dm^{-3}$) in hydroponia was 49.3 mg Pb per plant compared to the control (1.2 mg Pb per plant) suggesting that Pb accumulation was supported by saline conditions. Pb was predominantly allocated in roots (103.4 mg Pb $kg^{-1}$ d.m.) compared to shoots and leaves (17.5 mg Pb $kg^{-1}$ d.m. and 13.6 mg Pb $kg^{-1}$ d.m., respectively) and also BCF related to roots was 4–6 times higher than that of shoots [220]. Seenivasan et al. [221] investigated restoration of sodic soils involving chemical and biological amendments and phytoremediation by *E. camaldulensis* in a semiarid region and at the end of the third year estimated a reduction of 10% in soil pH, 33% in electrical conductivity, and 20% in exchangeable sodium percentage compared to the initial values. Also three- to fourfold increase in organic carbon content was detected, and considerable amelioration in the available major micronutrients of soil, microbial growth, and enzyme activity indicated phytoremediation potential of *E. camaldulensis* at restoration of degraded lands.

AMF which are frequently root colonizers of trees and shrubs in temperate and tropical habitats show beneficial effect on these plants by enhancing plant nutrient and water uptake, protecting plants from root herbivores and pathogens, and improving soil structure, and they were also reported to induce physiological drought tolerance, involving both increased dehydration avoidance and dehydration tolerance; however, in the majority of the experiments, it was found that the improvement of host drought resistance at symbiosis is realized by aiding drought avoidance [222].

The 1-year-old rooted cuttings of *Populus alba* cultivated in sandy soil and watered with solutions containing a mixture of NaCl and $CaCl_2$ (2000 mg $dm^{-3}$ or 5000 mg $dm^{-3}$, respectively) showed approximately three times higher total Na uptake than the control plants, whereby treatment with higher salt concentration resulted in about double level of Na compartmentalized in fallen leaves, dead leaves, and dead branches than at treatment with lower salt concentration. *P. alba* plants were found to accumulate 90% of $Na^+$ in their roots, and only low $Na^+$ levels were translocated into the shoots indicating that this species is suitable for phytostabilization purposes [223].

Zhang et al. [224] reported that phytoremediation of saline–alkali wasteland using *Caragana* shrubs resulted in the reduced soil pH, sodium adsorption ratio, exchangeable sodium percentage, salinity, and $Na^+$ concentration around the shrubs.

*Atriplex nummularia* was recommended for phytoremediation of salt-affected soils in arid and semiarid environments due to its high adaptability to salinity and water deficiency by Silva et al. [225]. This plant accumulated about 82% Na in leaves, and planting at 1 × 1 m crop density was found to be suitable for salt phytoextraction per area, while crop density of 2 × 2 m was satisfying for improvement of soil physical condition.

## 14.9 Ecological Restoration of Disturbed Vegetation, Habitats, and Ecosystems by Woody Plants

In the 1980s emerged restoration ecology as a separate field in ecology. It is ecological approach supporting the practice of ecological restoration, which is the practice of renewing and restoring degraded, damaged, destroyed vegetation, sites, habitats, and ecosystems in the environment by active human intervention and action. It should be mentioned that **restoration ecology** is the academic study of the process, whereas **ecological restoration** is the actual approach by restoration practitioners. This intentional activity initiates or accelerates the recovery of an ecosystem with respect to its health, integrity, and sustainability. The practice of ecological restoration includes a wide scope of projects such as erosion control, reforestation, removal of non-native species (e.g., invasive species) and weeds, or reintroduction of native species. So, introduction of plant species (including woody species) that can survive on contaminated areas is also considered [226, 227]. **Phytorestoration** is revegetation of barren areas by fast-growing resistant species that efficiently cover the soil, thus preventing the migration of contaminated soil particles and soil erosion by wind and surface water run-off [8]. Costantini et al. [228] emphasized that success of restoration process relies on a proper understanding of their ecology, namely, the relationships between soil, plants, environmental conditions (e.g., climate, hydrology), and land management at different scales, which are particularly complex due to the heterogeneous pattern of ecosystem functioning.

Restoring of degraded sites is a worldwide issue. Progress made with restoration could compensate the impacts of degradation, stressing the importance of a quantitative evaluation process. Studies and attempts to implement restoration strategies in different environments are numerous, from rangelands to shrub and forest stands and from agricultural ecosystems to mining sites and brownfields. Moreover, it should be considered soil characteristics because soil is a key part of the Earth that controls the hydrological, erosion, geochemical, and biological cycles (in detail see [228]).

The failure of restoration plans is often caused by the choice of plant species or practices that are not convenient to the site with the respect of soil contamination. The success of restoration plans instead relies on a proper and detailed knowledge of the both plant-specific features and relationships between soil and plant properties and environmental condition. One of the main challenges is to select the suitable species to be used for restoration of contaminated habitats which have a pattern of the root system matching the horizon characteristics of the soil profile and can survive under specific environmental conditions of the site.

We would like to mention that restoration processes are closely connected with both **succession** and biological diversity (**biodiversity**). The restoration of deforested or degraded areas can contribute to biodiversity conservation and global resilience given the current and projected impacts of climate change. In recent years, a robust array of ecological restoration frameworks has been generated to address restoration challenges at large scales in different ecosystems around the world. Unfortunately, the costs associated with restoration at such scales greatly challenge the implementation of such frameworks [229].

Plant responses to the environment are specific and are manifested through phenological, morphological, anatomical, and physiological characteristics. High plasticity of these traits contributes to the plant competition within succession of vegetation and is a keystone for plant biodiversity, too (in detail see [230]). Succession is the process by which the component species of a community changes over the time. Following a disturbance (e.g., after contamination of the site), an ecosystem generally progresses from a simple level of organization (i.e., only few dominant pioneer species) to a more complex community (i.e., many interdependent species forming plan community with high biodiversity) over the time (in details [231]). For many people the biodiversity has an intrinsic value that humans have a responsibility toward other living things and an obligation to future generations. Though restoration ecologists and other conservation biologists generally agree that habitat is the most important locus of biodiversity protection, the disciplines themselves have different focuses. The concept of biodiversity represents the approach to understanding of living nature through its variability, structure, and function on different levels, reflecting current knowledge of ecology, population ecology, molecular biology, and genetics. This concept reflects also the current stage of living nature conservation and attempts to find/develop new strategy for preservation of living nature and life in changing environmental condition [232].

Depending on the severity of the disturbance, restoration often consists of initiating, assisting, or accelerating ecological succession processes. However, **spontane-**

**ous succession** should not be neglected, because it is generally appeared to be an ecologically suitable way of ecosystem restoration of disturbed sites by reason that target species became dominant over time.

## 14.10 Conclusion

Fundamental questions for ecologists under negative environmental changes are how plant populations and ecosystems will respond to these new conditions and what mechanisms will be involved in this process. Thus, the understanding of physiological and production processes will be basic for predicting changes in species distribution, community composition, and plant production in ecosystems that are under harmful environmental attack including both inorganic and organic contaminants. Special interest of scientists is in the long term devoted to the woody plants and forest ecosystems seeing them to have extraordinarily important not only production but also many non-production functions. New biotechnological approach showed that woody species have significant application in many phytoremediation technologies (especially phytoextraction, phytostabilization, and rhizodegradation) to clean contaminated soils, waters, as well as air. It should be mentioned that in different countries with specific climatic and edaphic conditions, autochthonous species including woody plants are successfully applied as phytoremediators. These plants have perennial character, long life span, high transpiration rate, quick regeneration of removed aboveground parts, and easy vegetative reproduction. Many of them belong to the fast-growing trees that have an extensive and massive root system penetrating deeply into the soil and ensuring efficient uptake of water containing the pollutants from the substrate. All these facts are extraordinarily important because of crucial for each human population is living in health sustainable and stabile conditions with sufficient, healthy food and overall and comfort assurance, as well.

**Acknowledgement** This contribution was financially supported by the "code ITMS 26240120004, funded by the ERDF".

## References

1. Brosse J (1989) Mythologie des Arbres. Plon, Paris, p 360 ISBN 978-2-228-88711-3
2. Masarovičová E, Kráľová K (2017) Essential elements and toxic metals in some crops, medicinal plants and trees. In: Ansari A, Gill S, Gill RR, Lanza G, Newman L (eds) Phytoremediation. Springer International Publishing AG, Cham, pp 183–255. https://doi.org/10.1007/978-3-319-52381-1_7
3. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529–540. https://doi.org/10.1016/S0160-4120(02)00152-6
4. Pajević S, Borišev M, Nikolić N, Arsenov DD, Orlović S, Županski M (2016) Phytoextraction of heavy metals by fast-growing trees: a review. In: Ansari A, Gill S, Gill R, Lanza G, Newman

L (eds) Phytoremediation. Springer International Publishing, Cham, pp 29–64. https://doi.org/10.1007/978-3-319-40148-5_2

5. Stomp AM, Han KH, Wilbert S, Gordon MP (1993) Genetic improvement of tree species for remediation of hazardous wastes. In Vitro Cell Dev Biol Plant 29:227–232. https://doi.org/10.1007/BF02632039
6. Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamra M, Matloob A, Rehim A, Hussai S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116
7. Pandey VC, Bajpai O, Singh N (2016) Energy crops in sustainable phytoremediation. Renew Sustain Energy Rev 54:58–73. https://doi.org/10.1016/j.rser.2015.09.078
8. Kráľová K, Masarovičová E (2006) Plants for the future. Ecol Chem Eng 13:1179–1207 https://www.ceceurope.org/wp-content/uploads/2015/08/CSC%20plantsbioethics.pdf
9. Masarovičová E, Kráľová K, Peško M (2009) Energetic plants—cost and benefit. Ecol Chem Eng S 16:263–276. http://tchie.uni.opole.pl/freeECE/S_16_3/Masarovicova_16(3).pdf
10. Baker AJM (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654. https://doi.org/10.1080/01904168109362867
11. 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
12. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. Nature 411:438. https://doi.org/10.1038/35054664
13. Tu C, Ma LQ (2002) Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. J Environ Qual 31:641–647
14. Dzantor EK (2007) Phytoremediation: the state of rhizosphere ‘engineering’ for accelerated rhizodegradation of xenobiotic contaminants. J Chem Technol Biotechnol 82:228–232. https://doi.org/10.1002/jctb.1662
15. Le X, Hui D, Dzantor EK (2011) Characterizing rhizodegradation of the insecticide bifenthrin in two soil types. J Environ Prot 2:940–946. https://doi.org/10.4236/jep.2011.27107
16. Ashraf MY, Ashraf M, Mahmood K, Akhter J, Hussain F, Arshad M (2010) Phytoremediation of saline soils for sustainable agricultural productivity. In: Ashraf M, Ozturk M, Ahmad M (eds) Plant adaptation and phytoremediation. Springer, Dordrecht, pp 335–355. https://doi.org/10.1007/978-90-481-9370-7_15
17. Šottníková A, Lunáčková L, Masarovičová E, Lux A, Streško V (2003) Changes in the rooting and growth of willows and poplars induced by cadmium. Biol Plant 46:129–131. https://doi.org/10.1023/A:1022395118998
18. Lunáčková L, Masarovičová E, Kráľová K, Streško V (2003) Response of fast growing woody plants from family Salicaceae to cadmium treatment. Bull Environ Contam Toxicol 70:576–585. https://doi.org/10.1007/s00128-003-0024-2
19. Lunáčková L, Šottníková A, Masarovičová E, Lux A, Streško V (2003) Comparison of cadmium effect on willow and poplar in response to different cultivation conditions. Biol Plant 47:403–411. https://doi.org/10.1023/B:BIOP.0000023884.54709.09
20. Tozser D, Magura T, Simon E (2017) Heavy metal uptake by plant parts of willow species: a meta-analysis. J Hazard Mater 336:101–109. https://doi.org/10.1016/j.jhazmat.2017.03.068
21. Magdziak Z, Mleczek M, Rutkowski P, Golinski P (2017) Diversity of low-molecular weight organic acids synthesized by *Salix* growing in soils characterized by different Cu, Pb and Zn concentrations. Acta Physiol Plant 39:137. https://doi.org/10.1007/s11738-017-2434-5
22. Arsenov D, Zupunski M, Borisev M, Nikolic N, Orlovic S, Pilipovic A, Pajevic S (2017) Exogenously applied citric acid enhances antioxidant defense and phytoextraction of cadmium by willows (*Salix* spp.). Water Air Soil Pollut 228:221. https://doi.org/10.1007/s11270-017-3405-6

23. Cao YN, Ma CX, Chen GC, Zhang JF, Xing BS (2017) Physiological and biochemical responses of *Salix integra* Thunb. under copper stress as affected by soil flooding. Environ Pollut 225:644–653. https://doi.org/10.1016/j.envpol.2017.03.040
24. Shi X, Wang SF, Sun HJ, Chen YT, Wang DX, Pan HW, Zou YZ, Liu JF, Zheng LY, Zhao XL, Jiang ZP (2017) Comparative of *Quercus* spp. and *Salix* spp. for phytoremediation of Pb/Zn mine tailings. Environ Sci Pollut Res 24:3400–3411. https://doi.org/10.1007/s11356-016-7979-0
25. Mleczek M, Rutkowski P, Golinski P, Kaczmarek Z, Szentner K, Waliszewska B, Stolarski M, Szczukowski S (2017) Biological diversity of *Salix* taxa in Cu, Pb and Zn phytoextraction from soil. Int J Phytoremediation 19:121–132. https://doi.org/10.1080/15226514.2016.1207597
26. Salam MMA, Kaipiainen E, Mohsin M, Villa A, Kuittinen S, Pulkkinen P, Pelkonen P, Mehtatalo L, Pappinen A (2016) Effects of contaminated soil on the growth performance of young *Salix* (*Salix schwerinii* E. L. Wolf) and the potential for phytoremediation of heavy metals. J Environ Manage 183:467–477. https://doi.org/10.1016/j.jenvman.2016.08.082
27. Shi X, Sun HJ, Chen YT, Pan HW, Wang SF (2016) Transcriptome sequencing and expression analysis of cadmium (Cd) transport and detoxification related genes in Cd-accumulating *Salix integra*. Front Plant Sci 7:1577. https://doi.org/10.3389/fpls.2016.01577
28. Zemleduch-Barylska A, Lorenc-Plucinska G (2016) Response of leaf and fine roots proteomes of *Salix viminalis* L. to growth on Cr-rich tannery waste. Environ Sci Pollut Res 23:18394–18406. https://doi.org/10.1007/s11356-016-7026-1
29. Bart S, Motelica-Heino M, Miard F, Joussein E, Soubrand M, Bourgerie S, Morabito D (2016) Phytostabilization of As, Sb and Pb by two willow species (*S. viminalis* and *S.purpurea*) on former mine technosols. Catena 136:44–52. https://doi.org/10.1016/j.catena.2015.07.008
30. Zarubova P, Hejcman M, Vondrackova S, Mrnka L, Szakova J, Tlustos P (2015) Distribution of P, K, Ca, Mg, Cd, Cu, Fe, Mn, Pb and Zn in wood and bark age classes of willows and poplars used for phytoextraction on soils contaminated by risk elements. Environ Sci Pollut Res 22:18801–18813. https://doi.org/10.1007/s11356-015-5043-0
31. Van Slycken S, Witters N, Meiresonne L, Meers E, Ruttens A, Van Peteghem P, Weyens N, Tack FMG, Vangronsveld J (2013) Field evaluation of willow under short rotation coppice for phytomanagement of metal-polluted agricultural soils. Int J Phytoremediation 15:677–689. https://doi.org/10.1080/15226514.2012.723070
32. Guo BH, Dai SX, Wang RG, Guo JK, Ding YZ, Xu YM (2015) Combined effects of elevated $CO_2$ and Cd-contaminated soil on the growth, gas exchange, antioxidant defense, and Cd accumulation of poplars and willows. Environ Exp Bot 115:1–10. https://doi.org/10.1016/j.envexpbot.2015.02.002
33. Greger M, Landberg T (2015) Novel field data on phytoextraction: pre-cultivation with *Salix* reduces cadmium in wheat grains. Int J Phytoremediation 17:917–924. https://doi.org/10.1080/15226514.2014.1003785
34. Wang QB, Chen GC, Fang J, Lou C, Zhand JF (2014) Characteristics of soil lead tolerance, accumulation and distribution in *Salix babylonica* Linn. and *Salix jiangsuensis* J172. Bull Bot Res 34:626–633. https://doi.org/10.7525/j.issn.1673-5102.2014.05.008
35. Ranieri E, Gikas P (2014) Effects of plants for reduction and removal of hexavalent chromium from a contaminated soil. Water Air Soil Pollut 225:1981. https://doi.org/10.1007/s11270-014-1981-2
36. Konlechner C, Turktas M, Langer I, Vaculik M, Wenzel WW, Puschenreiter M, Hauser MT (2013) Expression of zinc and cadmium responsive genes in leaves of willow (*Salix caprea* L.) genotypes with different accumulation characteristics. Environ Pollut 178:121–127. https://doi.org/10.1016/j.envpol.2013.02.033
37. Tognetti R, Cocozza C, Marchetti M (2013) Shaping the multifunctional tree: the use of Salicaceae in environmental restoration. FOREST 6:37–47. https://doi.org/10.3832/ifor0920-006

38. Hrynkiewicz K, Baum C (2013) Selection of ectomycorrhizal willow genotype in phytoextraction of heavy metals. Environ Technol 34:225–230. https://doi.org/10.1080/09593330.2012.689369
39. Dimitriou I, Mola-Yudego B, Aronsson P, Eriksson J (2012) Changes in organic carbon and trace elements in the soil of willow short-rotation coppice plantations. Bioenergy Res 5:563–572. https://doi.org/10.1007/s12155-012-9215-1
40. Chen GC, Liu ZK, Zhang JF, Owens G (2012) Phytoaccumulation of copper in willow seedlings under different hydrological regimes. Ecol Eng 44:285–289. https://doi.org/10.1016/j.ecoleng.2012.04.018
41. Puckett EE, Serapiglia MJ, DeLeon AM, Long S, Minocha R, Smart LB (2012) Differential expression of genes encoding phosphate transporters contributes to arsenic tolerance and accumulation in shrub willow (*Salix* spp.). Environ Exp Bot 75:248–257. https://doi.org/10.1016/j.envexpbot.2011.07.008
42. Mrnka L, Kuchar M, Cieslarova Z, Matejka P, Szakova J, Tlustos P, Vosatka M (2012) Effects of endo- and ectomycorrhizal fungi on physiological parameters and heavy metals accumulation of two species from the family Salicaceae. Water Air Soil Pollut 223:399–410. https://doi.org/10.1007/s11270-011-0868-8
43. Gomes MP, Marques TCLLDEM, Silva GH, Soares AM (2011) Utilization of willow (*Salix humboldtiana* Willd) as a species for phytoremediation of zinc industry waste. Sci For 39:117–123
44. Mihalik J, Tlustos P, Szakova J (2011) The influence of citric acid on mobility of radium and metals accompanying uranium phytoextraction. Plant Soil Environ 57:526–531
45. Zhivotovsky OP, Kuzovkina YA, Schulthess CP, Morris T, Pettinelli D (2011) Lead uptake and translocation by willows in pot and field experiments. Int J Phytoremediation 13:731–749. https://doi.org/10.1080/15226514.2010.525555
46. Li JH, Sun YY, Yin Y, Ji R, Wu JC, Wang XR, Guo HY (2010) Ethyl lactate-EDTA composite system enhances the remediation of the cadmium-contaminated soil by autochthonous Willow (*Salix x aureo-pendula* CL 'J1011') in the lower reaches of the Yangtze River. J Hazard Mater 181:673–678. https://doi.org/10.1016/j.jhazmat.2010.05.065
47. 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. https://doi.org/10.1016/j.envpol.2008.10.024
48. Zimmer D, Baum C, Leinweber P, Hrynkiewicz K, Meissner R (2009) Associated bacteria increase the phytoextraction of cadmium and zinc from a metal-contaminated soil by mycorrhizal willows. Int J Phytoremediation 11:200–213. https://doi.org/10.1080/15226510802378483
49. Utmazian MND, Wenzel WW (2007) Cadmium and zinc accumulation in willow and poplar species grown on polluted soils. J Plant Nutr Soil Sci 170:265–272. https://doi.org/10.1002/jpln.200622073
50. Nikolic N, Zoric L, Cvetkovic I, Pajevic S, Borisev M, Orlovic S, Pilipovic A (2017) Assessment of cadmium tolerance and phytoextraction ability in young *Populus deltoides* L. and *Populus x euramericana* plants through morpho-anatomical and physiological responses to growth in cadmium enriched soil. FOREST 10:635–644. https://doi.org/10.3832/ifor2165-010
51. Ariani A, Romeo S, Groover AT, Sebastiani L (2016) Comparative epigenomic and transcriptomic analysis of *Populus* roots under excess Zn. Environ Exp Bot 132:16–27. https://doi.org/10.1016/j.envexpbot.2016.08.005
52. Lin T, Wan X, Zhang F (2016) The short-term responses of glutathione and phytochelation synthetic pathways genes to additional nitrogen under cadmium stress in poplar leaves. Russ J Plant Physiol 63:754–762. https://doi.org/10.1134/S1021443716060066
53. Rome C, Huang XY, Danku J, Salt DE, Sebastiani L (2016) Expression of specific genes involved in Cd uptake, translocation, vacuolar compartmentalisation and recycling in *Populus alba* Villafranca clone. J Plant Physiol 202:83–91. https://doi.org/10.1016/j.jplph.2016.07.009

54. Chen LH, Zhang DJ, Yang WQ, Liu Y, Zhang L, Gao S (2016) Sex-specific responses of *Populus deltoides* to *Glomus intraradices* colonization and Cd pollution. Chemosphere 155:196–206. https://doi.org/10.1016/j.chemosphere.2016.04.049
55. Ding LP, Wang HZ, Wei JH (2016) Progress and prospect of research in transgenic poplar. iForest Res 29:124–132
56. Houda Z, Bejaoui Z, Albouchi A, Gupta DK, Corpas FJ (2016) Comparative study of plant growth of two poplar tree species irrigated with treated wastewater, with particular reference to accumulation of heavy metals (Cd, Pb, As, and Ni). Environ Monit Assess 188:99. https://doi.org/10.1007/s10661-016-5102-0
57. Kubatova P, Hejcman M, Szakova J, Vondrackova S, Tlustos P (2016) Effects of sewage sludge application on biomass production and concentrations of Cd, Pb and Zn in shoots of *Salix* and *Populus* clones: improvement of phytoremediation efficiency in contaminated soils. Bioenergy Res 9:809–819. https://doi.org/10.1007/s12155-016-9727-1
58. Samuilov S, Lang F, Djukic M, Djunisijevic-Bojovic D, Rennenberg H (2016) Lead uptake increases drought tolerance of wild type and transgenic poplar (*Populus tremula x P. alba*) overexpressing gsh 1. Environ Pollut 216:773–785. https://doi.org/10.1016/j.envpol.2016:06.047
59. Wang JH, Li XL, Yang JX (2015) Heavy metal enrichment characteristics of poplar. In: Yingying S, Gurian C, Zhen L (eds) Proceedings of the international conference on logistics, engineering, management and computer science (LEMCS 2015) book series: Advances in intelligent systems research, vol 117. Atlantis Press, Paris, pp 1687–1689
60. Shi WG, Li H, Liu TX, Polle A, Peng CH, Luo ZB (2015) Exogenous abscisic acid alleviates zinc uptake and accumulation in *Populus x canescens* exposed to excess zinc. Plant Cell Environ 38:207–223. https://doi.org/10.1111/pce.12434
61. He JL, Li H, Ma CF, Zhang YL, Polle A, Rennenberg H, Cheng XQ, Luo ZH (2015) Overexpression of bacterial γ-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. New Phytol 205:240–254. https://doi.org/10.1111/nph.13013
62. Hu YH, Nan ZR, Jin C, Wang N, Luo HZ (2014) Phytoextraction potential of poplar (*Populus alba* L. var. *pyramidalis* Bunge) from calcareous agricultural soils contaminated by cadmium. Int J Phytoremediation 16:482–495. https://doi.org/10.1080/15226514.2013.798616
63. Hu YH, Nan ZR, Su JQ, Wang SL (2014) Chelant-assisted uptake and accumulation of Cd by poplar from calcareous arable soils around Baiyin nonferrous metal smelters, Northern China. Arid Land Res Manag 28:340–354. https://doi.org/10.1080/15324982.2013.841305
64. Lingua G, Todeschini V, Grimaldi M, Baldantoni D, Proto A, Cicatelli A, Biondi S, Torrigiani P, Castiglione S (2014) Polyaspartate, a biodegradable chelant that improves the phytoremediation potential of poplar in a highly metal-contaminated agricultural soil. J Environ Manage 132:9–15. https://doi.org/10.1016/j.jenvman.2013.10.015
65. Cicatelli A, Torrigiani P, Todeschini V, Biondi S, Castiglione S, Lingua G (2014) Arbuscular mycorrhizal fungi as a tool to ameliorate the phytoremediation potential of poplar: biochemical and molecular aspects. iFOREST 7:333–341. https://doi.org/10.3832/ifor1045-007
66. Stobrawa K (2014) Poplars (*Populus* spp.): ecological role, applications and scientific perspectives in the 21st century (Review paper). Balt For 20:204–213
67. Radojčić Redovniković I, De Marco A, Proietti C, Hanousek K, Sedak M, Bilandžić N, Jakovljević T (2017) Poplar response to cadmium and lead soil contamination. Ecotoxicol Environ Saf 144:482–489. https://doi.org/10.1016/j.ecoenv.2017.06.011
68. Pottier M, de la Torre VSG, Victor C, David LC, Chalot M, Thomine S (2015) Genotypic variations in the dynamics of metal concentrations in poplar leaves: a field study with a perspective on phytoremediation. Environ Pollut 199:73–82. https://doi.org/10.1016/j.envpol.2015.01.010
69. Baldantoni D, Cicatelli A, Bellino A, Castiglione S (2014) Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. J Environ Manage 146:94–99. https://doi.org/10.1016/j.jenvman.2014.07.045

70. Hu YH, Nan ZR, Su JQ, Wang N (2013) Heavy metal accumulation by poplar in calcareous soil with various degrees of multi-metal contamination: implications for phytoextraction and phytostabilization. Environ Sci Pollut Res 20:7194–7203. https://doi.org/10.1007/s11356-013-1711-0
71. Rafati M, Khorasani N, Moattar F, Shirvany A, Moraghebi F, Hosseinzadeh S (2011) Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromuim and nickel absorption from polluted soil. Int J Environ Res 5:961–970. https://ijer.ut.ac.ir/article_453_3455397d2f9cb7936096b980cb8d5ce2.pdf
72. Wang Q, Xiong D, Zhao P, Yu X, Tu B, Wang G (2011) Effect of applying an arsenic-resistant and plant growth-promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoides* LH05-17. J Appl Microbiol 111:1065–1074. https://doi.org/10.1111/j.1365-2672.2011.05142.x
73. Labidi S, Firmin S, Verdin A, Bidar G, Laruelle F, Douay F, Shirali P, Fontaine J, Sahraoui ALH (2017) Nature of fly ash amendments differently influences oxidative stress alleviation in four forest tree species and metal trace element phytostabilization in aged contaminated soil: a long-term field experiment. Ecotoxicol Environ Saf 138:190–198. https://doi.org/10.1016/j.ecoenv.2016.12.027
74. Pourrut B, Lopareva-Pohu A, Pruvot C, Garcon G, Verdin A, Waterlot C, Bidar G, Shirali P, Douay F (2011) Assessment of fly ash-aided phytostabilisation of highly contaminated soils after an 8-year field trial Part 2. Influence on plants. Sci Total Environ 409:4504–4510. https://doi.org/10.1016/j.scitotenv.2011.07.047
75. Kalubi KN, Mehes-Smith M, Omri A (2016) Comparative analysis of metal translocation in red maple (*Acer rubrum*) and trembling aspen (*Populus tremuloides*) populations from stressed ecosystems contaminated with metals. Chem Ecol 32:312–323. https://doi.org/10.1080/02757540.2016.1142978
76. Djukic M, Bojovic DD, Grbic M, Skocajic D, Obratov-Petkovic D, Bjedov I (2013) Effect of Cd and Pb on *Ailanthus altissima* and *Acer negundo* seed germination and early seedling growth. Fresen Environ Bull 22:524–530
77. Vandecasteele B, Samyn J, De Vos B, Muys B (2008) Effect of tree species choice and mineral capping in a woodland phytostabilisation system: a case-study for calcareous dredged sediment landfills with an oxidised topsoil. Ecol Eng 32:263–273. https://doi.org/10.1016/j.ecoleng.2007.12.002
78. Mertens J, Vervaeke P, De Schrijver A, Luyssaert S (2004) Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. Sci Total Environ 326:209–215. https://doi.org/10.1016/j.scitotenv.2003.12.010
79. Fernandez-Fuego D, Keunen E, Cuypers A, Bertrand A, Gonzalez A (2017) Mycorrhization protects *Betula pubescens* Ehr. from metal-induced oxidative stress increasing its tolerance to grow in an industrial polluted soil. J Hazard Mater 336:119–127. https://doi.org/10.1016/j.jhazmat.2017.04.065
80. Fernandez R, Bertrand A, Casares A, Garcia R, Gonzalez A, Tames RS (2008) Cadmium accumulation and its effect on the in vitro growth of woody fleabane and mycorrhized white birch. Environ Pollut 152:522–529. https://doi.org/10.1016/j.envpol.2007.07.011
81. Zloch M, Thiem D, Gadzala-Kopciuch R, Hrynkiewicz K (2016) Synthesis of siderophores by plant-associated metallotolerant bacteria under exposure to $Cd^{2+}$. Chemosphere 156:312–325. https://doi.org/10.1016/j.chemosphere.2016.04.130
82. Dmuchowski W, Gozdowski D, Bragoszewska P, Baczewska AH, Suwara I (2014) Phytoremediation of zinc contaminated soils using silver birch (*Betula pendula* Roth). Ecol Eng 71:32–35. https://doi.org/10.1016/j.ecoleng.2014.07.053
83. Theriault G, Nkongolo KK, Michael P (2014) Genetic and metal analyses of fragmented populations of *Betula papyrifera* (Marsh) in a mining reclaimed region: identification of population-diagnostic molecular marker. Ecol Evol 4:3435–3443. https://doi.org/10.1002/ece3.1195

84. Rodriguez-Seijo A, Arenas-Lago D, Lago-Vila M, Vega F, Couce LA (2014) Limitations for revegetation in lead/zinc minesoils (NW Spain). J Soil Sediment 14:785–793. https://doi.org/10.1007/s11368-013-0826-8
85. Alagic SC, Serbula SS, Tosic SB, Pavlovic AN, Petrovic JV (2013) Bioaccumulation of arsenic and cadmium in birch and lime from the Bor region. Arch Environ Contam Toxicol 65:671–682. https://doi.org/10.1007/s00244-013-9948-7
86. Migeon A, Richaud P, Guinet F, Chalot M, Blaudez D (2009) Metal accumulation by woody species on contaminated sites in the North of France. Water Air Soil Pollut 204:89–101. https://doi.org/10.1007/s11270-009-0029-5
87. Lievens C, Yperman J, Vangronsveld J, Carleer R (2008) Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis: part I. Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. Fuel 87:1894–1905. https://doi.org/10.1016/j.fuel.2007.10.021
88. Lievens C, Yperman J, Cornelissen T, Carleer R (2008) Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis: part II: characterisation of the liquid and gaseous fraction as a function of the temperature. Fuel 87:1906–1916. https://doi.org/10.1016/j.fuel.2007.10.023
89. Baltrenaite E, Butkus D (2007) Modelling of Cu, Ni, Zn, Mn and Pb transport from soil to seedlings of coniferous and leafy trees. J Environ Eng Landsc 15:200–207
90. Hung H, Mackay D (1997) A novel and simple model of the uptake of organic chemicals by vegetation from air and soil. Chemosphere 35:959–977. https://doi.org/10.1016/S0045-6535(97)00182-3
91. Soudek P, Petrova S, Benesova D, Tykva R, Vankova R, Vanek T (2007) Comparison of Ra-226 nuclide from soil by three woody species *Betula pendula*, *Sambucus nigra* and *Alnus glutinosa* during the vegetation period. J Environ Radioact 97:76–82. https://doi.org/10.1016/j.jenvrad.2007.03.008
92. Wislocka M, Krawczyk J, Klink A, Morrison L (2006) Bioaccumulation of heavy metals by selected plant species from uranium mining dumps in the Sudety Mts., Poland. Pol J Environ Stud 15:811–818
93. Klassen SP, McLean JE, Grossl PR, Sims RC (2000) Fate and behavior of lead in soils planted with metal-resistant species (river birch and small wing sedge). J Environ Qual 29:1826–1834
94. Qian Y, Gallagher FJ, Feng H, Wu MY, Zhu QZ (2014) Vanadium uptake and translocation in dominant plant species on an urban coastal brownfield site. Sci Total Environ 476:696–704. https://doi.org/10.1016/j.scitotenv.2014.01.049
95. Oh CY, Lee JC, Han SH, Kim PG (2004) Characteristics of Cd accumulation and phytoremediation among three half-sib families of *Betula schmidtii*. Korean J Agric For Meteor 6:204–209
96. Yeo JK, Kim IS, Koo YB, Lee JC (2001) Uptake and tolerance to lead in *Populus alba X glandulosa* and *Betula schmidtii*. J Korean Forestry Soc 90:600–607
97. Huang L, Zhang HQ, Song YY, Yang YR, Chen H, Tang M (2017) Subcellular compartmentalization and chemical forms of lead participate in lead tolerance of *Robinia pseudoacacia* L. with *Funneliformis mosseae*. Front Plant Sci 8:517. https://doi.org/10.3389/fpls.2017.00517
98. Huang SP, Jia X, Zhao YH, Chang YF, Bai B (2016) Response of *Robinia pseudoacacia* L. rhizosphere microenvironment to Cd and Pb contamination and elevated temperature. Appl Soil Ecol 108:269–277. https://doi.org/10.1016/j.apsoil.2016.09.002
99. Jia X, Zhao YH, Liu T, Huang SP (2016) Elevated $CO_2$ affects secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soils. Chemosphere 160:199–207. https://doi.org/10.1016/j.chemosphere.2016.06.089
100. Kaya G, Yaman M (2008) Trace metal concentrations in cupressaceae leaves as biomonitors of environmental pollution. Trace Elem Electroly 25:156–164
101. Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272. https://doi.org/10.1023/A:1026100707797

102. Pinheiro JC, Marques CR, Pinto G, Bouguerra S, Mendo S, Gomes NC, Goncalves F, Rocha-Santos T, Duarte AC, Roembke J, Sousa JP, Ksibi M, Haddioui A, Pereira R (2013) The performance of *Fraxinus angustifolia* as a helper for metal phytoremediation programs and its relation to the endophytic bacterial communities. Geoderma 202:171–182. https://doi.org/10.1016/j.geoderma.2013.03.014
103. Rabeda I, Bilski H, Mellerowicz EJ, Napieralska A, Suski S, Wozny A, Krzeslowska M (2015) Colocalization of low-methylesterified pectins and Pb deposits in the apoplast of aspen roots exposed to lead. Environ Pollut 205:315–326. https://doi.org/10.1016/j.envpol.2015.05.048
104. Krzeslowska M, Rabeda I, Basinska A, Lewandowski M, Mellerowicz EJ, Napieralska A, Samardakiewicz S, Wozny A (2016) Pectinous cell wall thickenings formation—a common defense strategy of plants to cope with Pb. Environ Pollut 214:354–361. https://doi.org/10.1016/j.envpol.2016.04.019
105. Langer I, Santner J, Krpata D, Fitz WJ, Wenzel WW, Schweiger PF (2012) Ectomycorrhizal impact on Zn accumulation of *Populus tremula* L. grown in metalliferous soil with increasing levels of Zn concentration. Plant Soil 355:283–297. https://doi.org/10.1007/s11104-011-1098-y
106. Hassinen V, Vallinkoski VM, Issakainen S, Tervahauta A, Karenlampi S, Servomaa K (2009) Correlation of foliar MT2b expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula x tremuloides*) grown in contaminated soil. Environ Pollut 157:922–930. https://doi.org/10.1016/j.envpol.2008.10.023
107. Weih M (2004) Intensive short rotation forestry in boreal climates: present and future perspectives. Can J For Res 34:1369–1378. https://doi.org/10.1139/X04-090
108. Dutton MV, Humphreys PN (2005) Assessing the potential of short rotation coppice (SRC) for cleanup of radionuclide-contaminated sites. Int J Phytoremediation 7:279–293. https://doi.org/10.1080/16226510500327137
109. He JL, Li H, Luo J, Ma CF, Li SJ, Qu L, Gai Y, Jiang XN, Janz D, Polle A, Tyree M, Luo ZB (2013) A transcriptomic network underlies microstructural and physiological responses to cadmium in *Populus x canescens*. Plant Physiol 162:424–439. https://doi.org/10.1104/pp.113.215681
110. 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 90:1478–1486. https://doi.org/10.1016/j.chemosphere.2012.09.044
111. Durand TC, Sergeant K, Planchon S, Carpin S, Label P, Morabito D, Hausman JF, Renaut J (2010) Acute metal stress in *Populus tremula x P. alba* (717-1B4 genotype): leaf and cambial proteome changes induced by cadmium(2+). Proteomics 10:349–368. https://doi.org/10.1002/pmic.200900484
112. Freitas H, Prasad MNV, Pratas J (2004) Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation—relevance to the management of mine environment. Chemosphere 54:1625–1642. https://doi.org/10.1016/j.chemosphere.2003.09.045
113. Parraga-Aguado I, Alvarez-Rogel J, Gonzalez-Alcaraz MN, Jimenez-Carceles FJ, Conesa HM (2013) Assessment of metal(loid)s availability and their uptake by *Pinus halepensis* in a Mediterranean forest impacted by abandoned tailings. Ecol Eng 58:84–90. https://doi.org/10.1016/j.ecoleng.2013.06.013
114. Alahabadi A, Ehrampoush MH, Miri M, Aval HE, Yousefzadeh S, Ghaffari HR, Ahmadi E, Talebi P, Fathabadi ZA, Babai F, Nikoonahad A, Sharafi K, Hosseini-Bandegharaei A (2017) A comparative study on capability of different tree species in accumulating heavy metals from soil and ambient air. Chemosphere 172:459–467. https://doi.org/10.1016/j.chemosphere.2017.01.045
115. Abbasi H, Pourmajidian MR, Hodjati SM, Fallah A, Nath S (2017) Effect of soil-applied lead on mineral contents and biomass in *Acer cappadocicum*, *Fraxinus excelsior* and *Platycladus orientalis* seedlings. iFOREST 10:722–728. https://doi.org/10.3832/ifor2251-010

116. Sugiura Y, Shibata M, Ogata Y, Ozawa H, Kanasashi T, Takenaka C (2016) Evaluation of radiocesium concentrations in new leaves of wild plants two years after the Fukushima Daiichi Nuclear Power Plant accident. J Environ Radioact 160:8–24. https://doi.org/10.1016/j.jenvrad.2016.04.015
117. Saba G, Parizanganeh AH, Zamani A, Saba J (2015) Phytoremediation of heavy metals contaminated environments: screening for native accumulator plants in Zanjan-Iran. Int J Environ Res 9:309–316
118. Liu J, Zhang XH, Li TY, Wu QX, Jin ZJ (2014) Soil characteristics and heavy metal accumulation by native plants in a Mn mining area of Guangxi, South China. Environ Monit Assess 186:2269–2279. https://doi.org/10.1007/s10661-013-3535-2
119. Wang Y, Bai S, Wu J, Chen J, Yang Y, Zhu X, Zhu T (2015) Plumbum/zinc accumulation in seedlings of six afforestation species cultivated in mine spoil substrate. J Trop For Sci 27:166–175. https://www.frim.gov.my/v1/JTFSOnline/jtfs/v27n2/166-175.pdf
120. Ozen SA, Yaman M (2016) Phytoextraction of lead and its relationship with histidine in six plant species using ICP-MS and HPLC-MS. Chem Ecol 32:346–356. https://doi.org/10.1080/02757540.2016.1142979
121. Lu Y, Li XR, He MZ, Zeng FJ, Li XY (2017) Accumulation of heavy metals in native plants growing on mining-influenced sites in Jinchang: a typical industrial city (China). Environ Earth Sci 76:446. https://doi.org/10.1007/s12665-017-6779-2
122. Yang YR, Liang Y, Ghosh A, Song YY, Chen H, Tang M (2015) Assessment of arbuscular mycorrhizal fungi status and heavy metal accumulation characteristics of tree species in a lead-zinc mine area: potential applications for phytoremediation. Environ Sci Pollut Res 22:13179–13193. https://doi.org/10.1007/s11356-015-4521-8
123. Kang W, Bao JG, Zheng J, Zou T, Min JH, Yang Y (2014) Analysis on heavy metal enrichment ability of woody plants at ancient copper mine site in Tonglushan of Hubei Province. J Plant Resour Environ 23:78–84. https://doi.org/10.3969/j.issn.1674-7895.2014.01.12
124. Antonijevic MM, Dimitrijevic MD, Milic SM, Nujkic MM (2012) Metal concentrations in the soils and native plants surrounding the old flotation tailings pond of the Copper Mining and Smelting Complex Bor (Serbia). J Environ Monit 14:866–877. https://doi.org/10.1039/c2em10803h
125. Monfared SH, Matinizadeh M, Shirvany A, Amiri GZ, Fard RM, Rostami F (2013) Accumulation of heavy metal in *Platanus orientalis*, *Robinia pseudoacacia* and *Fraxinus rotundifolia*. J For Res 24:391–395. https://doi.org/10.1007/s11676-012-0313-x
126. Shen J, Song LL, Muller K, Hu YY, Song Y, Yu WW, Wang HL, Wu JS (2016) Magnesium alleviates adverse effects of lead on growth, photosynthesis, and ultrastructural alterations of *Torreya grandis* seedlings. Front Plant Sci 7:1819. https://doi.org/10.3389/fpls.2016.01819
127. Shanker AK, Ravichandran V, Pathmanabhan G (2005) Phytoaccumulation of chromium by some multipurpose-tree seedlings. Agr Syst 64:83–87. https://doi.org/10.1007/s10457-005-2477-2
128. Meeinkuirt W, Kruatrachue M, Pichtel J, Phusantisampan T, Saengwilai P (2016) Influence of organic amendments on phytostabilization of Cd-contaminated soil by *Eucalyptus camaldulensis*. ScienceAsia 42:83–91. https://doi.org/10.2306/scienceasia1513-1874.2016.42.083
129. Madejon P, Maranon T, Navarro-Fernandez CM, Dominguez MT, Alegre JM, Robinson B, Murillo JM (2017) Potential of *Eucalyptus camaldulensis* for phytostabilization and biomonitoring of trace element contaminated soils. PLoS One 12:e0180240. https://doi.org/10.1371/journal.pone.0180240
130. Ouaryi A, Boularbah A, Sanguin H, Hafidi M, Baudoin E, Ouahmane L, Le Roux C, Galiana A, Prin Y, Duponnois R (2016) High potential of symbiotic interactions between native mycorrhizal fungi and the exotic tree *Eucalyptus camaldulensis* for phytostabilization of metal-contaminated arid soils. Int J Phytoremediation 18:41–47. https://doi.org/10.1080/15226514.2015.1058335
131. Aggangan NS, Aggangan BJS (2012) Selection of ectomycorrhizal fungi and tree species for rehabilitation of Cu mine tailings in the Philippines. J Environ Sci Manag 15:59–71

132. Guarino C, Conte B, Spada V, Arena S, Sciarrillo R, Scaloni A (2014) Proteomic analysis of eucalyptus leaves unveils putative mechanisms involved in the plant response to a real condition of soil contamination by multiple heavy metals in the presence or absence of mycorrhizal/rhizobacterial additives. Environ Sci Technol 48:11487–11496. https://doi.org/10.1021/es502070m
133. Melo RF, Dias LE, de Assis IR (2016) Behavior of *Eucalyptus urophylla* and *Eucalyptus citriodora* seedlings grown in soil contaminated by arsenate. Rev Bras Ciênc Solo 40:e0150310. https://doi.org/10.1590/18069657rbcs20150310
134. Melo RF, Dias LE, de Mello JWV, Oliveira JA (2010) Behavior of *Eucalyptus grandis* and *E. cloeziana* seedlings grown in arsenic-contaminated soil. Rev Bras Ciênc Solo 34:985–992. http://www.scielo.br/pdf/rbcs/v34n3/40.pdf
135. Sanchez-Palacios JT, Callahan D, Baker AJM, Woodrow IE, Doronila AI, Wang YD, Collins RN (2012) Arsenic response in roots of *Eucalyptus* spp. In: Ng JC, Noller BN, Naidu R, Bundschuh J, Bhattacharya P (eds) Understanding the geological and medical interface of arsenic. AS 2012 Book series: Arsenic in the environment. CRC Press, Boca Raton, FL, pp 332–334
136. King DJ, Doronila AI, Feenstra C, Baker AJM, Woodrow IE (2008) Phytostabilisation of arsenical gold mine tailings using four *Eucalyptus* species: growth, arsenic uptake and availability after five years. Sci Total Environ 406:35–42. https://doi.org/10.1016/j.scitotenv.2008.07.054
137. Luo J, Qi SH, Peng L, Wang JJ (2016) Phytoremediation efficiency of CD by *Eucalyptus globulus* transplanted from polluted and unpolluted sites. Int J Phytoremediation 18:308–314. https://doi.org/10.1080/15226514.2015.1094446
138. Mughini G, Alianiello F, Benedetti A, Gras LM, Gras MA, Salvati L (2013) Clonal variation in growth, arsenic and heavy metal uptakes of hybrid *Eucalyptus* clones in a Mediterranean environment. Agr Syst 87:755–766. https://doi.org/10.1007/s10457-013-9594-0
139. Marchiol L, Fellet G, Boscutti F, Montella C, Mozzi R, Guarino C (2013) Gentle remediation at the former "Pertusola Sud" zinc smelter: evaluation of native species for phytoremediation purposes. Ecol Eng 53:343–353. https://doi.org/10.1016/j.ecoleng.2012.12.072
140. Meeinkuirt W, Pokethitiyook P, Kruatrachue M, Tanhan P, Chaiyarat R (2012) Phytostabilization of a Pb-contaminated mine tailings by various tree species in pot and field trial experiments. Int J Phytoremediation 14:925–938. https://doi.org/10.1080/15226514.2011.636403
141. Shukla OP, Juwarkar AA, Singh SK, Khan S, Rai UN (2011) Growth responses and metal accumulation capabilities of woody plants during the phytoremediation of tannery sludge. Waste Manag 31:115–123. https://doi.org/10.1016/j.wasman.2010.08.022
142. Arriagada C, Pereira G, Garcia-Romera I, Ocampo JA (2010) Improved zinc tolerance in *Eucalyptus globulus* inoculated with *Glomus deserticola* and *Trametes versicolor* or *Coriolopsis rigida*. Soil Biol Biochem 42:118–124. https://doi.org/10.1016/j.soilbio.2009.10.011
143. Arriagada C, Aranda E, Sampedro I, Garcia-Romera I, Ocampo JA (2009) Interactions of *Trametes versicolor*, *Coriolopsis rigida* and the arbuscular mycorrhizal fungus *Glomus deserticola* on the copper tolerance of *Eucalyptus globulus*. Chemosphere 77:273–278. https://doi.org/10.1016/j.chemosphere.2009.07.042
144. Dhillon KS, Dhillon SK, Thind HS (2008) Evaluation of different agroforestry tree species for their suitability in the phytoremediation of seleniferous soils. Soil Use Manag 24:208–216
145. Arriagada CA, Herrera MA, Borie F, Ocampo JA (2007) Contribution of arbuscular mycorrhizal and saprobe fungi to the aluminum resistance of *Eucalyptus globulus*. Water Air Soil Pollut 182:383–394. https://doi.org/10.1007/s11270-007-9349-5
146. Raju D, Kumar S, Mehta UJ, Hazra S (2008) Differential accumulation of manganese in three mature tree species (*Holoptelea*, *Cassia*, Neem) growing on a mine dump. Curr Sci 94:639–643

147. Yang N, Zhou FR, Wang JX (2016) Eco-toxicological effects of two kinds of lead compounds on forest tree seed in alkaline soil. Environ Monit Assess 188:201. https://doi.org/10.1007/s10661-016-5198-2
148. Zong K, Huang J, Nara K, Chen YH, Shen ZG, Lian CL (2015) Inoculation of ectomycorrhizal fungi contributes to the survival of tree seedlings in a copper mine tailing. J For Res 20:493–500. https://doi.org/10.1007/s10310-015-0506-1
149. Sousa NR, Ramos MA, Marques APGC, Castro PML (2014) A genotype dependent-response to cadmium contamination in soil is displayed by *Pinus pinaster* in symbiosis with different mycorrhizal fungi. Appl Soil Ecol 76:7–13. https://doi.org/10.1016/j.apsoil.2013.12.005
150. Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater 250:477–483. https://doi.org/10.1016/j.jhazmat.2013.02.014
151. Yang JX, Yao DX, Li XL, Zhang ZG (2011) Research on effect of woody plants remediation heavy metal. In: Jin D, Lin S (eds) Advances in computer science, intelligent system and environment. Book series: Advances in intelligent and soft computing, vol 106, pp 679–684
152. de Carcer DA, Martin M, Karlson U, Rivilla R (2007) Changes in bacterial populations and in biphenyl dioxygenase gene diversity in a polychlorinated biphenyl-polluted soil after introduction of willow trees for rhizoremediation. Appl Environ Microbiol 73:6224–6232. https://doi.org/10.1128/AEM.01254-07
153. Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW (2014) Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISME J 8:344–358. https://doi.org/10.1038/ismej.2013.163
154. Gao Y, Yang Y, Ling W, Kong H, Zhu X (2011) Gradient distribution of root exudates and polycyclic aromatic hydrocarbons in rhizosphere soil. Soil Sci Soc Am J 75:1694–1703. https://doi.org/10.2136/sssaj2010.0244
155. Xie XM, Liao M, Yang J, Chai JJ, Fang S, Wang RH (2012) Influence of root-exudates concentration on pyrene degradation and soil microbial characteristics in pyrene contaminated soil. Chemosphere 88:1190–1195. https://doi.org/10.1016/j.chemosphere.2012.03.068
156. Cook RL, Landmeyer JE, Atkinson B, Messier JP, Nichols EG (2010) Field note: successful establishment of a phytoremediation system at a petroleum hydrocarbon contaminated shallow aquifer: trends, trials, and tribulations. Int J Phytoremediation 12:716–732. https://doi.org/10.1080/15226510903390395
157. da Cunha ACB, Sabedot S, Sampaio CH, Ramos CG, da Silva AR (2012) *Salix rubens* and *Salix triandra* species as phytoremediators of soil contaminated with petroleum-derived hydrocarbons. Water Air Soil Pollut 223:4723–4731. https://doi.org/10.1007/s11270-012-1228-z
158. Ferro AM, Adham T, Berra B, Tsao D (2013) Performance of deep-rooted phreatophytic trees at a site containing total petroleum hydrocarbons. Int J Phytoremediation 15:232–244. https://doi.org/10.1080/15226514.2012.687195
159. Argus GW (2007) *Salix* (Salicaceae) distribution maps and a synopsis of their classification in North America, North of Mexico. Harvard Pap Bot 12:335–368. https://doi.org/10.3100/1043-4534(2007)12[335:SSDMAA]2.0.CO;2
160. Jia H, Wang H, Lu HL, Jiang S, Dai MY, Liu JC, Yan CL (2016) Rhizodegradation potential and tolerance of *Avicennia marina* (Forsk.) Vierh in phenanthrene and pyrene contaminated sediments. Mar Pollut Bull 110:112–118. https://doi.org/10.1016/j.marpolbul.2016.06.075
161. Page AP, Yergeau E, Greer CW (2015) *Salix purpurea* stimulates the expression of specific bacterial xenobiotic degradation genes in a soil contaminated with hydrocarbons. PLoS One 10:e0132062. https://doi.org/10.1371/journal.pone.0132062
162. Yang XH, Garnier P, Wang SZ, Bergheaud V, Huang XF, Qiu RL (2014) PAHs sorption and desorption on soil influenced by pine needle litter-derived dissolved organic matter. Pedosphere 24:575–584. https://doi.org/10.1016/S1002-0160(14)60043-6
163. Wang YY, Fang L, Lin L, Luan TG, Tam NFY (2014) Effects of low molecular-weight organic acids and dehydrogenase activity in rhizosphere sediments of mangrove plants on

phytoremediation of polycyclic aromatic hydrocarbons. Chemosphere 99:152–159. https://doi.org/10.1016/j.chemosphere.2013.10.054

164. Oleszczuk P, Godlewska P, Reible DD, Kraska P (2017) Bioaccessibility of polycyclic aromatic hydrocarbons in activated carbon or biochar amended vegetated (*Salix viminalis*) soil. Environ Pollut 227:406–413. https://doi.org/10.1016/j.envpol.2017.04.064
165. Hultgren J, Pizzul L, Castillo MD, Granhall U (2010) 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. https://doi.org/10.1080/15226510902767122
166. Rezek J, der Wiesche C, Mackova M, Zadrazil F, Macek T (2009) Biodegradation of PAHs in long-term contaminated soil cultivated with European white birch (*Betula pendula*) and red mulberry (*Morus rubra*) tree. Int J Phytoremediation 11:66–81. https://doi.org/10.1080/15226510802363493
167. Sipila TP, Keskinen A, Akerman ML, Fortelius C, Haahtela K, Yrjala K (2008) High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of IE3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. ISME J 2:968–981. https://doi.org/10.1038/ismej.2008.50
168. Spriggs T, Banks MK, Schwab P (2005) Phytoremediation of polycyclic aromatic hydrocarbons in manufactured gas plant-impacted soil. J Environ Qual 34:1755–1762. https://doi.org/10.2134/jeq2004.0399
169. Bergeron JM, Crews D, McLachlan JA (1994) PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:780–781
170. Morgan M, Deoraj A, Felty Q, Roy D (2017) Environmental estrogen-like endocrine disrupting chemicals and breast cancer. Mol Cell Endocrinol 457:89–102. https://doi.org/10.1016/j.mce.2016.10.003
171. Ancona V, Caracciolo AB, Grenni P, Di Lenola M, Campanale C, Calabrese A, Uricchio VF, Mascolo G, Massacci A (2017) Plant-assisted bioremediation of a historically PCB and heavy metal-contaminated area in Southern Italy. N Biotechnol 38B:65–73. https://doi.org/10.1016/j.nbt.2016.09.006
172. Ficko SA, Rutter A, Zeeb BA (2010) Potential for phytoextraction of PCBs from contaminated soils using weeds. Sci Total Environ 408:3469–3476. https://doi.org/10.1016/j.scitotenv.2010.04.036
173. Meggo RE, Schnoor JL (2013) Cleaning polychlorinated biphenyl (PCB) contaminated garden soil by phytoremediation. Environ Sci (Ruse) 1:33–52. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3883314/pdf/nihms514018.pdf
174. Zhai G, Lehmler HJ, Schnoor JL (2010) Hydroxylated metabolites of 4-monochlorobiphenyl and its metabolic pathway in whole poplar plants. Environ Sci Technol 44:3901–3907. https://doi.org/10.1021/es100230m
175. Zhai G, Lehmler HJ, Schnoor JL (2010) Identification of hydroxylated metabolites of 3,3′,4,4′-tetrachlorobiphenyl and metabolic pathway in whole poplar plants. Chemosphere 81:523–528. https://doi.org/10.1016/j.chemosphere.2010.07.036
176. Zhai G, Lehmler HJ, Schnoor JL (2011) New hydroxylated metabolites of 4-monochlorobiphenyl in whole poplar plants. Chem Cent J 5:87. https://doi.org/10.1186/1752-153X-5-87
177. Zhai G, Lehmler HJ, Schnoor JL (2013) Sulfate metabolites of 4-monochlorobiphenyl in whole poplar plants. Environ Sci Technol 47:557–562. https://doi.org/10.1021/es303807f
178. Zhai G, Hu D, Lehmler HJ, Schnoor JL (2011) Enantioselective biotransformation of chiral PCBs in whole poplar plants. Environ Sci Technol 45:2308–2316. https://doi.org/10.1021/es1033662
179. Zhai G, Gutowski SM, Lehmler HJ, Schnoor JL (2014) Enantioselective transport and biotransformation of chiral hydroxylated metabolites of polychlorinated biphenyls in whole poplar plants. Environ Sci Technol 48:12213–12220. https://doi.org/10.1021/es503443e

180. Ma CX, Zhai GS, Wu HM, Kania-Korwel I, Lehmler HJ, Schnoor JL (2016) Identification of a novel hydroxylated metabolite of 2,2′,3,5′,6-pentachlorobiphenyl formed in whole poplar plants. Environ Sci Pollut Res 23:2089–2098. https://doi.org/10.1007/s11356-015-5939-8
181. Macci C, Peruzzi E, Doni S, Poggio G, Masciandaro G (2016) The phytoremediation of an organic and inorganic polluted soil: a real scale experience. Int J Phytoremediation 18:378–386. https://doi.org/10.1080/15226514.2015.1109595
182. Meggo E, Schnoor JL, Hu DF (2013) Dechlorination of PCBs in the rhizosphere of switchgrass and poplar. Environ Pollut 178:312–321. https://doi.org/10.1016/j.envpol.2013.02.035
183. Slater H, Gouin T, Leigh MB (2011) Assessing the potential for rhizoremediation of PCB contaminated soils in northern regions using native tree species. Chemosphere 84:199–206. https://doi.org/10.1016/j.chemosphere.2011.04.058
184. Leigh MB, Prouzova P, Mackova M, Macek T, Nagle DP, Fletcher JS (2006) Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. Appl Environ Microbiol 72:2331–2342. https://doi.org/10.1128/AEM.72.4.2331-2342.2006
185. Moore FP, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD, Moore ERB (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29:539–556. https://doi.org/10.1016/j.syapm.2005.11.012
186. Collins C, Laturnus F, Nepovim A (2002) Remediation of BTEX and trichloroethene—current knowledge with special emphasis on phytoremediation. Environ Sci Pollut Res 9:86–94. https://doi.org/10.1007/BF02987319
187. Schoeftner P, Watzinger A, Holzknecht P, Wimmer B, Reichenauer TG (2016) Transpiration and metabolisation of TCE by willow plants—a pot experiment. Int J Phytoremediation 18:686–692. https://doi.org/10.1080/15226514.2015.1131228
188. Lewis J, Qvarfort U, Sjostrom J (2015) *Betula pendula*: a promising candidate for phytoremediation of TCE in northern climates. Int J Phytoremediation 17:9–15. https://doi.org/10.1080/15226514.2013.828012
189. El-Gendy AS, Svingos S, Brice D, Garretson JH, Schnoor J (2009) Assessments of the efficacy of a long-term application of a phytoremediation system using hybrid poplar trees at former oil tank farm sites. Water Environ Res 81:486–498. https://doi.org/10.2175/106143008X357011
190. Gunderson JJ, Knight JD, Van Rees KCJ (2007) Impact of ectomycorrhizal colonization of hybrid poplar on the remediation of diesel-contaminated soil. J Environ Qual 36:927–934. https://doi.org/10.2134/jeq2006.0260
191. Agbogidi OM, Dolor ED, Okechukwu EM (2007) Evaluation of *Tectona grandis* (Linn.) and *Gmelina arborea* (Roxb.) for phytoremediation in crude oil contaminated soils. Agric Conspec Sci 72:149–152
192. Sun WHH, 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 11:260–266. https://doi.org/10.1065/espr2004.05.199.1
193. Jones RK, Sun WHH, Tang CS, Robert FM (2004) Phytoremediation of petroleum hydrocarbons in tropical coastal soils—II. Microbial response to plant roots and contaminant. Environ Sci Pollut Res 11:340–346. https://doi.org/10.1065/espr2004.05.199.2
194. Tesar M, Reichenauer TG, Sessitsch A (2002) Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. Soil Biol Biochem 34:1883–1892. https://doi.org/10.1016/S0038-0717(02)00202-X
195. Shan BQ, Zhang YT, Cao QL, Kang ZY, Li SY (2014) Growth responses of six leguminous plants adaptable in Northern Shaanxi to petroleum contaminated soil. Huanjing Kexue 35:1125–1130. https://doi.org/10.13227/j.hjkx.2014.03.043
196. McIntosh P, Kuzovkina YA, Schulthess CP, Guillard K (2016) Breakdown of low-level total petroleum hydrocarbons (TPH) in contaminated soil using grasses and willows. Int J Phytoremediation 18:656–663. https://doi.org/10.1080/15226514.2015.1109598

197. Leewis MC, Uhlik O, Fraraccio S, McFarlin K, Kottara A, Glover C, Macek T, Leigh MB (2016) Differential impacts of willow and mineral fertilizer on bacterial communities and biodegradation in diesel fuel oil-contaminated soil. Front Microbiol 7:837. https://doi.org/10.3389/fmicb.2016.00837
198. Dadrasnia A, Agamuthu P (2013) Organic wastes to enhance phyto-treatment of diesel-contaminated soil. Waste Manag Res 31:1133–1139. https://doi.org/10.1177/0734242X13502382
199. Best EPH, Kvesitadze G, Khatisashvili G, Sadunishvili T (2005) Plant processes important for the transformation and degradation of explosives contaminants. Z Naturforsch C 60:340–348
200. Van Aken B, Yoon JM, Schnoor JL (2004) Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, an 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 70:508–517. https://doi.org/10.1128/AEM.70.1.508-517.2004
201. van Dillewijn P, Couselo JL, Corredoira E, Delgado A, Wittich RM, Ballester A, Ramos JL (2008) Bioremediation of 2,4,6-trinitrotoluene by bacterial nitroreductase expressing transgenic aspen. Environ Sci Technol 42:7405–7410. https://doi.org/10.1021/es801231w
202. Schoenmuth BW, Pestemer W (2004) Dendroremediation of trinitrotoluene (TNT)—part 2: fate of radio-labelled TNT in trees. Environ Sci Pollut Res 11:331–339. https://doi.org/10.1065/espr2004.06.209.2
203. Hasanuzzaman M, Nahar K, Alam MM, Bhowmik PC, Hossain MA, Rahman MM, Prasad MNV, Ozturk M, Fujita M (2014) Potential use of halophytes to remediate saline soils. Biomed Res Int 2014:589341. https://doi.org/10.1155/2014/589341
204. Mirck J, Zalesny RS (2015) Mini-review of knowledge gaps in salt tolerance of plants applied to willows and poplars. Int J Phytoremediation 17:640–650. https://doi.org/10.1080/15226514.2014.950414
205. Qadir M, Oster JD, Schubert S, Noble AD, Sahrawat KL (2007) Phytoremediation of sodic and saline-sodic soils. Adv Agron 96:197–247. https://doi.org/10.1016/S0065-2113(07)96006-X
206. Liang L, Liu WT, Sun YB, Huo XH, Li S, Zhou QX (2017) Phytoremediation of heavy metal contaminated saline soils using halophytes: current progress and future perspectives. Environ Rev 25:269–281. https://doi.org/10.1139/er-2016-0063
207. Walter H (1961) Salinity problems in the acid zones. The adaptations of plants to saline soils. Arid Zone Res 14:65–68
208. Dagar JC (2009) Opportunities for alternative land uses in salty and water scarcity areas. Int J Ecol Environ Sci 35:53–66
209. Ladeiro B (2012) Saline agriculture in the 21st century: using salt contaminated resources to cope food requirements. J Bot 2012:310705. https://doi.org/10.1155/2012/310705
210. Ashraf MY, Awan AR, Mahmood K (2012) Rehabilitation of saline ecosystems through cultivation of salt tolerant plants. Pak J Bot 44:69–75
211. Doronila AI, Forster MA (2015) Performance measurement via sap flow monitoring of three *Eucalyptus* species for mine site and dryland salinity phytoremediation. Int J Phytoremediation 17:101–108. https://doi.org/10.1080/15226514.2013.850466
212. Shannon MC, Banuelos GS, Draper JH, Ajwa H, Jordahl J, Licht L (1999) Tolerance of hybrid poplar (*Populus*) trees irrigated with varied levels of salt, selenium, and boron. Int J Phytoremediation 1:273–288. https://doi.org/10.1080/15226519908500020
213. Major JE, Mosseler A, Malcolm JW, Heartz S (2017) Salinity tolerance of three *Salix* species: survival, biomass yield and allocation, and biochemical efficiencies. Biomass Bioenergy 105:10–22. https://doi.org/10.1016/j.biombioe.2017.06.014
214. Major JE, Mosseler A, Malcolm JW (2017) *Salix* species variation in leaf gas exchange, sodium, and nutrient parameters at three levels of salinity. Can J For Res 47:1045–1055. https://doi.org/10.1139/cjfr-2017-0028
215. Chen SL, Li JK, Wang SS, Fritz E, Huttermann A, Altman A (2003) Effects of NaCl on shoot growth, transpiration, ion compartmentation, and transport in regenerated plants of *Populus*

*euphratica* and *Populus tomentosa*. Can J For Res 33:967–975. https://doi.org/10.1139/X03-066

216. Chen SL, Lia JK, Fritz E, Wang SS, Huttermann A (2002) Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. For Ecol Manag 168:217–230. https://doi.org/10.1016/S0378-1127(01)00743-5
217. Mirck J, Volk TA (2010) Response of three shrub willow varieties (*Salix* spp.) to storm water treatments with different concentrations of salts. Bioresour Technol 101:3484–3492. https://doi.org/10.1016/j.biortech.2009.12.128
218. Stephens W, Tyrrel SF, Tiberghien JE (2000) Irrigating short rotation coppice with landfill leachate: constraints to productivity due to chloride. Bioresour Technol 75:227–229. https://doi.org/10.1016/S0960-8524(00)00065-1
219. Dangi SR, Banuelos G, Buyer JS, Hanson B, Gerik J (2018) Microbial community biomass and structure in saline and non-saline soils associated with salt- and boron-tolerant poplar clones grown for the phytoremediation of selenium. Int J Phytoremediation 20:129. https://doi.org/10.1080/15226514.2017.1337073
220. Nawaz MF, Gul S, Tanvir MA, Akhtar J, Chaudary S, Ahmad I (2016) Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (*Eucalyptus camaldulensis* Dehnh.). Turk J Agric For 40:425–432. https://doi.org/10.3906/tar-1407-73
221. Seenivasan R, Prasath V, Mohanraj R (2015) Restoration of sodic soils involving chemical and biological amendments and phytoremediation by *Eucalyptus camaldulensis* in a semiarid region. Environ Geochem Health 37:575–586. https://doi.org/10.1007/s10653-014-9674-8
222. Nasim G (2010) The role of arbuscualr mycorrhizae in inducing resistance to drought and salinity stress in crops. In: Ashraf N, Ozturk M, NSA A (eds) Plant adaptation and phytoremediation. Phytoremediation, pp 119–141. https://doi.org/10.1007/978-90-481-9370-7_6
223. Imada S, Yamanaka N, Tamai S (2009) Effects of salinity on the growth, Na partitioning, and Na dynamics of a salt tolerant tree, *Populus alba* L. J Arid Environ 73:245–251
224. Zhang LZ, Fan JJ, Meng QX, Niu Y, Niu W (2013) *Caragana* Fabr. promotes revegetation and soil rehabilitation in saline-alkali wasteland. Int J Phytoremediation 15:38–50. https://doi.org/10.1080/15226514.2012.670314
225. Silva YJAB, Silva YJAB, Freire MBGS, Lopes EAPL, Santos MA (2016) *Atriplex nummularia* Lindl. as alternative for improving salt-affected soils conditions in semiarid environments: a field experiment. Chilean JAR 76:343–348. https://doi.org/10.4067/S0718-58392016000300012
226. Lister NME, Beier P (2012) Design approaches to ecological restoration. Ecol Restor 30:263. https://doi.org/10.3368/er.30.4.263
227. Handel SN (2013) Ecological restoration foundations to designing habitats in urban areas. In: Beardsley J (ed) Designing wildlife habitats. Garden and Landscape Studies, Dumbarton Oaks Research Library, Harvard University Press, Cambridge, MA, pp 169–186
228. Costantini EAC, Branquinho C, Nunes A, Schwilch G, Stavi I, Valdecantos A, Zucca C (2016) Soil indicators to assess the effectiveness of restoration strategies in dryland ecosystems. Solid Earth 7:397–414. https://doi.org/10.5194/se-7-397-2016
229. Stefanes M, Ochoa Quintero JM, de Oliveira Roque F, Moreira Sugai LS, Reverberi Tambosi L, Lourival R, Laurance S (2016) Incorporating resilience and cost in ecological restoration strategies at landscape scale. Ecol Soc 21(4):54. https://doi.org/10.5751/ES-08922-210454
230. Masarovičová E, Májeková M, Vykouková I (2016) Functional traits and plasticity of the plants. In: Pessarakli M (ed) Handbook of photosynthesis, 3rd edn. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp 487–505, ISBN 978-1-4822-3073-4
231. Masarovičová E, Májeková M, Vykouková I (2015) Functional traits and plasticity of the plants (in Slovak), 1st edn. Publishing House of the Comenius University, Bratislava, p 84, ISBN 978-80-223-4033-5
232. Eliáš P (2007) Biodiversity—conception and its application (in Slovak). Život Prostr 41:5–12

# Part VII
# Nano-phytoremediation Applications

# Chapter 15
# Overview of Nano-phytoremediation Applications

**Alfreda Ogochukwu Nwadinigwe and Emmanuel Chibuzor Ugwu**

## 15.1 Meaning of Nano-phytoremediation

Remediation is the science of removal or reduction of pollutants from the environment using chemical or biological means. Nanotechnology is the science of creation and utilization of “petite” particles with dimensions in the order of $10^{-9}$ m [1], characterized by use of very small manufactured particles, called nanoparticles (NPs) or ultrafine particles [2]. Phytoremediation, on the other hand, is an aspect of bioremediation that uses higher plants to remove, transfer, stabilize, and/or destroy contaminants (such as volatile and semi-volatile organic compounds, explosives, solvents, pesticides, radionuclides, metals, and crude oil with its derivatives) in soil, surface water, sediments, and groundwater. Nano-phytoremediation is a combined technology between nanotechnology and phytoremediation for remediation of contaminated environments [3]. Nanotechnology deals with structural behavior at both molecular and submolecular levels and can be used in optical, electrical, and magnetic procedures [4].

## 15.2 Nanoparticles

Nanoparticles (NPs) are atomic or molecular aggregates with dimension between 1 and 100 nm that can drastically modify their physicochemical properties compared with bulk material [2, 5]. They are broadly divided into two groups, namely, organic and inorganic NPs [2]. Organic NPs include carbon NPs (fullerenes), while some of the inorganic NPs include magnetic NPs, noble metal NPs such as gold and silver, and semiconductor NPs such as titanium dioxide ($TiO_2$) and zinc oxide (ZnO).

---

A. O. Nwadinigwe (✉) · E. C. Ugwu
Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria
e-mail: alfreda.nwadinigwe@unn.edu.ng

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_15

Ruffini-Castiglione and Cremonini [6] identified three types of NPs which include natural NPs such as volcanic dust, lunar dust, and mineral composites; incidental NPs resulting from anthropogenic activity such as diesel exhaust, coal combustion, and welding fumes; and engineered NPs. Engineered NPs include quantum metal-based materials such as nanogold, nanozinc, nanoaluminium, and $TiO_2$ [7]. Nanomaterials have been suggested as efficient, cost-effective, and environmentally friendly alternatives to existing treatment materials, in both resource conservation and environmental remediation [8].

## 15.3 Biological Synthesis of Nanoparticles

Biological synthesis of NPs has grown markedly to create novel materials that are eco-friendly, cost-effective, and stable. Although NPs can be synthesized through an array of conventional methods, the biological route of synthesis is advantageous because of ease of rapid synthesis, controlled toxicity, control of size characteristics, low cost, and eco-friendly approach [9]. Vascular plants and microorganisms such as bacteria, yeasts, algae, fungi, and actinomycetes can be used for biosynthesis of NPs [10]. Extracellular secretion of enzymes offers the advantage of producing large quantities of NPs of size 100–200 nm in a relatively pure state, free from other cellular proteins; and the resultant NPs are further purified by filtration [2]. Microbial enzymes or plant phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective NPs. Fungi and bacteria require a comparatively longer incubation time for reduction of metal ions, but water soluble phytochemicals do this in much less time. Bacteria are considered as potential “biofactory” for synthesis of NPs like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, cadmium sulfide, and so on [2].

## 15.4 Phytoremediation

Phytoremediation is a low-cost, effective, and environment-friendly green technology that is successful in dealing with many contaminants. The different types of mechanisms for phytoremediation are as follows:

1. Phytodegradation: In this type of phytoremediation, contaminants are metabolized or biotransformed within the plant tissues.
2. Rhizosphere biodegradation: Natural exudates such as enzymes are released through plant roots. In so doing, nutrients are supplied to microorganisms in soil around the roots (rhizosphere), while microorganisms enhance biological degradation of contaminants.

3. Phytostabilization: For this, the plant produces chemical compounds which can sequester, precipitate, or immobilize contaminants. This is also called phytosequestration [11].
4. Phytovolatilization: Plants absorb water-containing organic contaminants and release them into the atmosphere through their leaves during transpiration.
5. Phytoaccumulation (or phytoextraction): Plant roots absorb mineral salts and water as well as contaminants and store/accumulate the contaminants in stems and leaves of plants. Since they are stored and not destroyed, such plants are harvested, while metals, especially precious ones, are extracted from the plants. This is regarded as phytomining. Accumulated hazardous wastes are disposed of safely.
6. Rhizofiltration: This process is a hydroponic system for treating water streams. Contaminated groundwater is pumped to the surface to irrigate plants. Therefore, this can be used for ex situ groundwater treatment. The roots absorb and accumulate contaminants (phytoaccumulation), and upon saturation, plants are harvested and disposed of.
7. Phytohydraulics (hydraulic control): Many trees grow their dense roots down into groundwater and absorb large quantities of water. In doing so, water table contaminants like pesticides, toxic herbicides, fertilizers, explosives, and radioactive compounds are degraded, reduced, or eliminated from the groundwater [11].

There are several types of plants that can remedy and take up contaminants from soil, surface water, groundwater, and sediments. Examples of such plants are *Pennisetum glaucum* (millet) (Nwadinigwe and Obi-Amadi) [12], poplar tree (*Populus deltoides*), sunflower (*Helianthus annuus*), Indian mustard (*Brassica juncea*), *Sorghum vulgare*, *Cynodon dactylon* (Bermuda grass), water hyacinth (*Eichhornia crassipes*).

## 15.5 Nano-phytoremediation Applications

NPs are extensively used for removal of biological and chemical contaminants including organic pollutants [13]. The unique properties of these nanosized materials have resulted in their use in various fields like biomedicine, electronics, agriculture [14], pharmaceuticals, cosmetics, environment [1, 15], food and beverages, surface coating, and polymers [9]. Due to their large surface area and high surface energy, NPs have the ability to absorb large quantities of pollutants or catalyze reactions at a much faster rate, thus reducing energy consumption during degradation and help in preventing release of contaminants [1]. In order words, this technology is explored for combating pollution by reducing the release or preventing formation of pollutants [16, 17]. The nanosize of particles also makes it possible to reach otherwise inaccessible areas and hence promote in situ rather than ex situ remediation [15]. The ability of NPs to be coated with various ligands and control surface area to volume ratio by changing their shapes enables the design of sensors with high selectivity, sensitivity, and specificity. Generally, nano-phytoremediation presents a number of potential environmental benefits, and this could be divided into three categories: treatment and remediation, sensing and detection, and pollution prevention [1].

The removal or reduction of environmental contaminants such as heavy metals, organic, and inorganic pollutants from contaminated sites using NPs formed by algae, fungi, and bacteria with the help of nanotechnology is called nano-bioremediation [2]. If such environmental contaminants are reduced/removed using NPs produced by or involving higher plants, it is regarded as nano-phytoremediation. Thus, there may be nano-phytodegradation, nano-phytostabilization, nano-phytovolatilization, nano-phytoaccumulation, nano-rhizofiltration, and so on. Nanotechnology increases phytoremediation efficiency, and so, NPs can be used for remediation of soils, water contaminated with heavy metals, e-wastes, and organic and inorganic pollutants. NPs in enzyme-based bioremediation can also be used in combination with phytoremediation [18]. For example, several complex organic compounds, such as long-chain hydrocarbons and organochlorines, are particularly resistant to microbial and plant degradation. A combined approach involving nanotechnology and phytoremediation could overcome this limitation; hence a complex organic compound would be degraded into simpler compounds by nanoencapsulated enzymes, enhancing rapid degradation by joint activities of microbes and plants [18].

Iron NPs are the most commonly used remediation tool [19, 20]. Iron has an integral role in remediation of environmental pollutants due to its reducing properties as an electron donor. It is a powerful reductant making it possible to be employed in remediation of any contaminant that can be degraded by reduction [1]. Studies have shown that organic contaminants such as atrazine, molinate, and chlorpyrifos can be degraded with nanosized zerovalent iron (nZV1) [21]. Engineered nanosized iron oxides, manganese oxides, cerium oxides, titanium oxides, or zinc oxides have great affinity for metal/metalloid absorption. This affinity, in conjunction with their high number of active surface sites and their significant surface area, make them effective for remediation of contaminated soils [22]. Karn et al. [23] discussed the degradation of polyhalogenated organic compounds and heavy metals using iron NPs. During reaction, iron NPs are oxidized to ferric/ferrous ions, and halogenated organic contaminant is reduced. Also, because of their versatile deployment ability, their site remediation abilities cover wide range of contaminants. Waraporn et al. [24] discovered that 2,4,6-trinitrotoluene (TNT) uptake by roots in soil treated with nZVI was more effective than that without nZVI, particularly in the experiments with TNT concentration of 500 mg/kg. This nano-phytoaccumulation experiment also showed that TNT which was found in roots was higher than that in shoots, in all experimental groups. In another study, nZVI combined with *Impatiens balsamina* was used to remediate e-waste-contaminated soil. The contaminated soil contained toxic pollutants such as polychlorinated biphenyls (PCBs) and heavy metals released during crude recycling of e-wastes. In this work, nZVI facilitated the growth of *I. balsamina* by 30.3% under clean soil and 53.5% under high pollution. Plants with nZVI treatment had significantly higher PCB accumulation efficiency compared with plants without addition of nZVI. This nano-phytoaccumulation may be attributed to improvement of soil quality and adjustment of soil pH by nZVI [25]. Harikumar and Jesitha [26] reported a decrease in accumulation of endosulfan by *Alpinia calcarata* in nano-phytoremediation experiment when compared with phytoremediation without NPs, because the added nZVIs might have promoted reductive dechlorination of endosulfan. Waraporn et al. [3] indicated that nano-phytoremediation using *Panicum maximum* was more effective than either nano-remediation (without *P. maximum*) or phytoremediation (using *P. maximum* without NPs) as a method for degradation and removal of TNT-contaminated soil. This is a case of nano-phytodegradation. In a nano-phytoaccumulation potted transplantation experiment, *Panicum maximum* was more tolerant than *Helianthus annuus* in TNT- and nZVI-contaminated soil [27]. Fariba and Abdolkarim [28, 29] reported that *Euphorbia macroclada* and *Noaea mucronata* (respectively) were effective phytoaccumulator plants and their NPs were useful for watery media detoxification and bioremediation in critical conditions. Tariq et al. [30] reported that *Eichhornia crassipes* accumulated heavy metals which were subsequently recovered from the contaminated plant and utilized for production of metallic NPs, and this added value to use of the plant. Thus, *E. crassipes* was used for nano-phytomining.

It should be noted that a number of processes can take place at the same time in one plant. For example, nano-phytodegradation, nano-phytostabilization, nano-phytovolatilization, and nano-phytoaccumulation can occur in the same plant. More research is needed in this area. There is also the need to determine toxicity and bioavailability of products of nano-biodegradation. It is necessary to ensure that toxic products of nano-phytoremediation do not enter the food chain or groundwater. These toxic by-products should not accumulate in leaves, stems, firewood, mulch, litter, etc.; otherwise herbivores and humans may be adversely affected.

## References

1. Poorva M, Arushi J, Sudha S, Nidhi G (2013) Environmental pollution and nanotechnology. Environ Pollut 2(2):49–58
2. Yadav KK, Singh JK, Gupta N, Kumar V (2017) A review of nanobioremediation technologies for environmental cleanup: a novel biological approach. J Mater Environ Sci 8(2):740–757
3. Waraporn J, Preeda P, Chongrak P, Rachain K (2012) Enhanced phytoremediation efficiency of TNT-contaminated soil by nanoscale zero valent iron. In: Second International Conference on Environment and Industrial Innovation, vol 35. IACSIT Press, Singapore, pp 82–86
4. Hasan M (2015) A review on nanoparticles: their synthesis and types. J Recent Sci 4:9–11
5. National Nanotechnology Initiative (NNI) (2005) What is nanotechnology? http://www.nano.gov
6. Ruffini-Castiglione M, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62:161–165
7. Lin DH, Xing BS (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollut 150:243–250
8. Dimitrov D (2006) Interactions of antibody-conjugated nanoparticles with biological surfaces. Colloids Surf A Physicochem Eng Asp 8:282–283

9. Ingale AG, Chaudhari AN (2013) Biogenic synthesis of nanoparticles and potential application: an eco-friendly approach. J Nanomed Nanotechnol 4(2):165. https://doi.org/10.4172/2157-7439.1000165
10. Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr Sci 85:162–170
11. Phillips T (2017) Phytoremediation. The balance. https://www.thebalance.com/six-types-of-phytoremediation-375529
12. Nwadinigwe AO, Obi-Amadi A (2014) Crude oil degrading potential of *Pennisetum glaucum* (L.) R. Br. Afr J Biotechnol 13(34):3489–3495
13. Okhovat N, Hashemi M, Golpayegani AA (2015) Phytocatalytic decomposition of metronidazole in space in aqueous solutions using titanium dioxide nanoparticles. J Mater Environ Sci 6(3):792–799
14. Kavitha KS, Syed B, Rakshith D, Kavitha HU, Yashwantha Rao HC, Harini BP, Satish S (2013) Plants as green source towards synthesis of nanoparticles. Int Res J Biol Sci 2(6):66–76
15. Watlington K (2005) Emerging nanotechnologies for site remediation and wastewater treatment. United States Environmental Protection Agency, Washington, DC, p 47 www.epa.gov
16. Karn B (2004) Nanotechnology and the environment: applications and implications. Oxford University Press, New York, NY, p 890
17. Masciangioli TWZ (2003) Environmental technologies at the nanoscale. Environ Sci Technol 37(5):102A–108A
18. Singh BK (2010) Exploring microbial diversity for biotechnology: the way forward. Trends Biotechnol 28:111–116
19. Kanel SR, Manning B, Charlet L, Choi H (2005) Removal of Arsenic (III) from groundwater by nanoscale zero-valent iron. Environ Sci Technol 39:1291–1298
20. Shipley HJ, Engates KE, Guttner AM (2010) Study of iron oxide nanoparticles in soil for remediation of arsenic. J Nanopart Res 13:2387–2397
21. Zhang W (2003) Nanoscale iron particles for environmental remediation: an overview. J Nanopart Res 5:323–332
22. Martínez-Fernández D, Vítková M, Míchálková Z, Komárek M (2017) Engineered nanomaterials for phytoremediation of metal/metalloid-contaminated soils: implications for plant physiology. In: Asari A, Gill S, Gill R, Lanza G, Newman L (eds) Phytoremediation. Springer, Cham, pp 369–403
23. Karn B, Kuiken T, Otto M (2009) Nanotechnology and *in situ* remediation: a review of the benefits and potential risks. Environ Health Perspect 117:1823–1831
24. Waraporn J, Preeda P, Chongrak P, Rachain K (2013) Trinitrotoluene and its metabolites in shoots and roots of *Panicum maximum* in nano-phytoremediation. Int J Environ Sci Dev 4(1):7–10
25. Gao YY, Zhou QX (2013) Application of nanoscale zero valent iron combined with *Impatiens balsamina* to remediation of e-waste contaminated soils. Adv Mater Res 790:73–76
26. Harikumar PSP, Jesitha K (2016) Nano-phytotechnological remediation of endosulfan using zero valent iron nanoparticles. J Environ Protect 7:734–744
27. Jiamjitrpanich W, Parkpian P, Polprasert C, Laurent F, Kosanlavit R (2012) The tolerance efficiency of *Panicum maximum* and *Helianthus annuus* in TNT-contaminated soil and nZVI-contaminated soil. J Environ Sci Health A Tox Hazard Subst Environ Eng 47(11):1506–1513
28. Fariba M, Abdolkarim CR (2011) Application of nano-particles of *Euphorbia macroclada* for bioremediation of heavy metal polluted environments. In: International Conference on Nanotechnology and Biosensors, vol 25. IACSIT Press, Singapore, pp 16–20
29. Fariba M, Abdolkarim CR (2012) Bioremediation of heavy metal pollution by nanoparticles of *Noaea mucronata*. Int J Biosci Biochem Bioinform 2(2):85–89
30. Tariq M, Salman AM, Syed TH, Shazia A (2012) Metallic phytoremediation and extraction of nanoparticles. Int J Phys Sci 7(46):6105–6116

# Chapter 16
# Nano-phytoremediation of Pollutants from Contaminated Soil Environment: Current Scenario and Future Prospects

**Akansha Srivastav, Krishna Kumar Yadav, Sunita Yadav, Neha Gupta, Jitendra Kumar Singh, Ravi Katiyar, and Vinit Kumar**

## 16.1 Introduction

Soil pollution involves the contamination of soil by various anthropogenic (agricultural, industrial, wastewater) activities which involve the addition of nutrients, pesticides, and sediments to soil, and on the other hand, industry and urbanization pollute the soil with solid wastes, heavy metals, solvents, and several other slow degrading organic and inorganic substances [1]. The pollution includes point sources, such as emission, effluents, and solid discharge from industries, vehicle exhaustion, and metals from smelting and mining, and nonpoint sources, such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers [2–5]. Dispersal of waste from its source can be through the atmosphere, via the water bodies and water channels, or directly into the soil itself, and from there it enters the food chain and adversely affects the human life directly or indirectly [6]. Metals, like copper, iron, manganese, and zinc, are essential for life processes, whereas others, like cadmium, nickel, and mercury, have no physiological function but often result in harmful disorders at a higher concentration [7]. High heavy metal ingestion such as cadmium, chromium, mercury, and lead can cause renal and lung dysfunction, bone degeneration, liver damage, etc. in human and animal health [7–9]. Although soil acts as a physical filter by its sieving action; a chemical filter by

---

A. Srivastav · S. Yadav · R. Katiyar
Center for Environmental Science and Climate Resilient Agriculture,
Indian Agriculture Research Institute, New Delhi, India

K. K. Yadav (✉) · N. Gupta · V. Kumar
Institute of Environment and Development Studies, Bundelkhand University,
Jhansi, Uttar Pradesh, India

J. K. Singh
School of Environment and Sustainable Development, Central University of Gujarat,
Gandhinagar, India

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_16

adsorption, precipitation, and transformations of chemical substances; and a biological filter by decomposing organic materials, it does not have infinite capacity to perform these functions [10]. Unlike air pollution which has a direct impact on human lives, soil pollution causes an indirect damage to humans and other animals. Any addition of a substance that may exert adverse effect on its functioning can be defined as soil contamination and those substances known as contaminants or pollutants. Phytoremediation has now emerged as a promising strategy for in situ removal of many contaminants [11]. Microbe-assisted phytoremediation including rhizoremediation appears to be particularly effective for removal and/or degradation of organic contaminants from impacted soils, particularly when used in conjunction with appropriate agronomic management and cultivation techniques [12]. Phytoremediation is widely viewed as the ecologically responsible alternative to the environmentally destructive physical and chemical remediation methods currently practiced. Significant progress has been made in recent years in developing native or genetically modified plants for the remediation of environmental contaminants. Phytoremediation has been defined as the use of plants directly or indirectly to degrade or remove contaminants from soil and water [13]. It is an emerging technology that utilizes plants and their associated rhizosphere microorganisms to remove and transform the toxic chemicals located in soils, sediments, groundwater, surface water, and even the atmosphere. Phytoremediation is an effective, nonintrusive, and inexpensive means of remediating soils [14].

Nanotechnology is a broad-based field of study focused on materials and applications occurring at a very small scale. In general, most people accept that nanotechnology deals with structures that are less than 100 nanometers (nm) and involves developing materials or devices within that size [15]. Nanoparticles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects [9]. These are unique chemical and physical properties; hence they received much attention by the researchers working in many fields of science such as allotropic forms of carbon, fillers, and efficient gas adsorbents for environmental remediation and as support medium for different inorganic and organic catalysts [16]. Nanoparticles can be broadly grouped into two: namely, organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) or be produced from the dead organic matter such as orange peel, potato peel, etc., while some of the inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver), and semiconductor nanoparticles (like titanium dioxide and zinc oxide). According to Ruffini-Castiglione and Cremonini [17], there were identified three types of NPs (nanoparticles): natural (e.g., volcanic or lunar dust, mineral composites), incidental (resulting from anthropogenic activity, e.g., diesel exhaust, coal combustion, welding fumes), and engineered. The last type of NPs belongs also to metal-based materials—quantum dots, nanogold, nanozinc, nanoaluminium, $TiO_2$, ZnO, and $Al_2O_3$ [18]. Environmental applications of nanoparticles such as cleanup of pollutants from air, water and soil have been done by various approaches. Cleanup process of pollutants is mainly known as remediation. If biological agents are involved in the process to degrade the pollutants in less/nontoxic forms, it is known as bioremediation and if living plants are involved in the remediation process, that is known as phytoremediation. Nano-phytoremediation is a technique which involves nanotechnology and phytotechnology for the remediation of environmental pollutants. Nano-phytoremediation for degradation and removal of TNT (2,4,6-trinitrotoluene)-contaminated soil is more effective than either nanoremediation or phytoremediation. Use of nanoparticles for environmental applications is increasing very rapidly. This is due to the large surface area of the particles as compared to bulk-size particles; therefore its reactivity is greatly enhanced for the chemical- or biological-mediated reactions for environmental applications [19]. Application of nanomaterials for remediation is rapidly progressing in the research field. Nanomaterials have the potential to absorb/adsorb or reduce the contaminants from the environment. Iron-based nanoparticles are widely used nanoparticles for the remediation process. For example, nanoscale zerovalent iron (nZVI) has been used to reduce the environmental contaminants such as cis-1,2-dichloroethylene (c-DCE), tetrachloroethylene (PCE), and trichloroethylene (TCE) (https://clu-in.org/download/remed/nano-fact-sheet-2011.pdf). Recent researches focused on improving the efficiency of the nanomaterials, and in addition the researchers also focus on understanding the processes by which the nanomaterials have been taken up and transported to check of any toxicological effects on the system. Conventional remediation techniques involve disposal on landfills or immobilization of the contaminated soils. This technique is very costly, while phytoremediation techniques are cheaper. NPs tend to adsorb the pollutants due to large surface area. NPs adhere to the roots and translocated to the harvestable part of the plants which are used for the phytoremediation.

This chapter includes the nanotechnology-based phytoremediation process to reduce the contaminants from the environment.

## 16.2 Need of Phytoremediation for Contaminant Cleanup

Phytoremediation has been defined as the use of green plants and their associated microorganisms, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental pollutants. It is the direct use of living green plants, waste of the fruit/vegetable, etc. for in situ removal or degradation of contaminants in soils, sludge sediments, surface water, and groundwater. It is allowing cost- and solar energy-driven cleanup technique. It is a cost-effective technique for remediation process which involves the plants to metabolize the molecules in the tissues and to degrade the elements from the environment. It is the natural ability of plants to accumulate, degrade, or concentrate the contaminants from soil, water, and air. Pollutants and toxic metals are the major goal for the phytoremediation. In recent studies, physiological and molecular mechanisms of phytoremediation are designed to improve the phytoremediation process for environmental cleanup. By using the food waste, it is also supportive to manage the utilization of food/vegetable/fruit waste.

### *16.2.1 Mechanism of Phytoremediation*

Unlike heavy metals, organic pollutants can be remediated in plants through several natural biophysical and biochemical processes. It has been suggested that faster-growing floras like grass species are effective plants for phytoremediation of PAHs in contaminated soils [20–22]. Trees like poplar (*Populus* spp.) or willow (*Salix* spp.), with extensive root systems and high transpiration rates, hold particular promise for phytoremediation [23]. The action of plants on these compounds is multifarious: they can be immobilized, stored, volatilized, and transformed into various extents (even mineralized) or a combination of them, depending on the specific compound, environmental conditions, and plant genotypes involved. The mechanisms used by plants to facilitate remediation include phytoextraction, phytodegradation, phytostabilization, phytovolatilization, and rhizodegradation. These mechanisms are shown in Fig. 16.1 and Table 16.1.

#### 16.2.1.1 Phytoextraction

Phytoextraction refers to the ability of plants to take up contaminants into the roots and translocate them to the aboveground shoots or leaves. Phytoextraction is the removal of a contaminant from the soil, groundwater, or surface water by live plants. Plants to be used for phytoextraction should have (a) tolerance to high-concentration metals, (b) high metal accumulation capability, (c) heavy biomass, (d) ability to grow fast, and (e) a profuse root system. The success of phytoextraction depends especially on the plant's ability (a) to accumulate biomass rapidly and (b) to store large quantities of the uptaken metals in the shoot tissue [29, 30].

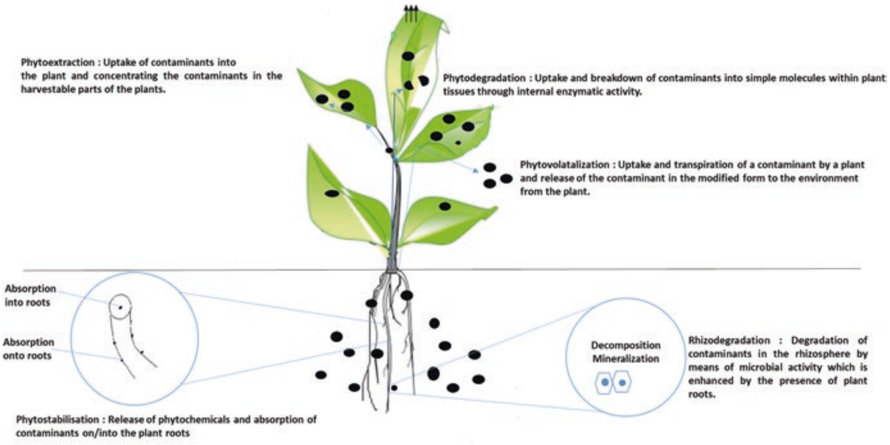


**Fig 16.1** Schematic representation of phytotechnologies

**Table 16.1** Phytotechnology mechanisms and significance

| Phytotechnology | Levels | Mechanism | Plants | Pollutants | References |
|---|---|---|---|---|---|
| Phytoextraction | Whole plant | Hyperaccumulation in plants | *Helianthus annuus* and *Brassica juncea* | Organic pollutants and metals (Cd, Cr, Co, Pb, Hg, Ni, Ag, Zn) and radionuclides | [24, 25] |
| Phytodegradation | Whole plant | Breakdown and eradication of contaminants | Algae, poplars, and stonewort | Chlorinated solvents, petroleum products | [24] |
| Phytovolatilization | Shoot | Volatilization of contaminants in modified form by leaves through transpiration process | Poplars, alfalfa, and *Brassica juncea* | Chlorinated solvents, metals (Se, Hg, and As) | [24, 26] |
| Phytostabilization | Root | Sorption of contaminants | Grasses, *Brassica juncea*, and poplars | Inorganics (Cd, Cu, Cr, Pb) | [24, 27] |
| Rhizodegradation | Root | Decomposition of contaminants by presence of microbes in rhizosphere | *Agropyron smithii* and *Bouteloua gracilis* | Chlorinated solvents, petroleum products | [24, 28] |

#### 16.2.1.2 Phytodegradation

The phytodegradation is defined as the metabolic degradation or breakdown of organic contaminants by plant enzymes or enzyme cofactors [31]. A contaminant can be eliminated via phytodegradation or phytotransformation by plant enzymes or enzyme cofactors [32, 33]. Enzymes, like dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase, have been discovered in plant sediments and soils.

#### 16.2.1.3 Phytostabilization

Phytostabilization is the mechanism that can be used to minimize the migration of contaminants in soils. This application aims to prevent the dispersion of contaminated sediments and soil by using plants (mainly grasses) to minimize erosion by wind or rain action. By choosing and maintaining an appropriate cover of plant species, coupled with appropriate soil amendments, it may be possible to stabilize certain contaminants (particularly metals) in the soil [34] and reduce the interaction of these contaminants with associated biota.

#### 16.2.1.4 Phytovolatilization

Phytovolatilization is a mechanism by which plants convert a contaminant into a volatile form, thereby removing the contaminant from the soil or water [35] at a contaminated site. It is the natural ability of a plant to volatilize a contaminant that has been taken up through its roots which can be exploited as a natural air-stripping pump system. Volatile pollutants diffuse from the plant into the atmosphere through open stomata in leaves where gas exchange occurs. Additionally, processes such as plant uptake and phytovolatilization, contaminant accumulation, and metabolic transformation may be relevant for some plants and organic chemicals [31]. Direct volatilization and phytovolatilization are expected to be moderate for hydrophilic compounds such as acetone [36] and phenol [37]. In contrast, volatilization may be an important removal process for volatile hydrophobic compounds such as lower chlorinated benzenes ([38]), chlorinated ethenes [39, 40], and BTEX compounds [41].

#### 16.2.1.5 Rhizodegradation

Rhizodegradation is a biological treatment of a contaminant by enhanced bacterial and fungal activity in the rhizosphere of certain vascular plants. The rhizosphere is a zone of increased microbial density and activity at the root/surface and was described originally for legumes by Lorenz Hiltner in 1904. Plants and microorganisms often have symbiotic relationships making the root zone or rhizosphere an area of very active microbial activity [42–46]. Plants have several miles of roots per acre, suggesting the potential of pollutant degradation in the rhizosphere. Sugars, organic acids, and larger organic compounds which constitute about 10–50% of plant's photosynthate are deposited in soils [47]. Rhizoremediation, an integral component of phytoremediation, can occur naturally or can be triggered by introducing specific pollutant-degrading microbes or plant growth-promoting microorganisms [48].

### *16.2.2 Advancements in Phytotechnological Remediation*

The removal of environmental contaminants (such as heavy metals) from contaminated sites using nanoparticles/nanomaterial formed by plant, fungi, and bacteria with the help of nanotechnology is called nanobioremediation (NBR). NBR is the new emerging technique for the removal of pollutants for environmental cleanup. Current technologies for remediation of contaminated sites are chemical and physical remediation, incineration, and bioremediation having their own advantage and disadvantage. With recent advancements, bioremediation offers an environmentally friendly and economically feasible option to remove contaminants from the environment [49]. Earlier researches were focused upon the metal accumulation in the plants in search of hyperaccumulators; after that the concept of using the plants for remediation process has been developed. Many researchers investigated other plant-contaminant interactions, rhizosphere degradation, and uptake of metals. The progression of research led to full-scale phytoremediation process to various contaminated sites (Joel G. [50]). Nowadays air pollution is the major and complex problem which directly affects the human health. Development of phytotechnologies for removing the pollutants from the environment to improve the air quality is highly required for quality life. Recent researches focused on mechanism by which plants may inhibit the airborne pollutants from the environment. Due to anthropogenic activities, concentration of elements and its compounds is continuously increasing in soils. Manual analysis and eradication of all the soil contaminants are very expensive and time-consuming. Therefore, it is very important to know the soil-contaminant interaction to prevent the uptake of contaminants from the soil to plant [51].

## 16.3 Nano-phytoremediation

Nano-phytoremediation involves both nanotechnology and phytoremediation together to clean up the environment. Nanomaterials are widely used nowadays in the field of cosmetics, paint, medicines, textiles, etc. Nanotechnology increases phytoremediation efficiency, and nanoparticles can also be used for remediation of soils and water which are contaminated with heavy metal, organic, and inorganic pollutants. Recent studies have shown that organic contaminants such as atrazine, molinate, and chlorpyrifos can be degraded with nanosized zerovalent irons. Nanoparticles in enzyme-based bioremediation can also be used in combination with phytoremediation [9, 52]. Many nanomaterials have been developed for the environmental application. Nanomaterials are useful for the remediation process because of their large surface area, hence these materials are more reactive than their bulk form, and they can easily penetrate the contaminated sites [53]. Use of nanomaterials for the environmental cleanup is progressing rapidly. Iron nanomaterials are most widely used for the remediation process (https://clu-in.org/download/remed/nano-fact-sheet-2011.pdf). Some researchers investigated that nanomaterials decontaminated the organic pollutants (PCBs, PAHs, PPCPs, and organic solvents) in the soil [54]. According to Jiamjitrpanich et al. [55], a combination of nanotechnology and phytotechnology for TNT remediation from soil is more effective than the soil without nanomaterials.

### *16.3.1 Factors Affecting the Nano-phytoremediation of Contaminant*

The factors affecting chemical uptake and distribution within living plants include:

1. Physical and chemical properties of the compounds (e.g., water solubility, vapor pressure, and molecular weight)

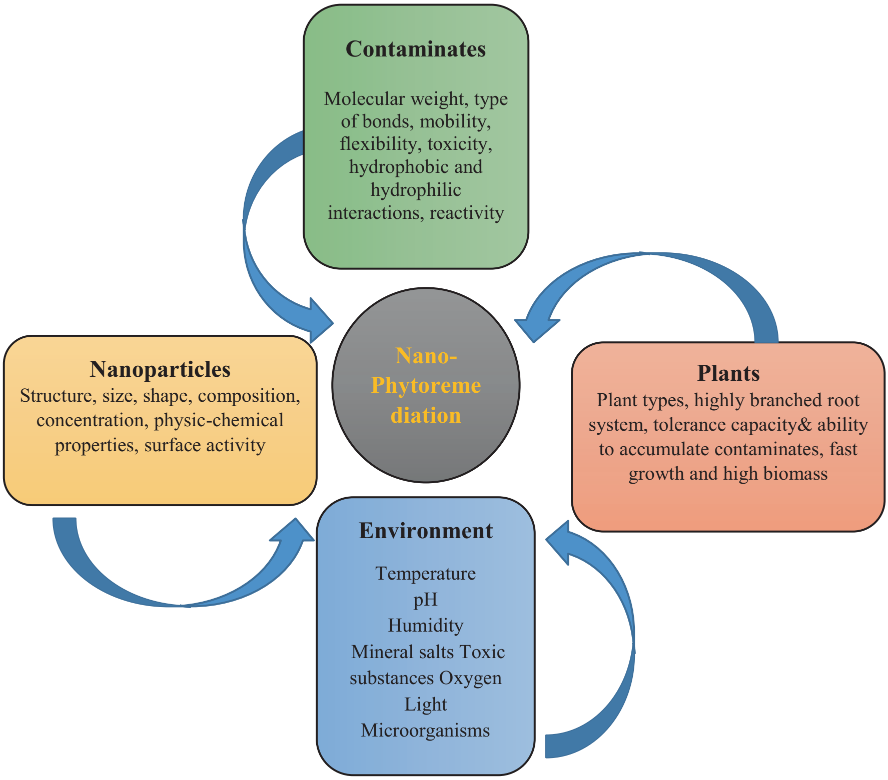


**Fig 16.2** Factor affecting the nano-phytoremediation of contaminates

2. Environmental characteristics (e.g., temperature, pH, organic matter, and soil moisture content)
3. Plant characteristics (e.g., type of root system and type of enzymes)

Schematic diagram of factors affecting of nano-phytoremediation is shown in Fig. 16.2.

### *16.3.2 Nano-phytoremediation of Pollutants in Soil*

Nano-phytoremediation technology for the remediation of contaminated soils is based on the combined application of nanoparticles and phytoremediation technique. The integration of phytoremediation and application of nanoparticles comprise an important step in the progress of soil decontamination. Numerous studies have demonstrated that various nanoparticles/nanomaterials significantly detoxify or remediate the organic, inorganic, and heavy metal pollutants in soils. The nano zerovalent iron (nZVI), magnetite nanoparticles ($n\mathrm{Fe}_3\mathrm{O}_4$), and bimetallic nanoparticles (Pd/Fe) could rapidly degrade organic pollutants such as chlorpyrifos, atrazine, lindane, pentachlorophenol, trichloroethylene (TCE), pyrene, polychlorinated biphenyls (PCBs), 2,4-dinitrotoluene, and ibuprofen from polluted soil environment [56–58]. Nano $TiO_2$ ($n$$TiO_2$) degrade the organic pollutants (diuron, *p*,*p*′-DDT, pyrene, phenanthrene) in soil and reduce the half lives of pyrene and phenanthrene [59], through redox reaction, photocatalysis, and thermal destruction under irradiation [60]. Recent study also performed the significant degradation of residues of the pesticide atrazine [2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine] through PEI-copper nanoparticles deposited onto montmorillonite and sand [61]. The indirect impact on the soil microorganisms may partially explain some mixed positive and negative results on the plants caused by NPs applied to the soil. Positive responses of soil bacteria have been reported in some cases, as was the case when 1 or 10 mg $kg^{-1}$ of NPs of FeO were applied to the soil, although the application of NPs of Ag (0.1–10 mg $kg^{-1}$) had a negative effect [62]. Nano-phytoremediation application has a wide range in terms of soil contaminants removal. Contaminants ranging from heavy metals to volatile organic compounds can be easily treated by this technology with high efficiency of its uptake by the plant. Nanomaterial-assisted phytoremediation is particularly effective for organic pollutants and more efficient removal of site contaminants. Recent studies have reported that the application of nanoparticles can also improve the stress tolerance of plants in ex situ and in situ conditions thereby promoting phytoremediation potential [63–65].

### *16.3.3 Interactions Between Plants and Nanoparticles*

Uptake of nanoparticles in the plants mainly depends on the size, type, and chemical composition. Size is the major factor of the nanoparticle to penetrate inside the plants and translocate from roots to other parts of the plants. Nanomaterials penetration process is also a factor to determine how efficiently plant takes up the nanomaterials [66]. After entering in the plant roots, there are two ways to move inside the plant tissues. Apoplastic transport; this type of transport takes place outside the plasma membrane, xylem vessels [67]. The other is symplastic transport; it involves the movement of water between the cytoplasm and sieve plates [68]. Uptake is affected by the environmental conditions [69]. The temperature affects growth substances and consequently roots length. Root structure under field conditions differs from that under greenhouse condition. The success of phytoremediation, more specifically phytoextraction, depends on a contaminant-specific hyperaccumulator.

### *16.3.4 Ideal Plant Characteristics for Nano-phytoremediation*

An ideal plant for removing contaminants from the soil environment should possess a series of the following characteristics:

- Fast growth of plants and produce large biomass (growth and productivity).
- Well-developed root systems (widespread highly branched root system) increased root surface area.
- Abilities to tolerate or accumulate contaminants [70] (toxicity tolerance limit)
- More capacity for hyperaccumulation of contaminants (inorganic, organic, and heavy metals) preferably in the aboveground parts (sink potential).
- Easy to harvest (based on utility of the sink organ).
- Nonconsumable by humans and animals
- Susceptible to genetic manipulation.

#### 16.3.4.1 Selection of Nanoparticles Used for Phytoremediation

Nanoparticles suitable for phytoremediation should have the following characteristics:

- Nanoparticles should be nontoxic for plant.
- Increased germination, seedling growth, root-shoot elongation, plant height, and biomass.
- Significantly increased phytoenzymes production in plants.
- Ability to enhance the plant growth hormones.
- Capabilities to bind contaminants and increased bioavailability for plant.
- Enhanced phytoremediation process.

A potential application of a combined treatment of nano- and phytoremediation (phyto-nano-treatment) could be used effectively for the remediation of contaminated soils. Nano-phytoremediation technologies (nanoparticle- and plant-based technology) use either naturally occurring or genetically engineered plants assisted with nanomaterials/nanoparticles for cleaning contaminated environments. The application of selected nanoparticles resulted in significantly enhanced plant growth, and nanoaugmentation increased phytoremediation potential which resulted in significant removal of contaminants from soil environment. Many nanoparticles were also identified as a plant growth promoter due to its ability to enhance the plant growth hormones and better uptake of contaminants by the plant species. Various plant species were also found to increase growth from the nanotreatment procedures. Several studies also confirmed positive effects of metal and metal oxide nanoparticles on growth of higher plants. In spite of the advantages of nanocarbon, a big challenge of its application to soil remediation is its potential toxicity to plant and soil microbes [71].

### *16.3.5 Nanomaterials Eco-Friendly Application for Nano-phytoremediation*

Nanomaterials application is site specific, and its efficiency is influenced by pollutant types, concentration, size, charge/non-charge material, and organic content and also by other physical and chemical properties of soil such as soil aggregates,

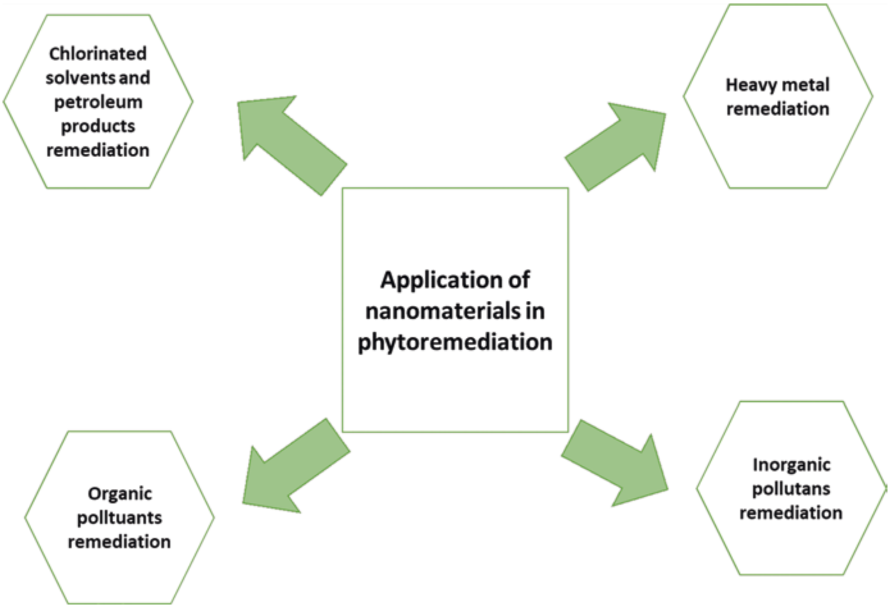


**Fig 16.3** Application of nanomaterials in phytoremediation

texture, etc. along with the nanomaterial size. There are various methods which are used for the application of nanomaterials to the soil for the remediation process. These methods are injection under pressure, recirculation, pressure pulse technology, and hydraulics. Schematic diagram representing the application of nanomaterials in phytoremediation is given in Fig. 16.3.

### *16.3.6 Plant Assisted Remediation Using Nanomaterial in Contaminated Soil*

Plant species to be used in the nano-phytotechnological remediation (nano-phytoremediation) technique for decontaminating contaminated soil is based on the application of nanoparticles and phytoremediation methods (e.g., phytostabilization, phytoextraction, and phytodegradation) by using plants. This technology has exhibited a great potential for environmental remediation and decontaminating organic pollutants in sediments. Plant species accelerated the removal of pollutants from the contaminated soil which takes less time than the normal. Earlier researches have proven the combination of nanoparticles and plant species performed significant contaminants removal from contaminated soil [63–65]. The various plant species and varieties of nanoparticles are used in nano-phytoremediation experiments of contaminants, and their removal efficiency is listed in Table 16.2.

**Table 16.2** List of plant species and nanoparticles used for removal of contaminants in nano-phytoremediation system

| Plant species | Used NPs | Contaminants | Removal efficiency | References |
|---|---|---|---|---|
| *Alpinia calcarata* | nZVIs | Endosulfan | ≈100 (%) | Pillai and Kottekottil [64] |
| *Ocimum sanctum* | nZVIs | Endosulfan | 76.28 ± 0.19 (%) | Pillai and Kottekottil [64] |
| *Cymbopogon citratus* | nZVIs | Endosulfan | 86.16 ± 0.09 (%) | Pillai and Kottekottil [64] |
| *Populus deltoides* | Fullerene ($nC_{60}$) | Trichloroethylene | 82 (%) | Ma and Wang [63] |
| *Isatis cappadocica* | SANPs | Arsenic | 705 ppm and 1188 ppm As accumulate in roots and shoots, respectively | [65] |

Nanoparticles interact with plants causing many physiological and morphological changes (describe in Table 16.3), and the plants' response will be strongly depending on the NP type, dose, and speciation as well as on the plant species involved [75]. Effectiveness of NPs is determined by their physical-chemical composition, size, shape, surface covering, reactivity, and most importantly the concentration on which they are effective which is varies from plant to plant [87, 88]. In many studies, increasing evidence suggests that various nanoparticles increase plant development and growth. Silver nanoparticle significantly (>90%) increased ABA and GA phytohormones production in plants, which helps plants to endure stresses as well as increase the uptake of nutrients and water for improved growth [72]. Salicylic acid nanoparticles also improve the plant growth and enhance phytoremediation efficiency of plant under arsenic (As) stress [65]. Nitrogen uptake, its assimilation, and metabolism of plant increased after MnNP soil application, and positive effects on root and shoot elongation were observed by Pradhan et al. [78, 79]. Nanoparticles including nano-fertilizers also help to regulate nutrient release in soil system [89], catalyze soil fertility [90], and promote enzymatic activity in plants [91], increasing chlorophyll content and seed germination.

## 16.4 Challenges of Nano-phytoremediation

- Nanomaterial-assisted phytoremediation studies are very scarce.
- Only microcosm experiments have been done up to now so there is a need to use more realistic studies in future researches, and better understanding at the field level is highly needed.
- Long-term experiments are also required to see the actual effects of nanomaterials in phytoremediation process and change in the soil fertility status.

**Table 16.3** Summary of potential nanoparticles in phytoremediation process

| Nanoparticles | Plant species | Beneficiary concentration(s) | Effects | References |
|---|---|---|---|---|
| AgNPs | *Zea mays* L. | | Increased production of ABA and GA phytohormones | Khan and Bano [72] |
| AgNPs | *Boswellia ovalifoliolata* | 10–30 g $mL^{-1}$ | Increased germination and seedling growth | [73] |
| SANPs | *Isatis cappadocica* | | Improve growth | [65] |
| $SiO_2$ NPs | *Changbai larch* | 500 μL $L^{-1}$ | Increased shoot height, root collar diameter, root length | Bao-shan et al. [74] |
| CuO NPs | *Common wheat* | 500 mg $kg^{-1}$ | Increased biomass | Dimkpa et al. [75] |
| $Fe_2O_3$ NPs | *Arachis hypogaea* | 2–1000 mg $kg^{-1}$ | Increased root length, plant height, biomass, | Rui et al. [76] |
| MnOx NPs | *Lactuca sativa* | 10 mg $L^{-1}$ | Improved the growth of lettuce seedlings by enhancing root elongation | Liu et al. [77] |
| MnNPs | *Vigna radiata* | 50–1000 mg $L^{-1}$ | Increased nitrogen uptake, root and shoot elongation | Pradhan et al. [78]; Pradhan et al. [79] |
| MWCNTs | *Lycopersicon esculentum* | 10–40 mg $L^{-1}$ | Enhanced the seed germination and growth | [80] |
| SWCNTs | *Allium cepa, Cucumis sativus* | 9, 56, 315, and 1750 mg $L^{-1}$ | Root elongation | Canas et al. [81] |
| Graphene oxide | *Vicia faba* L. | 400 and 800 mg $L^{-1}$ | Germination | Anjum et al. [82] |
| ZnO NPs | *Arachis hypogaea* | 1000 ppm | Germination, stem, root growth, and yield | Prasad et al. [83] |
| GNPs | *Arabidopsis thaliana* | 10 and 80 μg $mL^{-1}$ | Germination, shoot and root growth | Kumar et al. [84] |
| $TiO_2$ NPs | *Arabidopsis thaliana* | 400 mg $L^{-1}$ | Root length | Lee et al. [85] |
| Aluminum oxide NPs | *Arabidopsis thaliana* | 400–4000 mg $L^{-1}$ | Root length | Lee et al. [85] |
| $CO_3O_4$ NPs | *Raphanus sativus* L. | 5 g $L^{-1}$ | Root elongation | Wu et al. [86] |

- Nanomaterials have the ability to aggregate this and may reduce the mobility of the nanomaterials, so polymer or other coatings are required to enhance the bioavailability or mobility.
- Evaluation of impacts and safety of nanomaterials application in contaminated soil is required.
- Sustainable nano-phytoremediation depends mainly on the meteorological conditions; thus environmentally stable nanomaterial should be identified.

- Nano-phytoremediation is an appropriate method for sites which have moderate levels of pollution due to unsustainable plant growth in highly contaminated soils.
- A better understanding of contaminants uptake by plants from soil will also support in stimulating agro-mining, which can be used for extraction of contaminants even from harvestable plant biomass.

## 16.5 Conclusion and Future Perspectives

As discussed earlier, the nano-phytoremediation technology is comparatively a new field for the environmental cleanup process. Most of the research is limited to lab-scale and pot culture study and only a few studies have been done on full scale. Ji P *et al* (2011) [92] observed different behaviors of the lab/pot culture experiments and field experiments due to difference in temperature, pH, soil type, nutrients and moisture content in the soil which plays their role in the actual field environment [93].

Nanotechnology can provide green and eco-friendly alternatives for environmental cleanup and management without harming the nature. Several plants, fungi, and bacteria which have more ability to accumulate very large concentrations of metals have also been identified and are termed as hyperaccumulators. Such types of plant, fungi, and bacteria species are of interest for bioremediation of heavy metal-polluted areas. Nanomaterials in different forms can be used for the removal of environment pollutants. Nanoparticles from these types of plant, fungi, and bacteria species were used for removing some heavy metals from polluted sites. There is a need to understand the mechanism behind the transport of nanomaterials to the environment to check whether they have any toxicological effect to the plants or the environment. Selection of suitable plant species and nanomaterials for the uptake of contaminants is necessary along with the agronomic management optimization for high resolution cleanup process. The breath of anticipated opportunities, cross-disciplinary nature, potential for innovation, historical track record, and the impact of the potential advantages of nanotechnology lead to the recognition of this area as of increasing importance.

## References

1. Kanwar JS (1994) Relevance of soil management in sustainable agriculture in dryland areas. Bull Ind Soc Soil Sci 16:1–11
2. Bhatia A, Singh SD, Kumar A (2015) Heavy metal contamination of soil, irrigation water and vegetables in peri urban agricultural areas and markets of Delhi. Water Environ Res 87(11):2027–2034
3. McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere process involved in phytoremediation of metal-contaminated soils. Plant and Soil 232(1/2):207–214

4. Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air water and soils by trace metals. Nature 333(6169):134–139
5. Schalscha BE, Ahumada TI (1998) Heavy metals in rivers and soils of central Chile. Water Sci Technol 37(8):251–255
6. Gupta N, Yadav KK, Kumar V (2015) A review on current status of municipal solid waste management in India. J Environ Sci 37:206–217
7. Yadav KK, Gupta N, Kumar V, Singh JK (2017a) Bioremediation of heavy metals from contaminated sites using potential species: a review. Ind J Environ Prot 37(1):65–84
8. Jain N, Johnson TA, Kumar A, Mishra S, Gupta N (2015) Biosorption of Cd(II) on jatropha fruit coat and seed coat. Environ Monit Assess 187:411
9. Yadav KK, Singh JK, Gupta N, Kumar V (2017b) A review of nanobioremediation technologies for environmental cleanup: a novel biological approach. J Mater Environ Sci 8(2):740–757
10. Singh B, Shan YH, Beeebout SEJ, Singh Y, Buresh RJ (2008) Crop residue management for lowland rice-based cropping systems in Asia. Adv Agron 98:118–199
11. Greenberg BM (2006) Development and field tests of a multi-process phytoremediation system for decontamination of soils. Can Reclam 1:27–29
12. Frick CM, Farrell RE, Germida JJ (2008) Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Petroleum Technology Alliance of Canada Report. https://clu-in.org/download/remed/phyas%20sess.pdf
13. Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719
14. Wiltse CC, Rooney WL, Chen Z, Schwab AP, Bank MK (1998) Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. J Environ Qual 27:169–173
15. Yadav KK, Gupta N, Kumar V, Khan SA, Kumar A (2018) A review of emerging adsorbents and current demand for defluoridation of water: bright future in water sustainability. Environ Int 111:80–108
16. Khan I, Saeed K, Khan T (2017) Nanoparticles: properties, applications and toxicities. Arabian J Chem. http://sci-hub.tw/10.1016/j.arabjc.2017.05.011
17. Ruffini-Castiglione M, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62(2):161–165
18. Lin DH, Xing BS (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollut 150(2):243–250
19. Sozer N, Kokini JL (2009) Nanotechnology and its applications in the food sector. Trends Biotechnol 27(2):82–89
20. Chiapusio G, Pujol S, Toussaint ML, Badot PM, Binet P (2007) Phenanthrene toxicity and dissipation in rhizosphere of grassland plants (*Lolium perenne* L. and *Trifolium pratense* L.) in three spiked soils. Plant and Soil 294:103–112
21. Fang C, Radosevich M, Fuhrman JJ (2001) Atrazine and phenanthrene degradation in grass rhizosphere soil. Soil Biol Biochem 33:671–678
22. Wang J, Zhang Z, Su Y, He W, He F, Song H (2008) Phytoremediation of petroleum polluted soil. Pet Sci 5:167–171
23. Jansson S, Douglas CJ (2007) Populus: a model system for plant biology. Annu Rev Plant Biol 58:435–458
24. Subhashini V, Swamy AVVS (2013) Phytoremediation of Pb and Ni contaminated soils using *Catharanthus roseus* (L.). Univer J Environ Res Technol 3:465–472
25. Rafati M, Khorasani N, Moattar F, Shirvany A, Moraghebi F, Hosseinzadeh S (2011) Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromium and nickel absorption from polluted soil. Int J Environ Res 5:961–970
26. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut 184(1-4):105–126
27. Barcelo J, Poschenrieder C (2003) Phytoremediation: principles and perspectives. Contrib Sci 2:333–344

28. Mukhopadhyay S, Maiti SK (2010) Phytoremediation of metal enriched mine waste: a review. Glo J Environ Res 4:135–150
29. 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. John Wiley and Sons, New York, NY, pp 53–70
30. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, New York, NY, pp 109–128
31. Susarla S, Medina VF, McCutcheon SC (2002) Phytoremediation: an ecological solution to organic chemical contamination. Ecol Eng 18:647–658
32. Dec J, Bollag JM (1994) Use of plant material for the decontamination of water polluted with phenols. Biotechnol Bioeng 44:1132–1139
33. Strand SE, Newman L, Ruszaj M, Wilmoth J, Shurtleff B, Brandt M, Choe N, Ekuan G, Duffy J, Massman JW, Heilman PE, Gordon MP (1995) Removal of trichloroethylene from aquifers using trees. In: Vidic RD, Pohland FG (eds) Innovative technologies for site remediation and hazardous waste management, Proceedings of the national Conference of the Environmental Engineering. Division of the American Society of Civil Engineers, New York, NY
34. Cunningham SD, Anderson TA, Schwab P, Hsu FC (1996) Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56:55–114
35. Terry N, Zayed A, Pilon-Smits E, Hansen D (1995) Can plants solve the selenium problem? In: Proc. 14th Annu.Symp. Curr. Top. Plant Biochem. Physiolo Mol Biol Will Plants have a role in Bioremediation? Univer. Missour., Columbia. April 19-22, pp 63–64
36. Grove JK, Stein OR (2005) Polar organic solvent removal in microcosm constructed wetlands. Water Res 39:4040–4050
37. Polprasert C, Dan NP, Thayalakumaran N (1996) Application of constructed wetlands to treat some toxic wastewaters under tropical conditions. Water Sci Technol 34(11):165–171
38. MacLeod CJA (1999) The fate of chlorinated organic pollutants in a reed-bed system. In: Leeson A, Alleman BC (eds) Phytoremediation and innovative strategies for specialized remedial applications: the Fifth International *In situ* and On-Site Bioremediation Symposium. San Diego, CA. Batelle Press, Columbus, OH, pp 19–22
39. 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
40. Ma X, Burken JG (2003) TCE diffusion to the atmosphere in phytoremediation applications. Environ Sci Technol 37:2534–2539
41. Wallace SD (1999) On-site remediation of petroleum contact wastes using subsurface-flow wetlands. In: Wetlands & Remediation II: Second International Conference on Wetlands and Remediation. Battelle Press, Columbus, OH, pp 125–132
42. Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630–2636
43. Anderson TA, Kruger EL, Coats JR (1994) Enhanced degradation of a mixture of three herbicides in the rhizosphere of a herbicide-tolerant plant. Chemosphere 28:1551–1557
44. Jordahl JL, Foster L, Schnoor JL, Alvarez PJJ (1997) Effect of hybrid poplar trees on microbial populations important to hazardous waste bioremediation. Environ Toxicol Chem 16:1318–1321
45. Schwab AP, Banks MK, Arunachalam M (1995) Biodegradation of polycyclic aromatic hydrocarbons in rhizosphere soil. In: Hinchee RE, Anderson DB, Hoeppel RE (eds) Bioremediation of recalcitrant organics. Battelle Press, Columbus, OH
46. Siciliano SD, Germida JJ (1998) Bacterial inoculants of forage grasses enhance degradation of 2-chlorobenzoic acid in soil. Environ Toxicol Chem 16:1098–1104
47. Kumar R, Pandey S, Pandey A (2006) Plant roots and carbon sequestration. Curr Sci 91:885–890
48. Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176:20–30

49. Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30:428–471
50. Burken JG (2001) Advancement of phytoremediation. Pract Period Hazard Toxic Radioact Waste Manag 5(3):120. http://sci-hub.tw/10.1061/(ASCE)1090-025X(2001)5:3
51. Henry HF, Burken JG, Maier RM, Newman LA, Rock S, Schnoor JL, Suk WA (2013) Phytotechnologies: preventing exposures, improving public health. Int J Phytoremediation 15:889–899
52. Singh BK (2009) Organophosphorus-degrading bacteria: ecology and industrial applications. Nat Rev Microbiol 7(2):156–164
53. Davis AS, Prakash P, Thamaraiselvi K (2017) Nanobioremediation technologies for sustainable environment. In: Prashanthi M (ed) Bioremediation and sustainable technologies for cleaner environment. Environmental Science. Springer International Publishing AG, Cham, pp 13–33
54. Crane RA, Scott TB (2012) Nanoscale zero-valent iron: future prospects for an emerging water treatment technology. J Hazard Mater 211:112–125
55. Jiamjitrpanich W, Parkpian P, Polprasert C, Kosanlavit R (2013) Trinitrotoluene and its metabolites in shoots and roots of *Panicum maximum* in nano-phytoremediation. Int J Environ Sci Dev 4(1):7
56. Reddy A, Madhavi V, Reddy KG, Madhavi G (2012) Remediation of chlorpyrifos-contaminated soils by laboratory-synthesized zero-valent nano iron particles: effect of pH and aluminium salts. J Chem 2013:7. http://sci-hub.tw/10.1155/2013/521045
57. Satapanajaru T, Anurakpongsatorn P, Pengthamkeerati P, Boparai H (2008) Remediation of atrazine-contaminated soil and water by nanozerovalent iron. Water Air Soil Pollut 192:349–359
58. Singh R, Misra V, Mudiam MKR, Chauhan LKS, Singh RP (2012) Degradation of γ-HCH spiked soil using stabilized Pd/Fe0 bimetallic nanoparticles: pathways, kinetics and effect of reaction conditions. J Hazard Mater 237–238:355–364
59. Li Q, Chen X, Zhuang J, Chen X (2016) Decontaminating soil organic pollutants with manufactured nanoparticles. Environ Sci Pollut Res 23(12):11533–11548
60. Makarova OV, Rajh T, Thurnauer MC, Martin A, Kemme PA, Cropek D (2000) Surface modification of TiO2 nanoparticles for photochemical reduction of nitrobenzene. Environ Sci Technol 34:4797–4803
61. Kalidhasan S, Dror I, Berkowitz B (2017) Atrazine degradation through PEI-copper nanoparticles deposited onto montmorillonite and sand. Sci Rep 7(1):1415
62. He S, Feng Y, Ni J, Sun Y, Xue L, Feng Y, Yang L (2016) Different responses of soil microbial metabolic activity to silver and iron oxide nanoparticles. Chemosphere 147:195–202
63. Ma X, Wang C (2010) Fullerene nanoparticles affect the fate and uptake of trichloroethylene in phytoremediation systems. Environ Eng Sci 27(11):989–992
64. Pillai HPS, Kottekottil J (2016) Nano-phytotechnological remediation of endosulfan using zero valent iron nanoparticles. J Environ Prot 7:734–744
65. Souri Z, Karimi N, Sarmadi M, Rostami E (2017) Salicylic acid nanoparticle (SANPs) improves growth and phytoremediation efficiency of *Isatis cappadocica Desv*. under arsenic stress. IET Nanobiotechnol 11(6):650–655
66. Schwab F, Zhai G, Kern M, Turner A, Schnoor JL, Wiesner MR (2015) Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants-critical review. Nanotoxicology 10:257–278
67. Sattelmacher B (2001) The apoplast and its significance for plant mineral nutrition. New Phytol 149:167–192. http://sci-hub.tw/10.1046/j.1469-8137.2001.00034.x
68. Roberts AG, Oparka KJ (2003) Plasmodesmata and the control of symplastic transport. Plant Cell Environ 26:103–124
69. Burken JG, Schnoor JL (1996) Phytoremediation: plant uptake of atrazine and role of root exudates. J Environ Sci 122(11):958–963
70. Ahmadpour P, Ahmadpour F, Mahmud TMM, Abdu A, Soleimani M, Tayefeh FH (2012) Phytoremediation of heavy metals: a green technology. Afr J Biotechnol 11(76):14036–14043

71. Sajid M, Ilyas M, Basheer C, Tariq M, Daud M, Baig N, Shehzad F (2014) Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects. Environ Sci Pollut Res 22:4122–4143
72. Khan N, Bano A (2016) Modulation of phytoremediation and plant growth by the treatment with PGPR, Ag nanoparticle and untreated municipal wastewater. Int J Phytoremediation 18(12):1258–1269
73. Savithramma N, Ankanna S, Bhumi G (2012) Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. Nano Vision 2(1):2
74. Bao-shan L, Chun-hui L, Li-jun F, Shu-chun Q, Min Y (2004) Effect of TMS (nanostructured silicon dioxide) on growth of Changbai larch seedlings. J For Res 15(2):138–140
75. Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, Anderson AJ (2012) CuO and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. J Nanopart Res 14(9):1125
76. Rui MM, Ma CX, Hao Y, Guo J, Rui YK, Tang XL, Zhao Q, Fan X, Zhang ZT, Hou TQ, Zhu SY (2016) Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). Front Plant Sci 7:1–10
77. Liu R, Zhang H, Lal R (2016) Effects of stabilized nanoparticles of copper, zinc, manganese, and iron oxides in low concentrations on lettuce (*Lactuca sativa*) seed germination: nanotoxicants or nanonutrients? Water Air Soil Pollut 227(1):42
78. Pradhan S, Patra P, Das S, Chandra S, Mitra S, Dey KK, Goswami A (2013) Photochemical modulation of biosafe manganese nanoparticles on Vigna radiata: a detailed molecular, biochemical, and biophysical study. Environ Sci Technol 47(22):13122–13131
79. Pradhan S, Patra P, Mitra S, Dey KK, Jain S, Sarkar S, Goswami A (2014) Manganese nanoparticles: impact on non-nodulated plant as a potent enhancer in nitrogen metabolism and toxicity study both *in vivo* and *in vitro*. J Agric Food Chem 62(35):8777–8785
80. Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, Biris AS (2009) Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano 3(10):3221–3227
81. Canas JE, Long M, Nations S, Vadan R, Dai L, Luo M, Ambikapathi R, Lee EH, Olszyk D (2008) Effects of functionalized and nonfunctionalized single walled carbon nanotubes on root elongation of select crop species. Environ Toxicol Chem 27(9):1922–1931
82. Anjum NA, Singh N, Singh MK, Sayeed I, Duarte AC, Pereira E, Ahmad I (2014) Single-bilayer graphene oxide sheet impacts and underlying potential mechanism assessment in germinating faba bean (*Vicia faba* L.). Sci Total Environ 472:834–841
83. Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TSP, Sajanlal R, Pradeep T (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutr 35(6):905–927
84. Kumar V, Guleria P, Kumar V, Yadav SK (2013) Gold nanoparticle exposure induces growth and yield enhancement in *Arabidopsis thaliana*. Sci Total Environ 461:462–468
85. Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, Alvarez PJJ (2010) Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. Environ Toxicol Chem 29:669–675
86. Wu SG, Huang L, Head J, Chen DR, Kong IC, Tang YJ (2012) Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. J Pet Environ Biotechnol 3:126
87. Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagarcia H (2012) Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano 6(3):2128–2135
88. Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ 408(16):3053–3061
89. Subramanian KS, Manikandan A, Thirunavukkarasu M, Rahale CS (2015) Nano-fertilizers for balanced crop nutrition. In: Rai M, Ribeiro C, Mattoso L, Duran N (eds) Nanotechnol food agricul. Springer, Cham, pp 69–80

90. Varma A, Khanuja AM (2017) Role of nanoparticles on plant growth with special emphasis on *Piriformospora indica*: a review. In: Ghorbanpour M, Manika K, Varma A (eds) Nanoscience and plant–soil systems. Soil biology, vol 48. Springer, Cham, pp 387–403
91. Yang J, Cao W, Rui Y (2017) Interactions between nanoparticles and plants: phytotoxicity and defense mechanisms. J Plant Interact 12(1):158–169
92. Ji P, Sun T, Song Y, Ackland ML, Liu Y (2011) Strategies for enhancing the phytoremediation of cadmium-contaminated agricultural soils by *Solanum nigrum* L. Environ Pollut 159:762–768
93. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, Van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794

# Chapter 17
# Impact of Engineered Nanoparticles on the Phytoextraction of Environmental Pollutants

**Xingmao Ma and Xiaoxuan Wang**

## 17.1 Introduction

After more than 20 years of development, phytoremediation has become a mature technology widely used in the remediation of mildly contaminated soil and groundwater. The technology depends on plants and their associated microorganisms to break down or to extract pollutants from the environment [1]. Dependent upon the unique properties of environmental pollutants and the specific remediation goals, one or more phyto-related processes can be emphasized in the phytoremediation process. This can be achieved through the proper selection of plant species and the growing conditions [2]. At least seven phyto-related processes have been identified which are important for the success of phytoremediation, including phytosequestration [3], phytoextraction [4], phytostabilization [5], phytotransformation [6], phytovolatilization [7], rhizosphere degradation [8], and rhizofiltration [9]. Phytoextraction, which involves the uptake and accumulation of environmental pollutants in plant tissues so that contaminants from the environment can be removed after the plant biomass is harvested [10], is one of the most frequently adopted techniques for phytoremediation. Hyperaccumulators play a considerable role in the phytoextraction process, which are defined as plants capable of accumulating significantly higher concentrations of environmental pollutants than the background concentrations of interested chemicals [11]. Over 500 hyperaccumulators have been identified for different environmental contaminants, mostly heavy metals [12]. In addition to those hyperaccumulators aiming to remove environmental pollutants from contaminated sites, accumulation of environmental pollutants in agricultural crops has been extensively studied by the phytoremediation community as a result of concerns on food safety. While the purposes of investigating the plant uptake of environmental pollutants by these two different groups of plants differ, the

---

X. Ma (✉) · X. Wang
Zachry Department of Civil Engineering, Texas A&M University, College Station, TX, USA
e-mail: xma@civil.tamu.edu

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_17

processes involved in their interactions with surrounding environmental pollutants are similar.

There have been numerous studies exploring the uptake mechanisms of environmental pollutants for both organics and heavy metals. For organic pollutants, the hydrophobicity of these chemicals indicated by the $\mathrm{Log}K_{ow}$ of these compounds plays a crucial role in the phytoextraction process. Very hydrophilic compounds ($\mathrm{Log}K_{ow} < 0.5$) are unlikely to be taken up by plants; alternatively, highly hydrophobic compounds ($\mathrm{Log}K_{ow} > 5.0$) can be taken up by plant roots, but are unlikely to be translocated up to the shoots because the phospholipid double layer functions as a regulator on what compounds can get into the plant roots and shoots [13]. Indeed, previous research has shown that compounds with intermediate hydrophobicity show the highest efficiency of translocation from the environment to the shoot tissues of plants [14]. For heavy metals, the phytoextraction process is relatively more complicated due to the complex chemistry involved in the rhizosphere. For example, dissolved metal ions can form complexes with various ligands in plant exudates or in the environment, and numerous studies have shown that the transporters involved in different species of metal uptake differ [15]. Take cadmium ($Cd^{2+}$) as an example, freestanding $Cd^{2+}$ was shown to be mainly transported through the specific $Cd^{2+}$ ZIP (zinc-regulated transporter/iron-regulated transporter-like proteins) transporters and less selective calcium-permeable channels, while chelated Cd is primarily transported in the cell through YSL (yellow stripe-like proteins) [16]. Their fate inside the plant cell cytoplasm also differs because chelated Cd is more likely to be compartmented in the vacuole [17]. In addition to these intrinsic processes involved in the phytoextraction of these environmental pollutants, heavy metal uptake and accumulation are greatly affected by various environmental conditions such as the amount and properties of organic matter in soil, soil properties, and coexisting environmental pollutants [18]. One emerging group of environmental chemicals, engineered nanoparticles (ENPs), has demonstrated significant impacts on the fate and transport of coexisting environmental pollutants, including the plant uptake efficiency of these chemicals [19]. This book chapter will provide a brief review on recent literature regarding the impact of these emerging ENPs on the plant uptake of both organic pollutants and heavy metals. Before we get into the topic though, a brief introduction of engineered nanoparticles (ENPs) will be provided. The introduction of ENPs will be followed by a discussion on the impact of ENPs on organic compounds and heavy metals separately. The book chapter will be concluded with some discussion on the potential mechanisms involved in the altered phytoextraction of environmental pollutants and some insights into the performance of phytoremediation as a result of the co-occurring ENPs.

## 17.2 Engineered Nanoparticles (ENPs)

ENPs are defined as particles with at least two dimensions less than 100 nm. Particles at this size scale display unique optical, electrical, and chemical properties, primarily due to their very large specific surface area and the quantum nature of the energy state. These unique properties have lent numerous potentials for the applications of ENPs in consumer, industrial, agricultural, and biomedical products [20]. The Project on Emerging Nanotechnologies found that the number of commercial products containing ENPs has been steadily growing and is close to 2000 now (http://www.nanotechproject.org/cpi). The most frequently used ENPs are carbonaceous ENPs (primarily carbon nanotubes and fullerenes) and metallic ENPs (including both elemental ENPs and metal oxide ENPs). Both types of nanoparticles are prone to be released and built up in the environment due to their intentional and/or incidental releases [19]. Many previous studies have reported the detection of these ENPs in the wastewater streams [21–23]. Very recently, Ti-containing particles were first detected in a natural stream [24]. While there might still be some lingering doubts concerning the significance of the threat posed by ENPs, more evidence has surfaced indicating significant environmental implications of ENPs. The suspicion of low ENP risks can be traced to two reasons: perceived low concentrations of ENPs in the environment and ENP aggregation which reduces risks. The perceived low ENP concentrations are primarily derived from the low concentrations estimated by mass flow models. However, it should be cautioned that the output values from mass flow models are typically the average concentrations in certain media of a specific region. The low model output does not exclude the possibility of "hot spots" where significantly higher concentrations of ENPs can be detected. Such "hot spots" include the point of discharge and the primary sink of ENPs in the environment such as agricultural soils. As far as the aggregation is concerned, while ENPs have the tendency to aggregate, surface manipulation in ENP synthesis often reduces aggregation. In addition, many factors in the environment (e.g., natural organic matters) actually facilitate the stabilization of ENPs [24, 25]. More importantly, almost none of the previous studies concerning ENP-plant interactions completely avoided aggregation; however, significant plant accumulation of ENP elements has been reported, despite ENP aggregation in growth media [26–28]. In similar settings, ENPs also displayed strong effects on plant uptake and accumulation of coexisting environmental pollutants. While a precise inventory on the ENP concentrations in the environment is still not available and their concentrations and significance in the environment continue to be debated, the detection of ENPs in wastewater and natural streams underscores that the presence of ENPs in the environment is likely much broader than previously thought and their impact on the fate and transport of coexisting environmental pollutants needs to be understood.

Take cerium oxide nanoparticles ($CeO_2$NPs) as an example; it is now a popular component in a plethora of commercial products such as catalysts, sunscreens, fuel additives, microelectronics, polishing agents, and nanomedicine due to its capability to form oxygen vacancy [29]. In particular, its application as a fuel additive and fertilizers entails potentially substantial release of this nanoparticle into the environment. The Organization for Economic Cooperation and Development (OECD) has designated $CeO_2$NPs as a priority pollutant. As the most abundant rare earth element, cerium is unique in the lanthanide group because it is stabilized in both trivalent ($Ce^{3+}$) and tetravalent ($Ce^{4+}$) states [30]. The concentration of oxygen vacancy and the percentage of $Ce^{3+}$ on $CeO_2$NPs surface increase with the decrease of particle size [31]. An increase in $Ce^{3+}$ strains the lattice and results in the lattice expansion due to the relatively larger size of $Ce^{3+}$ compared with $Ce^{4+}$ (1.14 Å vs. 0.97 Å) and therefore enhances the reactivity of $CeO_2$NPs. This unique redox chemistry between $Ce^{3+}$ and $Ce^{4+}$ also makes $CeO_2$NPs a potentially attractive nanomedicine because some previous research has demonstrated that $CeO_2$NPs can act as radical scavengers and redox cycling antioxidants. Therefore, it is likely that when $CeO_2$NPs are released into the environment, it will interact closely with other coexisting environmental chemicals, and our research indeed has indicated that $CeO_2$NPs exert strong effects on the plant uptake and accumulation of co-present $Cd^{2+}$ by different plants [32, 33].

## 17.3 Phytoextraction of Organic Compounds in the Presence of Engineered Nanoparticles

Ma and Wang first reported that fullerenes $nC_{60}$ altered the uptake and accumulation of trichloroethylene (TCE) by a tree species: eastern cottonwood (*Populus deltoides*) and a wetland bush species (red osier dogwood) [34]. The impact, however, is greatly dependent upon the unique properties of fullerenes. The authors found that fullerenes synthesized through solvent exchange (SON/$nC_{60}$) significantly increased the TCE contents in plant roots, but reduced TCE contents in plant leaves and upper stems. In contrast, fullerene synthesized through prolonged mixture with water (Aqu/$nC_{60}$) resulted in lower TCE contents in all plant tissues. While the results appeared contradictory, the authors attributed the observation to the same mechanism: the high adsorption capacity of fullerene for TCE. What led to the different observations was the size differences of these two types of fullerenes. The average size of SON/$nC_{60}$ was about 40 nm, while the average size of Aqu/$nC_{60}$ was about 250 nm. Particles at the size of Aqu/$nC_{60}$ were unlikely to be taken up by plant roots.

In a subsequent study, it was found that the impact of fullerenes on the plant uptake of *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), a metabolite of a broadly used insecticide dichlorodiphenyltrichloroethane (DDT), was greatly dependent upon plant species [35]. For instance, the presence of 40 mg of fullerene (3333 mg fullerene/kg dry vermiculate) in their growth system significantly increased DDE in both the root and shoot tissues of zucchini (*Cucurbita pepo* L.), but decreased the DDE shoot content significantly in soybean (*Glycine max* L.), while the total DDE content in the roots increased. Fullerene also increased the total DDE in tomato (*Solanum lycopersicum* L.) roots but showed no impact on DDE contents in tomato shoots. The presence of humic substances drastically reduced the impact of fullerene on DDE uptake, and at the tested levels, humic substances exerted a greater impact on DDE plant uptake than fullerenes. No mechanistic insights were provided by the authors for the strong impact of humic substances, but it was likely due to the strong adsorption of humic substances on fullerene which might have occupied the most adsorption sites for DDE and even significantly changed the surface properties of fullerene $C_{60}$. In a follow-up study, the same authors explored the impact of fullerene and multiwalled carbon nanotubes (MWCNTs) on the plant uptake of weathered pesticides and insecticides, which provides a more realistic picture on the impact of carbonaceous nanoparticles on plant uptake of aged environmental organic contaminants [36, 37]. Unfortunately, the authors did not distinguish the content of weathered organochlorines in the roots and shoots in this study; instead, the total content of organochlorines in the whole plant tissues was reported. Nonetheless, the results were insightful because, first of all, it confirmed the results from the previous study that the impact of fullerene on plant uptake of organochlorines was plant species dependent. For example, the total content of trans-chlordane in zucchini was unaffected by the presence of 5000 mg/kg of fullerenes $nC_{60}$, but was significantly increased in tomato and significantly decreased in corn (*Zea mays*). The results also demonstrated that the physicochemical properties of concerned organic compounds play a role in the interactions with $nC_{60}$ in plant systems. For instance, while the uptake of trans-chlordane and cis-chlordane by zucchini was unaffected by the presence of $nC_{60}$, the uptake of trans-nonachlor was significantly reduced by fullerenes. Similarly, the uptake of DDT and its metabolites by corn and tomato was completely inhibited by fullerene but not for other compounds. Finally, as expected, the impact of $C_{60}$ and MWCNTs on plant uptake of these historical compounds differed due to their very different physicochemical structures. The long tubular structures of MWCNTs make it difficult for plants to take them up, while it has been reported that fullerenes can be taken up and accumulated in plant tissues [35]. Therefore, when environmental pollutants are adsorbed on the surface of MWCNTs, they will be more likely to be retained in the environment than taken up and accumulated in plant tissues.

Zhang et al. investigated the impact of MWCNTs on the phytoextraction of pyrene and 1-methylpyrene by corn and found that 3000 mg/kg of MWCNTs significantly reduced the concentrations of these compounds in both the root and shoot tissues of corn [38]. However, 50 mg/kg MWCNTs did not demonstrate any significant effects on plant uptake of these two compounds. The impact of MWCNTs was mitigated by the presence of the surfactant sodium dodecylbenzenesulfonate (SDBS). Importantly, MWCNTs also reduced the bioaccumulation factor of these two compounds in corn, suggesting that the upward transport of these compounds was inhibited by MWCNTs. This is likely due to the adsorption of these two compounds to CNT surfaces and the low transferability of CNTs from roots to shoots. Because the adsorption of environmental pollutants on MWCNTs surfaces is heavily affected by the surface properties of CNTs, it is understandable that functionalization of CNT surfaces will have a great impact on the effect of these carbonaceous nanoparticles on the plant accumulation of coexisting environmental pollutants.

Hamdi et al. investigated the impact of unfunctionalized and amino-functionalized MWCNTs on the uptake of four organochlorine insecticides (cis-chlordane, trans-chlordane, trans-nonachlor, and $p,p'$-dichlorodiphenyldichloroethylene ($p,p'$-DDE)) by lettuce (*Lactuca sativa*) [39]. The authors reported that unfunctionalized CNTs drastically reduced the concentrations of these compounds in both lettuce roots and shoots. Functionalization with amino functional group mitigated the effects of MWCNTs on the uptake of these compounds, and their concentrations were generally higher than those simultaneously exposed to unfunctionalized MWCNTs, but the concentrations in the co-presence of amino-MWCNTs in both plant root and shoot tissues were still significantly lower than plants exposed to the same concentrations of these four insecticides alone. As observed earlier, addition of 100 mg/L of humic substances to the growth media drastically reduced the effects of both MWCNTs on plant uptake and accumulation of coexisting organic compounds. The authors attributed the observation to the competitive adsorption of humic substances on CNTs. A recent study reported the impact of carboxyl-functionalized MWCNTs and pristine MWCNTs on the plant uptake of carbamazepine by collard greens (*Brassica oleracea*) in both hydroponic and soil growth systems [40]. Overall, the presence of MWCNTs increased the concentration of carbamazepine associated with roots but reduced the carbamazepine concentration in the shoots. Significantly lower translocation factor of carbamazepine from roots to shoots was observed in the presence of MWCNTs, indicating that adsorption of carbamazepine on CNT surfaces and lower CNT transfer efficiency from roots to shoots are the underlying mechanisms for the altered carbamazepine uptake in plant tissues. Further evidence to support that the altered uptake of carbamazepine was mainly due to the physical process was that neither CNTs affect the metabolism of carbamazepine in collard greens. As expected, surface functionalization with carboxyl functional group changed the efficiency of CNTs for altering carbamazepine plant uptake, possibly for two reasons: (1) the greater adsorption capacity of COOH-CNTs and (2) greater efficiency of COOH-CNTs in plant root penetration. It was also noted in this study that the growth medium played a significant role in the interaction of MWCNTs with carbamazepine in plant systems. For example, while the overall content of carbamazepine was also reduced in soil-grown plants, the difference between functionalized and pristine MWCNTs was less noticeable. Also, both MWCNTs reduced the concentration of carbamazepine in plant roots grown in soil, instead of increasing its concentration in the roots as noticed in the hydroponic system, suggesting that adsorption of CNTs on soil particles affects the bioavailability of carbamazepine to plants. Very interestingly, COOH-CNT enhanced the translocation factor of carbamazepine from roots to shoots even though the mechanisms are not clear.

So far, the investigations on the impact of co-present nanoparticles on plant uptake of organic compounds are mostly focused on carbonaceous nanomaterials. Only a few studies examined the effect of metallic ENPs on plant uptake of organic compounds. Wu et al. investigated the impact of bimetallic Ni/Fe nanoparticles on plant uptake of decabromodiphenyl ether (BDE209), a primary congener in the group of polybrominated diphenyl ethers (PBDEs) by Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), and found that total PCBEs in plant tissues were increased by the presence of 0.3 g/g bimetallic Ni/Fe nanoparticles even though the increase was insignificant [41]. A closer look of the results, however, indicated that the presence of bimetallic Ni/Fe decreased the content of mother compound BDE209 but increased the concentration of BDE209 metabolites with less bromines, suggesting that biometallic Ni/Fe enhanced the metabolism of BDE209 in plant rhizosphere, most likely outside the plant tissues. The conclusion is supported by the fact that aged bimetallic Ni/Fe nanoparticles led to much lower uptake of total PBDEs and the BDE209 metabolite, indicating that the reactivity of the fresh bimetallic nanoparticles is critical for the enhanced metabolism of BDE in the rhizosphere. The impact of silver nanoparticles (AgNPs) on the uptake of *p*,*p′*-DDE by zucchini and soybean was also reported recently [36, 37]. The results again demonstrated the plant species-dependent impact of AgNPs on plant DDE uptake. The presence of AgNPs significantly reduced DDE contents in all plant tissues in soybean including their roots, stems, and leaves. However, AgNPs increased the DDE content in zucchini leaves. AgNPs lowered DDE contents in zucchini roots and stems as well, but the reduction was much smaller than in soybeans. Noticeably, the authors found that AgNPs displayed much greater impact on plant DDE uptake than their bulk counterparts, highlighting the need for more studies on ENP-specific effects on the plant uptake of coexisting environmental pollutants.

## 17.4 Phytoextraction of Heavy Metals in the Presence of Engineered Nanoparticles

As observed for organic compounds, plant uptake of heavy metals has been shown to be affected by coexisting ENPs. Up to this point, most investigations on ENP impact on plant metal uptake concentrate on metallic ENPs. This is understandable because some metallic ENPs tend to dissolute in the rhizosphere, and their dissolved ions may compete with coexisting heavy metals for plant uptake through some shared channels, leading to changed plant uptake of coexisting heavy metals [32]. For example, citrate-coated magnetic NPs were shown to significantly reduce the concentrations of cadmium ($Cd^{2+}$) and chromium ($Cr^{6+}$) in the root and shoot tissues of wheat (*Triticum aestivum*) [42], resulting in reduced toxicity to plants. For the reduced toxicity of chromium ($Cr^{6+}$), the authors also suspected that magnetic NPs reduced the $Cr^{6+}$ to $Cr^{3+}$, which has lower solubility and less bioavailability and toxicity to wheat even though conclusive evidence was not provided. Tassi et al. also reported that $CeO_2$NPs reduced the concentrations of boron (B) in sunflower (*Helianthus annuus*) stems and leaves even though the concentration of B in sunflower roots was unaffected grown in a contaminated soil with B [43]. While detailed mechanisms were not provided, the authors hypothesized that some interactions between B and $CeO_2$NPs occurred in the roots including redox reactions between B and $CeO_2$NPs. It should be mentioned that the effect of $CeO_2$NPs on plant B uptake depends on the "age" of the boron in soil. $CeO_2$NPs displayed different effects on plant uptake of freshly spiked boron and background boron. For example, $CeO_2NPs$ significantly reduced the concentration of B in the sunflower roots grown in soils with freshly spiked boron, but showed no impact on the boron concentration in roots grown in soils with similar concentrations of background boron. This is likely due to the "aging"-related distribution and transformation of boron in soil. "Aging" is an important process for environmental pollutants; several processes have been identified for heavy metals which resulted in altered soil distribution and bioavailability, including adsorption, precipitation, and complexation with the functional groups on clay platelets [44].

In other studies, ZnONPs were found to significantly increase the accumulation of lead (Pb) and Cd in *Leucaena leucocephala* seedlings [45]. The authors did not distinguish the concentrations of these heavy metals in the roots and shoots. However, $TiO_2NPs$ were reported to significantly reduce the accumulation of Cd in both the root and shoot tissues of rice (*Oryza sativa*) [46]. Similarly, magnetic NPs significantly reduced the accumulation of Cd and $Cr^{6+}$ in wheat root and shoots as mentioned earlier. Interestingly, the translocation factor of these metals from root to shoots was largely unaffected by the magnetic NPs [42]. The authors' research group also examined the effect of $CeO_2NPs$ on the accumulation of co-occurring $Cd^{2+}$ by soybean and found that the accumulation of Cd was significantly changed by $CeO_2NPs$, but the effect varied depending on the growth medium of the soybeans (hydroponically grown vs. soil grown). In hydroponically grown soybeans, the co-presence of $CeO_2NPs$ did not affect Cd concentration in plant roots, but drastically reduced the concentration of Cd in soybean shoots [33]. In soil-grown soybeans, however, the concentration of Cd in plant tissues was unaffected by $CeO_2NPs$, indicating that soil plays an important role in the interactions of $CeO_2NPs$ and Cd in plant rhizosphere. We also showed that the altered Cd in soybean can be partially attributed to the modified "root apoplastic barriers" and enhanced root exudation in the co-presence of $CeO_2NPs$ and Cd, in addition to the strong adsorption of Cd on $CeO_2NPs$. Overall, the literature is extremely thin on the effect of ENPs on plant uptake of coexisting heavy metals, and mechanistic understanding on their interactions in plant rhizosphere is very limited. Considering the complex chemical and biological processes in plant rhizosphere which may all affect the plant uptake of heavy metals in the presence of ENPs, more research is needed to gain mechanistic insights into the altered heavy metal uptake by plants.

## 17.5 Mechanisms of the Impact of ENPs on the Phytoextraction of Environmental Pollutants

Even though detailed understanding on the modified phytoextraction of environmental pollutants by ENPs is still lacking, several physical, chemical, and biological processes stand out as important based on current literature. Also, it is clear that many concurrent processes at different levels occur simultaneously and they together affect the interactions of ENPs and co-present environmental pollutants in phytoremediation systems. The net alteration of plant uptake of environmental pollutants depends on the relative significance of each process in a specific system. Physiologically, plants may adjust at different levels in the co-presence of ENPs and environmental pollutants, including the modification of the root anatomical structures by forming new "root apoplastic barriers" [32], change of plant root exudates, and altered physiological processes. The physiological and biochemical alteration of plants in response to joint exposure to ENPs and other environmental pollutants is severely understudied.

In addition to the physiological processes altered at plant root and shoot level, a suite of physical and chemical processes also contribute to the altered plant uptake of environmental pollutants by ENPs. While it is likely that the predominant physical and chemical processes affecting organic and inorganic compounds will be different, for both compounds, the high adsorption capacity of ENPs may play a significant role. For CNTs, the unique tubular structure makes it behave somewhat differently from other ENPs [47]. In addition to the high adsorption capacity of nanotube surfaces for hydrophobic compounds, the unique structure of CNTs makes it more difficult for them to be accumulated in plant tissues; therefore, less environmental pollutants might be taken up by plants in the presence of CNTs. On the other hand, CNTs can physically pierce through cell walls and membranes, providing a direct channel for environmental pollutants to enter into plant tissues [48]. Therefore, the net alteration of plant uptake of environmental pollutants probably depends on the relative importance of these processes. Adsorption of heavy metals on ENP surfaces is an equally important mechanism for the altered bioavailability of heavy metals to plants. Wang et al. found that both $CeO_2$NPs and titanium oxide nanoparticles ($TiO_2$NPs) exhibited strong adsorption capacity for $Cu^{2+}$, leading to reduced bioavailability of $Cu^{2+}$ and phytotoxicity to rice (*Oryza sativa*) [49]. The presence of humic acid appeared to further increase the adsorption capacity of these two NPs and reduced the phytotoxicity of Cu in their study, contrary to what was observed for organic pollutants. Plant root exudates which include a suite of low molecular weight organic acids, proteins, amino acids, and polysaccharides may reduce the adsorption of heavy metals on ENPs due to competitive adsorption. However, the degree of effect depends on the specific composition of plant root exudates which is a function of plant species and their age, as well as the interested ENPs and heavy metals.

Chemical reactions are equally important for the altered accumulation of environmental pollutants by ENPs. A significant chemical reaction affecting heavy metal accumulation in plants is their complexation with molecules in plant root exudates, and our previous study has provided some evidence that the complexation of Cd with ligand molecules from plant exudates occurred in soybean rhizosphere which played a significant role in plant metal uptake [33]. Other important chemical reactions include the altered metabolism of organic compounds by ENPs and ENPs facilitated redox reactions for multivalent heavy metals. Neither of these two mechanisms has been explored in the literature, but the circumstantial evidences suggest that they may occur. Some ENPs, such as $CeO_2$NPs, possess active redox couple of

$Ce^{3+}/Ce^{4+}$ on ENP surfaces, and when they encounter redox-sensitive heavy metals (e.g., arsenic), these ENPs may lead to the oxidation or reduction of these metals, and it is well recognized that metals at different valence states display highly different environmental fate and toxicity to plants.

As the understanding of ENPs' phytotoxicity and accumulation improves, more attention should be dedicated to the understanding of ENPs' effects on the plant uptake of coexisting environmental pollutants. These effects have significant implications for food safety and the efficacy of phytoremediation. As mentioned above, the impact of ENPs on plant phytoextraction efficiency of coexisting environmental pollutants varies with the properties of environmental pollutants, the properties of ENPs, and specific plant species. Therefore, the specific effects of ENPs need to be evaluated based on the specific choices of plants, ENPs, and environmental pollutants. However, an important message from this chapter is that the efficacy of phytoextraction is affected by a variety of environmental factors including the entrance of emerging materials in the environment and the potential effects of these emerging ENPs need to be considered in the design and applications of phytoremediation.

## References

1. Karigar CS, Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Res 2011:11
2. Conesa HM, Evangelou MWH, Robinson BH, Schulin R (2012) A critical view of current state of phytotechnologies to remediate soils: still a promising tool? Sci World J 2012:173829
3. Jansson C, Wullschleger SD, Kalluri UC, Tuskan GA (2010) Phytosequestration: carbon biosequestration by plants and the prospects of genetic engineering. Bioscience 60(9):685–696
4. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236
5. Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Phytostabilization: a green approach to contaminant containment. In: Sparks DL (ed) Advances in agronomy, vol 112. Academic Press, New York, NY, pp 145–204
6. Bock C, Kolb M, Bokern M, Harms H, Mackova M, Chroma L et al (2002) Advances in phytoremediation: phytotransformation. NATO Sci Ser IV Earth Environ Sci 15:115–140
7. Ma XM, Burken JG (2003) TCE diffusion to the atmosphere in phytoremediation applications. Environ Sci Technol 37(11):2534–2539
8. McGuinness M, Dowling D (2009) Plant-associated bacterial degradation of toxic organic compounds in soil. Int J Environ Res Public Health 6(8):2226–2247
9. Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176(1):20–30
10. Nascimento CWA, Xing B (2006) Phytoextraction: a review on enhanced metal availability and plant accumulation. Sci Agric 63:299–311
11. 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(2):169–181
12. Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4(2):118–138
13. Cunningham SD, Berti WR (1993) Remediation of contaminated soils with green plants: an overview. In Vitro Cell Dev Biol Plant 29(4):207–212

14. Burken JG, Schnoor JL (1998) Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environ Sci Technol 32(21):3379–3385
15. Alvarez-Fernandez A, Diaz-Benito P, Abadia A, Lopez-Millan AF, Abadia J (2014) Metal species involved in long distance metal transport in plants. Front Plant Sci 5:20
16. Lux A, Martinka M, Vaculik M, White PJ (2011) Root responses to cadmium in the rhizosphere: a review. J Exp Bot 62(1):21–37
17. Cataldo DA, Garland TR, Wildung RE (1983) Cadmium uptake kinetics in intact soybean plants. Plant Physiol 73(3):844–848
18. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:20
19. Ma X, Geisler-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ 408:3053–3061
20. Kang H. (2010) A review of the emerging nanotechnology industry: materials, fabrications, and applications
21. Mody VV, Siwale R, Singh A, Mody HR (2010) Introduction to metallic nanoparticles. J Pharm Bioallied Sci 2(4):282–289
22. Roco MC (1999) Nanoparticles and nanotechnology research. J Nanopart Res 1(1):1–6
23. Zheng X, Chen YG, Wu R (2011) Long-term effects of titanium dioxide nanoparticles on nitrogen and phosphorus removal from wastewater and bacterial community shift in activated sludge. Environ Sci Technol 45(17):7284–7290
24. Donovan AR, Adams CD, Ma Y, Stephan C, Eichholz T, Shi H (2016) Single particle ICP-MS characterization of titanium dioxide, silver, and gold nanoparticles during drinking water treatment. Chemosphere 144:148–153
25. Cassee FR, van Balen EC, Singh C, Green D, Muijser H, Weinstein J et al (2011) Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. Crit Rev Toxicol 41(3):213–229
26. Ebbs S, Bradfield S, Kumar P, White JC, Musante C, Ma X (2016) Accumulation of zinc, copper, or cerium in carrot (Daucus carota) exposed to metal oxide nanoparticles and metal ions. Environ Sci Nano 3:114
27. Hyung H, Fortner JD, Hughes JB, Kim J-H (2007) Natural organic matter stabilizes carbon nanotubes in the aqueous phase. Environ Sci Technol 41(1):179–184
28. Schwertfeger DM, Velicogna JR, Jesmer AH, Scroggins RP, Princz JI (2016) Single particle-inductively coupled plasma mass spectroscopy analysis of metallic nanoparticles in environmental samples with large dissolved analyte fractions. Anal Chem 88(20):9908–9914
29. Benn TM, Westerhoff P (2008) Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol 42(11):4133–4139
30. Peralta-Videa JR, Zhao L, Lopez-Moreno ML, de la Rosa G, Hong J, Gardea-Torresdey JL (2011) Nanomaterials and the environment: a review for the biennium 2008–2010. J Hazard Mater 186(1):1–15
31. Perullini M, Bilmes SAA, Jobbágy M (2013) Cerium oxide nanoparticles: structure, applications, reactivity, and eco-toxicology. Nanomaterials: a danger or a promise? Springer, New York, NY, pp 307–333
32. Rossi L, Zhang W, Schwab AP, Ma X (2017) Uptake, accumulation, and in planta distribution of coexisting cerium oxide nanoparticles and cadmium in Glycine max (L.) Merr. Environ Sci Technol 51(21):12815–12824
33. Rossi L, Sharifan H, Zhang W, Schwab AP, Ma X (2018) Mutual effects and in planta accumulation of co-existing cerium oxide nanoparticles and cadmium in hydroponically grown soybean (Glycine max (L.) Merr.). Environ Sci Nano 5:150
34. Ma XM, Wang C (2010) Fullerene nanoparticles affect the fate and uptake of trichloroethylene in phytoremediation systems. Environ Eng Sci 27(11):989–992

35. De La Torre-Roche R, Hawthorne J, Deng YQ, Xing BS, Cai WJ, Newman LA et al (2012) Fullerene-enhanced accumulation of p,p′-DDE in agricultural crop species. Environ Sci Technol 46(17):9315–9323
36. De La Torre-Roche R, Hawthorne J, Deng Y, Xing B, Cai W, Newman LA et al (2013a) Multiwalled carbon nanotubes and C60 fullerenes differentially impact the accumulation of weathered pesticides in four agricultural plants. Environ Sci Technol 47(21):12539–12547
37. De La Torre-Roche R, Hawthorne J, Musante C, Xing BS, Newman LA, Ma XM et al (2013b) Impact of Ag nanoparticle exposure on p,p′-DDE bioaccumulation by Cucurbita pepo (Zucchini) and glycine max (Soybean). Environ Sci Technol 47(2):718–725
38. Zhang HY, Liu Y, Shen XF, Zhang M, Yang Y, Tao S et al (2017) Influence of multiwalled carbon nanotubes and sodium dodecyl benzene sulfonate on bioaccumulation and translocation of pyrene and 1-methylpyrene in maize (Zea mays) seedlings. Environ Pollut 220:1409–1417
39. Hamdi H, De La Torre-Roche R, Hawthorne J, White JC (2015) Impact of non-functionalized and amino-functionalized multiwall carbon nanotubes on pesticide uptake by lettuce (Lactuca sativa L.). Nanotoxicology 9(2):172–180
40. Deng YQ, Eitzer B, White JC, Xing BS (2017) Impact of multiwall carbon nanotubes on the accumulation and distribution of carbamazepine in collard greens (Brassica oleracea). Environ Sci Nano 4(1):149–159
41. Wu J, Xie YY, Fang ZQ, Cheng W, Tsang PE (2016) Effects of Ni/Fe bimetallic nanoparticles on phytotoxicity and translocation of polybrominated diphenyl ethers in contaminated soil. Chemosphere 162:235–242
42. Lopez-Luna J, Silva-Silva MJ, Martinez-Vargas S, Mijangos-Ricardez OF, Gonzalez-Chavez MC, Solis-Dominguez FA et al (2016) Magnetite nanoparticle (NP) uptake by wheat plants and its effect on cadmium and chromium toxicological behavior. Sci Total Environ 565:941–950
43. Tassi E, Giorgetti L, Morelli E, Peralta-Videa JR, Gardea-Torresdey JL, Barbafieri M (2017) Physiological and biochemical responses of sunflower (Helianthus annuus L.) exposed to nano-CeO2 and excess boron: modulation of boron phytotoxicity. Plant Physiol Biochem 110:50–58
44. Zhang WL, Dan YB, Shi HL, Ma XM (2016) Effects of aging on the fate and bioavailability of cerium oxide nanoparticles to radish (Raphanus sativus L.) in soil. ACS Sustain Chem Eng 4(10):5424–5431
45. Venkatachalam P, Jayaraj M, Manikandan R, Geetha N, Rene ER, Sharma NC et al (2017) Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in Leucaena leucocephala seedlings: a physiochemical analysis. Plant Physiol Biochem 110:59–69
46. Ji Y, Zhou Y, Ma CX, Feng Y, Hao Y, Rui YK et al (2017) Jointed toxicity of TiO2 NPs and Cd to rice seedlings: NPs alleviated Cd toxicity and Cd promoted NPs uptake. Plant Physiol Biochem 110:82–93
47. Jackson P, Jacobsen NR, Baun A, Birkedal R, Kuhnel D, Jensen KA et al (2013) Bioaccumulation and ecotoxicity of carbon nanotubes. Chem Cent J 7:21
48. Wild E, Jones KC (2009) Novel method for the direct visualization of in vivo nanomaterials and chemical interactions in plants. Environ Sci Technol 43(14):5290–5294
49. Wang Y, Peng C, Fang HX, Sun LJ, Zhang H, Feng JB et al (2015) Mitigation of Cu(II) phytotoxicity to rice (Oryza sativa) in the presence of TiO2 and CeO2 nanoparticles combined with humic acid. Environ Toxicol Chem 34(7):1588–1596

# Chapter 18
# Application of Nano-phytoremediation Technology for Soil Polluted with Pesticide Residues and Heavy Metals

**K. Jesitha and P. S. Harikumar**

## 18.1 Introduction

Nano-phytoremediation is a combined technology between nanotechnology and phytotechnology for remediation of contaminated environments. The steps involved in this technology should be chosen with utmost care before on-site application for remediation. Phytoremediation – a cost-effective "green" technique – has been used to remediate environmental media contaminated with crude oil, explosives, metals, pesticides, solvents, and other pollutants. Here, we focus on environmental cleanup and provide a background and overview of current practice, research findings, and future directions for nano-phytoremediation. This cost-effective technology can reduce the cleanup time, eliminate the need for treatment and disposal of contaminated soil, and degrade the toxic pollutants [1–3].

Nanomaterials can either react directly with a pollutant or support the transformation of the pollutant into less toxic forms and thus protect the environment through pollution prevention, treatment, and cleanup. Nanotechnology is a broad, interdisciplinary field dealing with structures and particles at the nanoscale. Nanotechnology involves the creation and use of structures, devices, and systems that have novel properties and functions because of their small size and the ability to control or manipulate matter on an atomic scale [4]. Nanoremediation studies have shown that Ag, Au, Mg, and Fe nanoparticles can dehalogenate halocarbon pesticides. Nanomaterials can either react directly with a pollutant or support the transformation of the pollutant into less toxic forms [5–7]. Nanoscale zero valent iron (nZVIs) are very reactive because of their small sizes, and they can be used in

---

K. Jesitha
SreeSankara College, Ernakulam, India

P. S. Harikumar (✉)
Centre for Water Resources Development and Management, Kozhikode, India
e-mail: hps@cwrdm.org

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_18

in situ treatments. nZVIs can reduce chlorinated organic contaminants and pesticides [8–10].

Phytoremediation is a cost-effective "green" technique that has been used to remediate environmental media contaminated with metals, pesticides, solvents, and other pollutants. Plants can act as hyperaccumulators, and they can remove pollutants or convert pollutants into harmless products by bioaccumulating, degrading, extracting, or immobilizing the pollutants. Phytoremediation offers advantages over traditional remediation methods such as chemical oxidation, excavation, and thermal treatments [1, 2]. Combining nanotechnology and phytoremediation techniques could allow endosulfan and other pollutants to be degraded quickly and effectively under natural environmental conditions. The aim of the study was to investigate the capability of nano-phytoremediation by plant species such as chittaratha (*Alpinia calcarata*), tulsi (*Ocimum sanctum*), and *lemon*grass (*Cymbopogon citratus*) using Nanoscale zero valent iron (nZVI) for endosulfan removal from contaminated soil. Decontamination study of soil polluted with heavy metals was conducted using selected plant species *Tradescantia spathacea* and *Alternanthera dentata*. There are many obstacles to overcome in implementing nano-phytoremediation technology for common usage for which science is constantly refining, developing, and making breakthroughs.

## 18.2 Decontamination of Soil Polluted with Pesticide Residues Utilizing Phytoremediation and Nano-phytoremediation Techniques

### *18.2.1 Materials and Methods*

#### 18.2.1.1 Selection of Plant Species

Easily harvested plants were selected based on their abilities to tolerate or accumulate contaminants, grow quickly, effectively accumulate large quantities of pollutants, and produce large amounts of biomass. The plants that were screened were *Aerva lanata*, *Ageratum conyzoides*, *Alpinia calcarata*, *Biophytum sensitivum*, *Cleome viscosa*, *Cymbopogon citratus*, *Ocimum sanctum*, *Phyllanthus amarus*, *Plumbago indica*, *Ruta chalepensis*, and *Vernonia cinerea*.

#### 18.2.1.2 Phytoremediation Experiments

Seedlings (2 weeks old) of the plants selected for screening were transplanted into rectangular growth chambers containing soil contaminated with endosulfan. The chambers were placed in a greenhouse that was kept at 30 °C during the day and 27 °C at night. Water was added to the soil in each chamber every day to maintain appropriate moisture content. The greenhouse study was performed with natural light.

**Plate 18.1** Plants used for phytoremediation. (**a**) *Ocimum sanctum*, (**b**) *Alpinia calcarata*, (**c**) *Cymbopogon citratus*

The study was continued after the screening period using *A. calcarata*, *C. citratus*, and *O. sanctum* (Plate 18.1) that grew well in the endosulfan-contaminated soil. Healthy plants, with similar heights and biomasses, of the selected species were transplanted into rectangular pots containing 4 kg of soil artificially spiked with 1200 μg/kg of endosulfan for the main study. All the experiments using these potted plants were conducted in triplicate. Samples of the soil were removed for analysis at regular intervals after 0, 7, 14, 21, and 28 days. The plants used in the experiment were also analyzed for endosulfan to confirm their roles in phytoremediation experiments.

#### 18.2.1.3 Nano-phytoremediation Experiments

Synthesis of Nanoscale zero valent iron

The nZVIs were synthesized by the reductive precipitation process between $FeCl_3$ (0.045 M) and sodium borohydride (0.25 M). The borohydride solution was added drop by drop in 1:1 volume ratio into iron chloride solution with vigorous stirring

[11, 12]. The vacuum filtration technique was used to separate the black iron nanoparticles from the liquid phase. The solid particles were washed 3 times with 25 mL portions of absolute ethanol. The synthesized nanoparticles were finally dried in oven at 323 K overnight. For storage, a thin layer of ethanol was added to preserve the nano iron particles from oxidation. Then the mixture was agitated for 5–6 h so that the nanoparticles became homogenously adsorbed on the surface [3, 13, 14].

### Column Experiments to Find Out the Efficiency of Nanoscale Iron Particles

The efficiency at which the nZVIs to be used in the nano-phytoremediation study degraded endosulfan was determined by passing an endosulfan solution through a column packed with nZVIs. To study the effectiveness of Nanoscale zero valent iron for the remediation of endosulfan, the test solution containing 105.19 μg/L endosulfan was passed through a column (27 × 1 cm) packed with nZVIs. All experiments were carried out at room temperature. Aliquots of the eluate were collected at regular intervals, and the decrease in endosulfan concentrations in the eluate was determined using gas chromatograph with electron capture detector. After the column test, the nZVIs from the column were also analyzed by scanning electron microscopy (SEM) energy-dispersive X-ray spectroscopy (EDS).

### Determination of Size and Distribution of Nanoscale zero valent iron

The sizes and shapes of the nano- and submicron particles were determined using a SU-6600 field emission SEM instrument equipped with a Horiba EDX analyzer (Hitachi High-Technologies, Tokyo, Japan). The size distribution of the nZVIs was further examined using a H07600 transmission electron microscope (Hitachi High-Technologies). The SEM-EDS spectra of nZVIs were recorded before and after passing endosulfan solution during column experiments.

### Addition of nZVIs to the Soil Spiked with Endosulfan

A 4 kg aliquot of soil spiked with 1200 μg/kg of endosulfan was added to each pot, and the selected plants were transplanted into the pots. The same amount of nZVIs was applied to the soil in each pot. A 40 mL aliquot of the surfactant Tween 80 was added to each pot to increase the mobilities of the nZVIs. A soil sample (10 g) was collected from each pot after 0, 7, 14, 21, and 28 days and analyzed for endosulfan. The plants used in the experiment were also extracted and analyzed for endosulfan to confirm their roles in nano-phytoremediation experiments. The sample extracts were analyzed by gas chromatography with electron capture detection.

Two sets of control experiments were performed, one without any treatment and one with only nZVIs added.

The impact of nZVIs to the microbes in the soil was also assessed. The bacterial colonies in the control soil (without any Nanoscale zero valent iron) and soil with added nanoparticles were assessed by plate count method. The soil samples were serially diluted, and the number of bacterial colonies formed was counted by plating out the sample of culture on a nutrient agar surface.

### Extraction and Analysis of Pesticide Residues

Pesticide residues in water samples were extracted using a liquid-liquid extraction method. Pesticide residues in soil samples were extracted using a mixture of chromatographic grade *n*-hexane and acetone. Pesticide residues in plant samples were extracted with ethyl acetate, using a procedure that has been described previously. The extracts were cleaned and concentrated and then analyzed using a Varian CP-3800 gas chromatograph with a $^{63}$Ni electron capture detector. Separation was achieved using a wall-coated fused silica capillary column 30 m long, with a 0.32 mm internal diameter and a film 0.25 µm thick. After processing the samples through the different extraction steps, the final concentrated and cleaned up-sample was analyzed using gas chromatograph using electron capture detector which is specific and highly sensitive for halogenated compounds. A Varian-made CP-3800 Gas Chromatograph equipped with Ni$^{63}$ ECD electron capture detector was used to analyze the pesticides. One microliter volume of each extract was injected into the injection port using the microsyringe. WCOT fused silica capillary column of length 30 m, 0.32 mm internal diameter, 0.25 µm film thickness was fitted, and standard temperature programs were used. Nitrogen (99.999% purity) was used as the carrier gas, and the gas inlet pressure was 80 psi corresponding to a flow rate of 2 mL/min. The temperature for injector and detector were 250 °C and 300 °C, respectively. The temperature column was programmed from 130 (hold 1 min) to 200 °C at 5 °C (hold 10 min) and then from 200 to 232 °C at 1 °C/min. The chromatograms were recorded and integrated using Star Workstation software. The pesticides detected were compared with that of the standards. Accuracy within-day and between-day precision were assessed using QC samples at three concentration levels of 50, 100, and 200 µg/L. The samples were all run in triplicate ($n = 3$) on 3 different days and the RSD and relative error (RE) were calculated for each. Acceptable precision here was considered to be an RSD of <5%. The overall accuracy was assessed by subtracting the theoretical concentration of each QC sample from the mean concentration determined from the 3 days of analyses. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated relative to the values for the blank at the retention times of the analytes (ten injections). The final degradation product was confirmed by Thermo Trace 1300 gas chromatograph with mass spectrometer (GC-MS).

For the extraction of pesticide residues from plant biomass, samples were placed in glass tubes and homogenized twice with 4 mL of ethyl acetate. Ethyl acetate (2 mL) was utilized for washing. The homogenized samples were centrifuged for 10 min at 4600 rev./min and the extract was transferred to another tube and concentrated to 1 mL. Cleanup was accomplished by passing the extract through a column

**Plate 18.2** Extraction of endosulfan from plant parts. (**a**) Different parts of the plants selected, (**b**) treated plant parts in centrifuging tubes, (**c**) extraction in separating funnel

containing a small amount of glass wool at the base and 3.5 g of aluminum oxide with a thin layer of anhydrous sodium sulfate on top. A hexane-ethyl acetate (80:20, v/v) mixture (10 mL) was used to elute the pesticides from the column. Finally, the extracts were concentrated to an appropriate volume (10 mL) and analyzed by GC-ECD. Different steps during the extraction are shown in Plate 18.2 [15].

## 18.2.2 Results and Discussion

The results of the phytoremediation, nano-phytoremediation, and control (no treatments) experiments are shown in Table 18.1.

### 18.2.2.1 Phytoremediation Experiments

*A. calcarata* proved to be the most effective hyperaccumulator of endosulfan of the species that were tested, removing 81.20 ± 0.20% of the endosulfan initially added to the soil within 28 days. Endosulfan was removed slowly in the first 14 days of the experiment and then removed quickly in the following 14 days. *C. citratus* was also a good remediator, removing 65.08 ± 0.13% of the endosulfan from the soil within 28 days, although the plants became withered and unhealthy in the final days of the

**Table 18.1** Endosulfan concentration in the soil and the proportion of the endosulfan in the soil that was removed in each phytoremediation and nano-phytoremediation experiment

| Treatment | Days | Total endosulfan (α + β-endosulfan) concentration (μg/kg) | Proportion of endosulfan removed (%) |
|---|---|---|---|
| Control (without any treatment) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 1053.14 ± 1.35 | 7.61 ± 0.12 |
| | 14 | 1010.95 ± 1.68 | 11.31 ± 0.15 |
| | 21 | 926.03 ± 5.31 | 18.76 ± 0.47 |
| | 28 | 910.42 ± 3.77 | 20.13 ± 0.33 |
| Control (with added nZVI) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 942.71 ± 1.75 | 17.29 ± 0.15 |
| | 14 | 905.93 ± 3.32 | 20.52 ± 0.29 |
| | 21 | 627.10 ± 1.13 | 44.98 ± 0.10 |
| | 28 | 511.87 ± 1.55 | 55.09 ± 0.14 |
| *Alpinia calcarata* (phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 550.10 ± 1.52 | 51.74 ± 0.13 |
| | 14 | 501.05 ± 0.32 | 56.04 ± 0.03 |
| | 21 | 232.14 ± 0.43 | 79.63 ± 0.04 |
| | 28 | 214.33 ± 2.23 | 81.20 ± 0.20 |
| *Alpinia calcarata* (nano-phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 202.64 ± 1.65 | 82.20 ± 0.14 |
| | 14 | 83.59 ± 1.09 | 92.67 ± 0.10 |
| | 21 | 57.94 ± 0.96 | 94.92 ± 0.08 |
| | 28 | BDL | ≈100 |
| *Ocimum sanctum* (phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 1045.80 ± 2.01 | 8.25 ± 0.18 |
| | 14 | 932.74 ± 1.57 | 18.17 ± 0.14 |
| | 21 | 920.42 ± 2.33 | 19.25 ± 0.20 |
| | 28 | 903.19 ± 1.70 | 20.76 ± 0.15 |
| *Ocimum sanctum* (nano-phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 1015.50 ± 2.02 | 10.91 ± 0.18 |
| | 14 | 732.25 ± 1.07 | 35.76 ± 0.09 |
| | 21 | 366.65 ± 1.17 | 67.83 ± 0.10 |
| | 28 | 270.42 ± 2.16 | 76.28 ± 0.19 |
| *Cymbopogon citratus* (phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 1085.06 ± 1.05 | 4.81 ± 0.09 |
| | 14 | 456.05 ± 0.86 | 59.99 ± 0.12 |
| | 21 | 433.13 ± 1.26 | 62.0 ± 0.08 |
| | 28 | 398.09 ± 1.51 | 65.08 ± 0.13 |
| *Cymbopogon citratus* (nano-phytoremediation) | 0 | 1139.84 ± 0.93 | 0 |
| | 7 | 427.11 ± 0.76 | 62.53 ± 0.07 |
| | 14 | 213.80 ± 1.02 | 81.24 ± 0.09 |
| | 21 | 183.63 ± 0.99 | 83.89 ± 0.09 |
| | 28 | 157.73 ± 1.07 | 86.16 ± 0.09 |

*BDL* below detection limit
Detection limit: 0.05 μg/kg

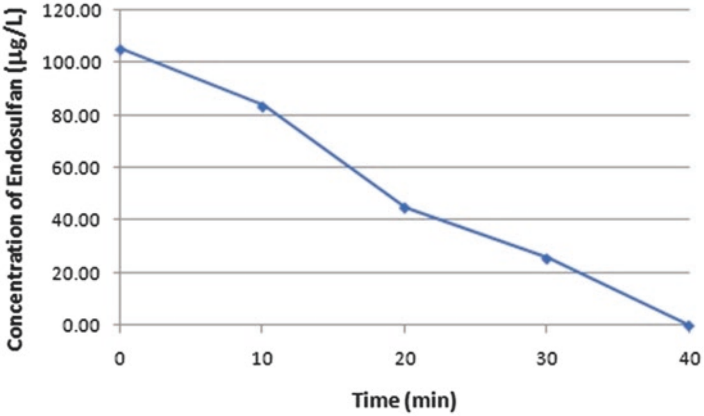


**Fig. 18.1** Decrease in the concentration of endosulfan with nZVIs

experiment. Of the selected plants, *O. sanctum* was the poorest remediator, removing only 20.76 ± 0.15% of the endosulfan in the soil within 28 days. The leaves of the *O. sanctum* plants became yellow and many were lost toward the end of the experiment.

All the plants used in the experiment were analyzed for endosulfan. The *A. calcarata* plants (which removed 81.20% of the endosulfan in the soil within 28 days, as stated above) accumulated, in various parts, 80% of the endosulfan removed from the soil, showing that this species is a good hyperaccumulator of endosulfan. The *C. citratus* and *O. sanctum* plants accumulated 62.13% and 14.06%, respectively, of the endosulfan they removed from the soil.

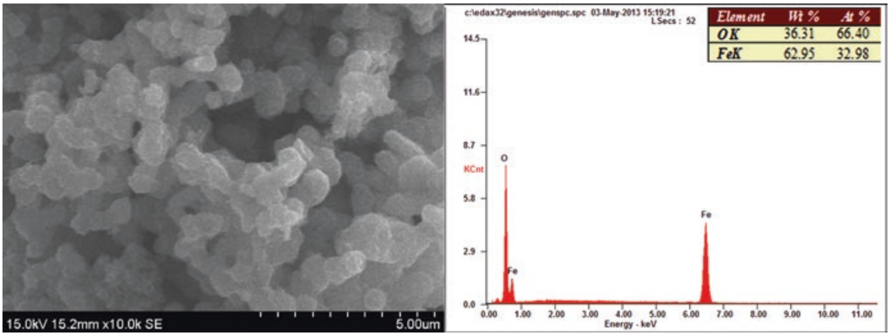
#### 18.2.2.2 Nano-phytoremediation Experiments

The results of column experiments to find out the efficiency of nZVIs in degrading endosulfan are given in Fig. 18.1.

A fast and substantial degradation of endosulfan was observed during the experiment with nZVI. The column experiment proved that endosulfan can be treated effectively by using iron nanoparticles at a residence time of less than 40 min.

The results of the systematic characterization of the nZVIs performed by SEM-EDS and transmission electron microscopy are shown in Figs. 18.2, 18.3, and 18.4.

The SEM image (Fig. 18.2) of the nZVIs showed that the nZVIs formed were nanospheres that were in contact with each other and formed chains. The linear chains would have formed because of the magnetic properties of iron. The EDS spectra showed that each nZVI had a core of zerovalent iron and a shell mainly composed of iron oxide (FeO). The dual properties of such nZVIs may allow them to be used to separate and transform many different contaminants [16]. The transmission electron microscopy image (Fig. 18.3) of the nZVIs that were synthesized showed that the nZVIs were mostly spherical and that most of the particles formed



| Element | Wt % | At % |
|---|---|---|
| OK | 36.31 | 66.40 |
| FeK | 62.95 | 32.98 |

**Fig. 18.2** Scanning electron microscopy-energy-dispersive X-ray spectroscopy image of the Nanoscale zero valent iron

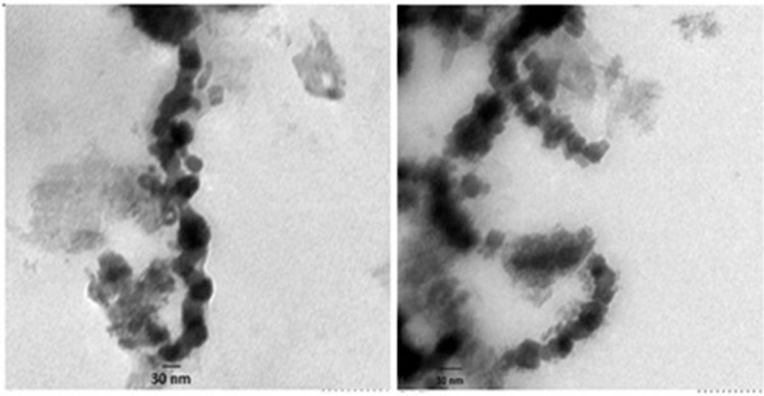
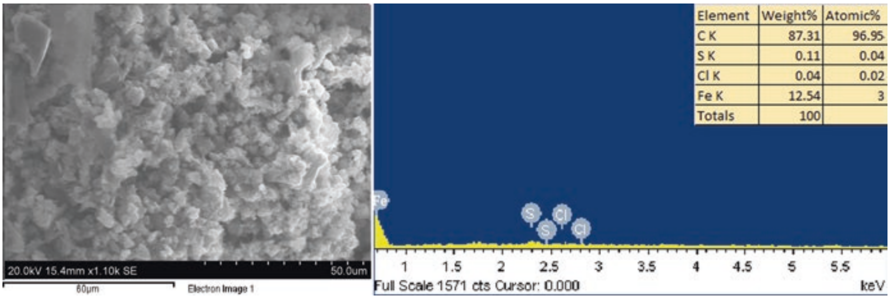


**Fig. 18.3** Transmission electron microscopy images of the Nanoscale zero valent iron



| Element | Weight% | Atomic% |
|---|---|---|
| C K | 87.31 | 96.95 |
| S K | 0.11 | 0.04 |
| Cl K | 0.04 | 0.02 |
| Fe K | 12.54 | 3 |
| Totals | 100 | |

**Fig. 18.4** Scanning electron microscopy-energy-dispersive X-ray spectroscopy image of the Nanoscale zero valent iron after they had been in contact with endosulfan

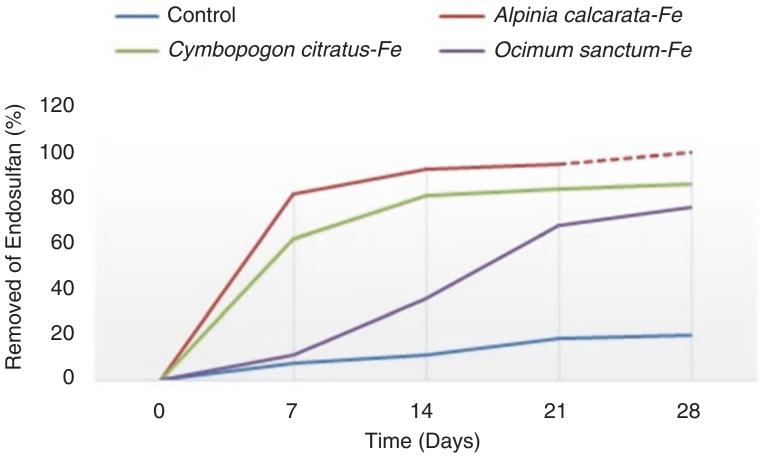


**Fig. 18.5** Percentage of the endosulfan in the soil removed in the control and nano-phytoremediation experiments

chain-like aggregates. A single particle was typically around 30 nm in diameter, and most of the particles were less than 100 nm in diameter. A SEM image of nZVIs after they had been in contact with endosulfan in the column experiment is shown in Fig. 18.4. Elements other than iron (e.g., sulfur and chlorine) were detected in the spectral data due to the release of those elements during the degradation of endosulfan by nZVIs. [17] found that nZVIs are highly reactive because they have high surface area to volume ratios and dense coverings of reactive sites and because the reactive sites have high intrinsic reactivities.

The results indicated that nano-phytoremediation is a more effective method for remediating endosulfan-contaminated soil than is phytoremediation alone (which is a long-term process). The proportions of the endosulfan in the spiked soil removed from the control experiments (without any treatment) and in the nano-phytoremediation experiments using *A. calcarata*, *C. citratus*, and *O. sanctum* are shown in Fig. 18.5. The endosulfan was removed efficiently in the first 7 days of the nano-phytoremediation experiments, and the endosulfan was completely removed within 28 days when *A. calcarata* was used and nZVIs were added to the soil.

The amount of endosulfan that was degraded naturally in the soil was determined from the results of the control soil samples. Only 20.13 ± 0.33% of the endosulfan had been removed from the soil in the control experiments after 28 days, and it is likely that the endosulfan that had been lost had been degraded. About 55% of the endosulfan was removed from the soil within 28 days in the control experiments with nZVIs added. Adding nZVIs to the experiments using plants caused more endosulfan to be removed than what was removed by the plants alone. Adding the nZVIs to the soil caused the amount of residual endosulfan in the soil to suddenly decrease. The endosulfan was removed more quickly and effectively in the experiments with *A. calcarata* and nZVIs than in the experiments with only *A. calcarata* or only nZVIs, and no endosulfan could be detected in the soil after 28 days. The endosulfan concentration decreased rapidly in the first 7 days of the experiments with *A. calcarata* and nZVIs, and the remaining endosulfan was removed more gradually in the following days. The activities of the nZVIs may have gradually decreased because of the Fe (II) being oxidized to form Fe (III). Fe (III) is insoluble and may precipitate and deposit a hydroxide film on the surfaces of nZVIs, decreasing their activities. The formation of hydrogen would also deactivate the reactive sites on nZVIs. The gradual loss of nZVI activity might have caused the gradual decrease in the efficiency at which the endosulfan was removed in the experiments [18].

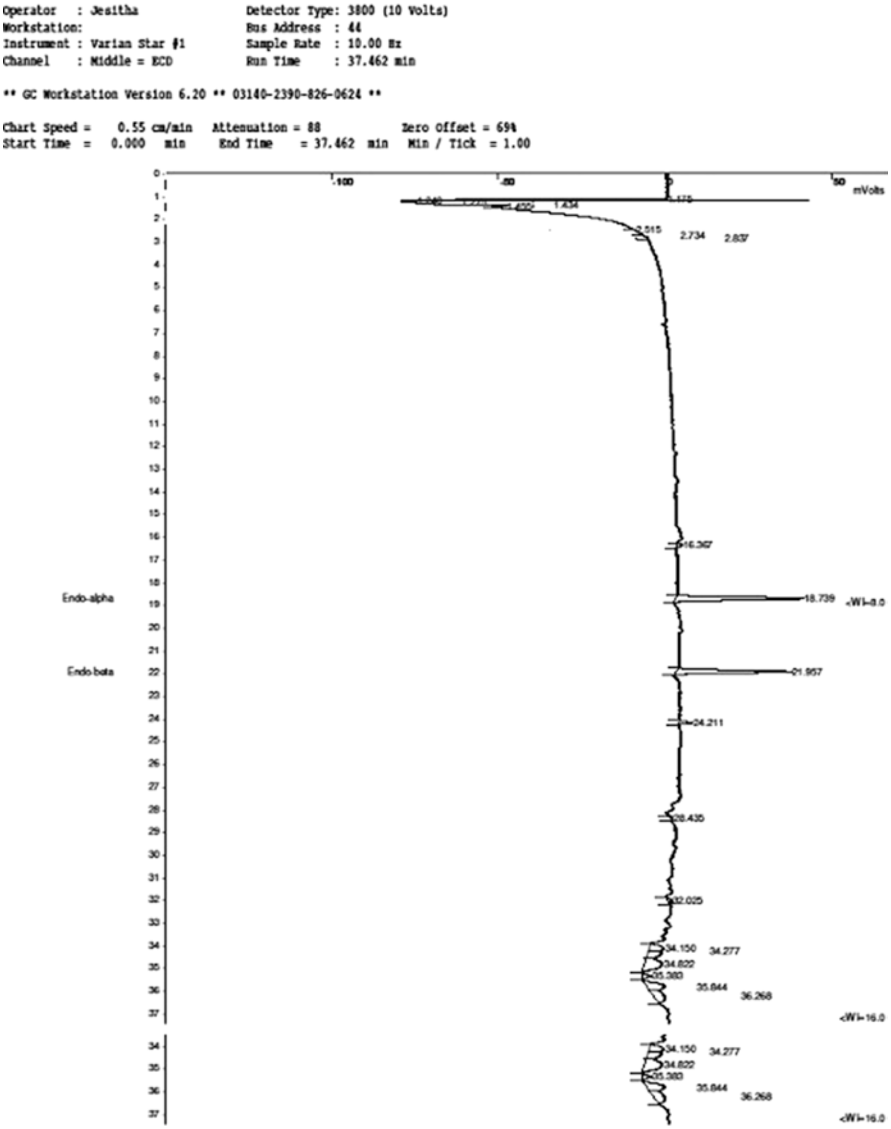
After 28 days, 86.16 ± 0.09% of the endosulfan in the soil had been removed in the experiments with *C. citratus* and nZVIs. Only 76.28 ± 0.19% of the endosulfan in the soil had been removed in the experiments with *O. sanctum* and nZVIs within 28 days. The results of impact of nZVI on the growth of microbial colonies indicated a growth in the number of colonies formed in the soil with added nZVI particles compared to the soil without any added nanoparticles. Thus, the addition of nanoparticles provided a suitable condition for the growth of bacteria [19]. Different parts of the plants were analyzed for endosulfan to determine if the plants had accumulated endosulfan from the soil. Endosulfan was found in the roots, shoots, and leaves of the plants. The nZVIs would have reduced the endosulfan in the soil, preventing the normal uptake of the endosulfan by the plants in the nano-phytoremediation experiments. Only 20.35% of the endosulfan (endosulfan alpha and beta) removed from the soil was found in the *A. calcarata* tissues, and 12.21% and 9.84% of the endosulfan removed from the soil were found in the *O. sanctum* and *C. citratus* tissues, respectively.

The Fe (0) in nZVIs can become oxidized to form Fe(II) and Fe(III) by reducing organic or inorganic species, and this means that the nZVIs in the experiments were able to remove endosulfan from the soil. Metallic iron ($Fe^0$) serves effectively as an electron donor [20] as shown in Eq. (18.1).

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \tag{18.1}$$

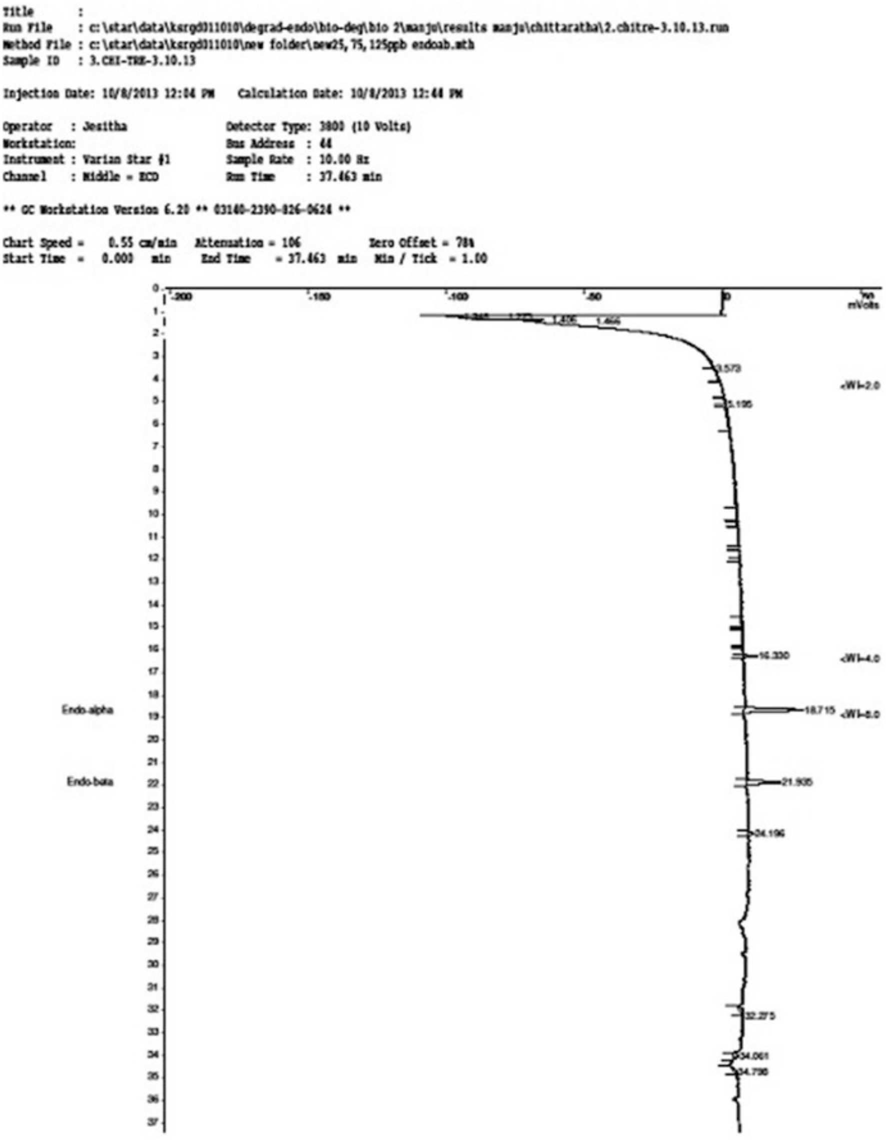
Chlorinated pesticides such as endosulfan can accept electrons and undergo reductive dechlorination. The coupling of the iron oxidation and pesticide reduction reactions is often highly energetically favorable from a thermodynamic perspective. The standard reduction potential ($E^0$) for the Fe(0) in nZVI (i.e., $Fe^{2+}/Fe$) is −0.44 V. This is lower than the $E^0$ for many organic compounds, including many chlorinated pesticides, so these organic compounds are able to be reduced by nZVIs. The pH increases, hydrogen is evolved, and oxidizable materials are consumed as nZVIs are oxidized to form ferrous and/or ferric iron, and the strong reducing conditions created favor the complete dechlorination of chlorinated pesticides [3, 21, 22]. The degradation of endosulfan by nZVIs involves hydrogenolysis through sequential dehalogenation. The presence of plants will have accelerated the removal of endosulfan from the soil by effectively phytoextracting it. The endosulfan isomers hyperaccumulated in the plant parts that were analyzed much less in the nano-phytoremediation experiments than in the phytoremediation experiments. This clearly proves that the nZVIs played an important role in the endosulfan detoxification process in the nano-phytoremediation experiments.

The chromatograms obtained during the nano-phytoremediation study using nZVI and *Alpinia calcarata* after 7 days, 14 days, 21 days, and 28 days of analysis of endosulfan from soil are shown in Figs. 18.6, 18.7, 18.8, and 18.9, respectively. A decrease in the concentration of endosulfan in the soil has occurred with increase in the number of days in nano-phytoremediation study. The peaks indicating toxic isomers have completely disappeared in Fig. 18.9 indicating complete degradation of endosulfan.



```
Operator   : Jesitha              Detector Type: 3800 (10 Volts)
Workstation:                      Bus Address  : 44
Instrument : Varian Star #1       Sample Rate  : 10.00 Hz
Channel    : Middle = ECD         Run Time     : 37.462 min

** GC Workstation Version 6.20 ** 03140-2390-826-0624 **

Chart Speed =    0.55 cm/min   Attenuation = 88          Zero Offset = 69%
Start Time  =   0.000   min     End Time    = 37.462  min   Min / Tick  = 1.00
```

**Fig. 18.6** Chromatogram obtained in the nano-phytoremediation study with *Alpinia calcarata* after 7 days

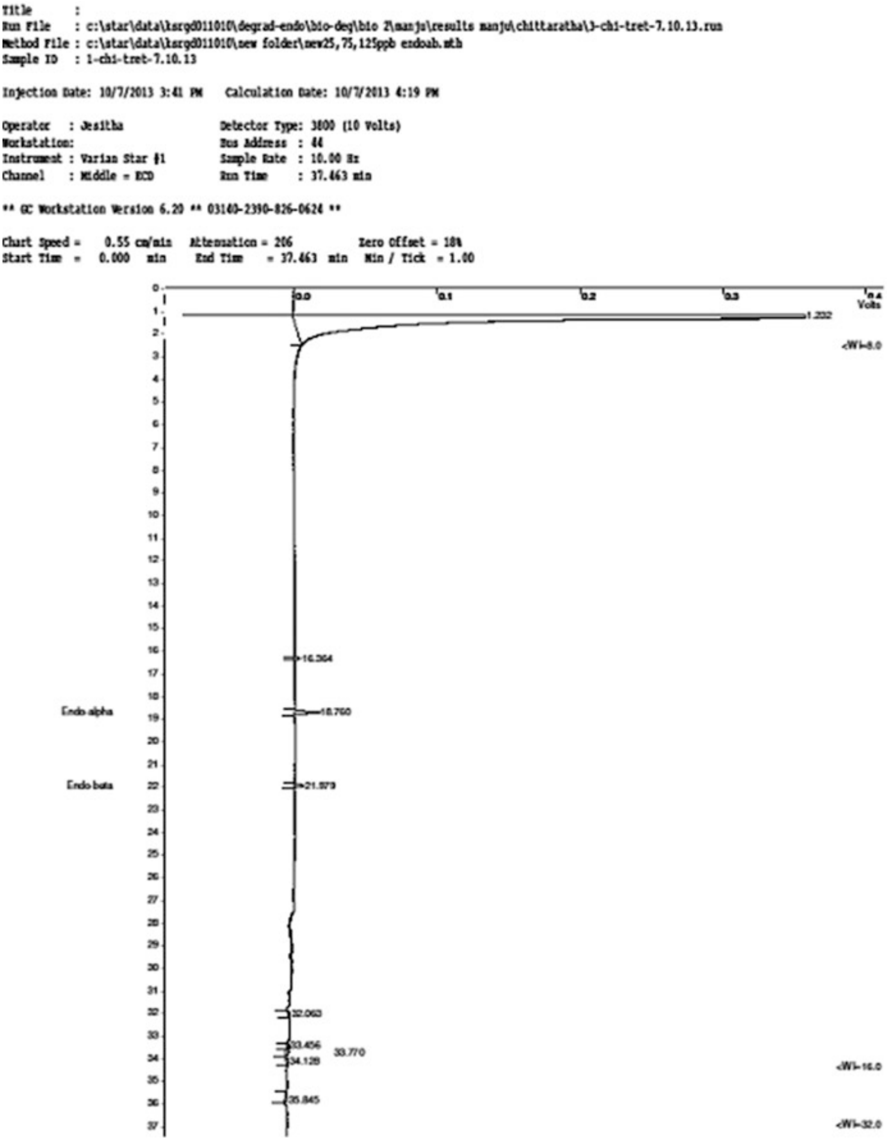


```
Title        :
Run File     : c:\star\data\ksrgd011010\degrad-endo\bio-deg\bio 2\manju\results manju\chittaratha\2.chitre-3.10.13.run
Method File : c:\star\data\ksrgd011010\new folder\new25,75,125ppb endoab.mth
Sample ID   : 3.CHI-TRE-3.10.13

Injection Date: 10/8/2013 12:04 PM    Calculation Date: 10/8/2013 12:44 PM

Operator    : Jesitha                 Detector Type: 3800 (10 Volts)
Workstation:                          Bus Address  : 44
Instrument : Varian Star #1           Sample Rate  : 10.00 Hz
Channel     : Middle = ECD            Run Time     : 37.463 min

** GC Workstation Version 6.20 ** 03140-2390-826-0624 **

Chart Speed =    0.55 cm/min   Attenuation = 106          Zero Offset = 78%
Start Time  =   0.000  min     End Time    = 37.463  min   Min / Tick  = 1.00
```

**Fig. 18.7** Chromatogram obtained in the nano-phytoremediation study with *Alpinia calcarata* after 14 days

### 18.2.2.3 Comparison of Endosulfan Accumulated in Plant Parts in Phytoremediation and Nano-phytoremediation Experiments

All the plants used in the experiment were extracted and the amount of endosulfan present in the plant parts were determined (Table 18.2). Extraction was done to

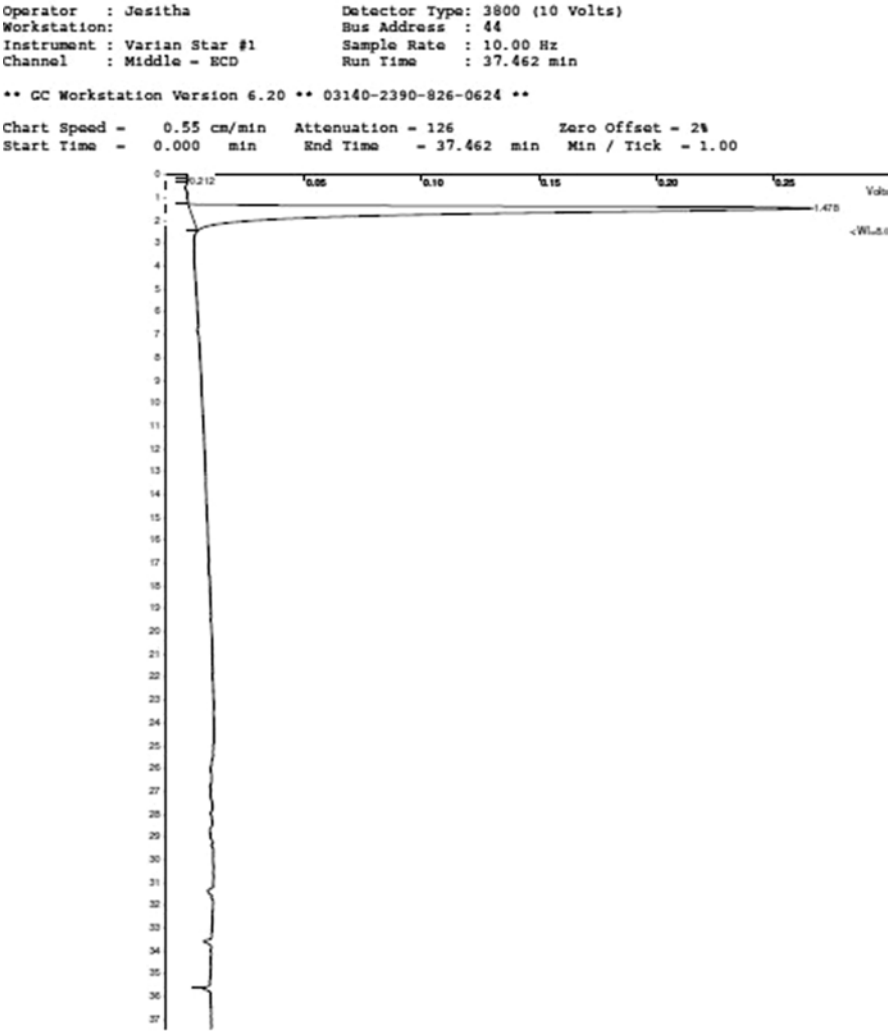


```
Title       :
Run File    : c:\star\data\ksrgd011010\degrad-endo\bio-deg\bio 2\manju\results manju\chittaratha\3-chi-tret-7.10.13.run
Method File : c:\star\data\ksrgd011010\new folder\new25,75,125ppb endoab.mth
Sample ID   : 1-chi-tret-7.10.13

Injection Date: 10/7/2013 3:41 PM    Calculation Date: 10/7/2013 4:19 PM

Operator   : Jesitha              Detector Type: 3800 (10 Volts)
Workstation:                      Bus Address  : 44
Instrument : Varian Star #1       Sample Rate  : 10.00 Hz
Channel    : Middle = ECD         Run Time     : 37.463 min

** GC Workstation Version 6.20 ** 03140-2390-826-0624 **

Chart Speed =    0.55 cm/min   Attenuation = 206          Zero Offset = 18%
Start Time  =   0.000   min     End Time    = 37.463  min  Min / Tick  = 1.00
```

**Fig. 18.8** Chromatogram obtained in the nano-phytoremediation study with *Alpinia calcarata* after 21 days

make sure that the plants had accumulated endosulfan taken up from the soil. Endosulfan was found to be deposited in plant parts like root, shoot, and leaves. Five grams of total plant parts was taken for extraction.

In the phytoremediation using *A. calcarata*, 81.20% of endosulfan was removed from the soil within 28 days. The residue present in the plant parts was 80.38% of



```
Operator   : Jesitha               Detector Type: 3800 (10 Volts)
Workstation:                       Bus Address  : 44
Instrument : Varian Star #1        Sample Rate  : 10.00 Hz
Channel    : Middle = ECD          Run Time     : 37.462 min

** GC Workstation Version 6.20 ** 03140-2390-826-0624 **

Chart Speed =    0.55 cm/min   Attenuation = 126          Zero Offset = 2%
Start Time  =   0.000    min     End Time    = 37.462  min   Min / Tick  = 1.00
```

**Fig. 18.9** Chromatogram obtained in the nano-phytoremediation study with *Alpinia calcarata* after 28 days

the initial concentration of endosulfan. This shows that this plant species is a good hyperaccumulator for endosulfan. In nano-phytoremediation the nanoparticles had prevented the normal uptake of toxic isomers of endosulfan by carrying out the reduction process. Hence the amount of endosulfan (endosulfan alpha and endosulfan beta) residue in plant parts used for nano-phytoremediation was found to be only 20.35%. The percentage of pesticide present in the parts of *C. citratus* in phytoremediation was 62.13%, while 9.8% endosulfan was found in parts of the plant used in nano-phytoremediation. *O. sanctum* used in phytoremediation had 14.06%

**Table 18.2** Presence of endosulfan in plant parts in phytoremediation and nano-phytoremediation experiments

| Treatment | Plant parts | Endosulfan alpha (μg/kg) | Endosulfan beta (μg/kg) | Total endosulfan (alpha + beta) (μg/kg) | Concentration of endosulfan inside the plant (μg/kg) | Percentage taken up by the plant (%) |
|---|---|---|---|---|---|---|
| *Alpinia calcarata* (phytoremediation) | Root | 202.01 ± 0.59 | 95.09 ± 0.93 | 297.10 ± 1.27 | 916.18 | 80.38 |
| | Shoot | 314.46 ± 0.58 | 171.22 ± 1.13 | 485.68 ± 1.48 | | |
| | Leaves | 120.51 ± 0.38 | 12.89 ± 0.73 | 133.40 ± 0.99 | | |
| *Alpinia calcarata* (nano-phytoremediation) | Root | 25.89 ± 0.80 | 20.74 ± 0.16 | 46.63 ± 0.69 | 231.93 | 20.35 |
| | Shoot | 152.69 ± 0.40 | 32.61 ± 0.20 | 185.3 ± 0.47 | | |
| | Leaves | BDL | BDL | BDL | | |
| *Cymbopogon citratus* (phytoremediation) | Root | 215.41 ± 0.94 | 124.13 ± 1.21 | 339.54 ± 1.93 | 708.17 | 62.13 |
| | Shoot | 111.86 ± 0.66 | 108.63 ± 0.45 | 220.49 ± 1.02 | | |
| | Leaves | 80.60 ± 0.92 | 67.54 ± 2.10 | 148.14 ± 2.87 | | |
| *Cymbopogon citratus* (nano-phytoremediation) | Root | 83.75 ± 1.64 | 19.72 ± 0.41 | 103.47 ± 1.25 | 112.11 | 9.84 |
| | Shoot | BDL | BDL | BDL | | |
| | Leaves | 3.04 ± 0.16 | 5.60 ± 0.56 | 8.64 ± 0.50 | | |
| *Ocimum sanctum* (phytoremediation) | Root | 15.90 ± 0.74 | 144.37 ± 0.74 | 160.27 ± 0.90 | 160.27 | 14.06 |
| | Shoot | BDL | BDL | BDL | | |
| | Leaves | BDL | BDL | BDL | | |
| *Ocimum sanctum* (nano-phytoremediation) | Root | 11.48 ± 0.99 | 127.70 ± 1.99 | 139.18 ± 1.75 | 139.18 | 12.21 |
| | Shoot | BDL | BDL | BDL | | |
| | Leaves | *BDL* | *BDL* | *BDL* | | |

*BDL* below detection limit
Detection limit: 0.05 μg/kg
Initial concentration of endosulfan applied to the soil: 1139.84 ± 0.93 μg/kg

residue in its plant parts, and 12.21% was present in the one which was used in nano-phytoremediation. In all the cases irrespective of the ability of the plant to remove endosulfan, nZVIs marked strongly its pesticide reduction efficiency.

## 18.3 Decontamination of Soil Polluted with Heavy Metals Utilizing Phytoremediation and Nano-phytoremediation Techniques

### *18.3.1 Materials and Methods*

The soil was taken from the surface layer (0–90 cm depth). For the experimental study, collected soil was bulked, well mixed, air-dried, and sieved through 2 mm sieve. Soil samples were taken from bulk soil for determination of pH, texture, electrical conductivity, and total organic carbon. The selected physical and chemical properties of the soil used in the study are presented in Table 18.3.

Commonly available terrestrial plant species such as *Tradescantia spathacea* (boat lily) and *Alternanthera dentata* were selected and screened for the study (Plate 18.1). The study was continued after the screening period using these plant species which grew well in the heavy metal-contaminated soil. *Tradescantia spathacea* has fleshy rhizomes and rosettes of waxy lance-shaped leaves. Leaves are dark to metallic green above, with glossy purple underneath. These will reach up to 1 ft (30 cm) long by 3 in. (7.5 cm) wide. They are very attractive foliage plants that will reach 1 ft (30 cm) tall. It spreads by seeds, which are dispersed by wind, and it also grows from cuttings and plant fragments. Once established, it is able to grow forming dense ground cover on the forest floor preventing the germination and establishment of native plants.

**Table 18.3** Physicochemical characteristics of soil

| | |
|---|---|
| PH | 5.38 |
| EC (µS/cm) | 31.00 |
| TDS (ppm) | 22.60 |
| Salinity (ppm) | 23.50 |
| Alkalinity (mg/L) | 12.00 |
| Chloride (mg/L) | 23.99 |
| Sulfate (mg/L) | 8.84 |
| Total hardness (mg/L) | 8.00 |
| Calcium hardness (mg/L) | 4.00 |
| Magnesium hardness (mg/L) | 4.00 |
| Calcium (ppm) | 1.60 |
| Magnesium (ppm) | 0.97 |
| Sand (%) | 72.5 |
| Clay (%) | 20 |
| Silt (%) | 7.5 |

*Alternanthera* is a genus of flowering plants in the amaranth family, Amaranthaceae. It is a widespread genus with most species occurring in the tropical Americas and others in Asia, Africa, and Australia. While some of the better-known species are aquatic plants, most are terrestrial. They take many forms, from prostrate to erect to floating. The leaves are oppositely arranged. The experiments were carried out in pots filled with garden soil and three replicates were used for each treatment. Soil was passed through 2 mm sieve and 7 kg soil was put into each pot. Medium-growing plants with extensive rooting system were selected for the study. Half-month-old seedlings were transplanted into the pots, which were then placed in a temperature-controlled greenhouse. Water was added daily to adjust the soil to appropriate moisture content. Natural light was used for the greenhouse study. The soil in each pot was artificially contaminated with Pb and Cd in the form of $Pb(NO_3)_2$ and $CdSO_4 \cdot 8H_2O$. Control experiments without the plant species were also laid to study natural degradation of the metals. The plant samples were collected from the experimental pots after 40 days.

A 7 kg of soil spiked with heavy metals [Pb (100 mg/kg) and Cd (50 mg/kg)] was added to each pot, and the selected plants were transplanted into the pots. The same amount (1000 mg/kg) of nZVIs was applied to one set of each plant pot, and another same amount (1000 mg/kg) of citric acid was added to another set of each plant pot. Plant sample was collected from each pot after 40 days. The plants used in the experiment were extracted and analyzed for heavy metals to confirm their roles in nano-phytoremediation and chelate-assisted phytoremediation experiments. The sample extracts were analyzed by ICP-OES.

#### 18.3.1.1 Synthesis of Nanoscale zero valent iron

The nZVIs were synthesized by the reductive precipitation process by mixing 1:1 volume ratio of $FeCl_3 \cdot 6H_2O$ (0.18 M) and sodium borohydride (0.8 M) [23, 24]. The reaction is taking place as per the following mechanism:

$$4Fe^{3+} + 3BH_4 + 9H_2O\,4Fe^0\left(s\right) + 3H_2BO_3 + 12H^+\left(aq\right) + 6H_2\left(g\right)$$

The borohydride solution was added drop by drop into iron chloride solution with vigorous stirring (400 rpm) (Poursaberi et al. 2012; Rahmani et al. 2011). The formed black solid iron nanoparticles were filtered. The synthesized iron nanoparticles were then washed several times with distilled water and absolute ethanol. The synthesized nanoparticles were finally dried in an oven at 500 °C overnight. For storage, a thin layer of ethanol was added to preserve the nano iron particles from oxidation. Then the mixture was agitated for 5–6 h so that the nanoparticles became homogenously adsorbed on the surface [13, 14]. The sizes and shapes of the nano- and submicron particles were determined using a SU-6600 field emission SEM instrument equipped with a Horiba EDX analyzer (Hitachi High-Technologies, Tokyo, Japan). The size distribution of the nZVIs was further examined using a H07600 transmission electron microscope (Hitachi High-Technologies).

#### 18.3.1.2 Extraction and Analysis of Heavy Metals in Plants

After 40 days, the plants were harvested and heavy metal concentrations in plants were determined. Plant samples were separated into the root and shoot and thoroughly washed with distilled water to remove soil particles adhered to the plants. After washing, plant samples were dried in an oven until constant weight was obtained and then ground to powders. Samples (1 g) of finely ground plant tissue were digested with 16 mL mixtures of 6:2 $HNO_3/H_2O_2$ mixtures on the hot plate. After cooling, 10 mL of distilled water was added to the sample and mixed. The residue was filtered through filter paper and then the sample was diluted to 50 mL with distilled water. Metal contents of final solution were determined by ICP-OES [25].

### *18.3.2 Accumulation and Translocation of Metals in Plants*

Both bioconcentration factors (BCF) and translocation factors (TF) were used to estimate the plant's potential for phytoremediation purpose. Bioconcentration factor (BCF) indicates the efficiency of a plant in uptake of heavy metals from soil and accumulating them into its tissues. It is a ratio of the heavy metal concentration in the plant tissue (root, stem, or leaves) to that in soil [26]. Higher BCF value indicates the increased suitability for phytoextraction (BCF values >2 were regarded as high values) [27, 28].

Bioconcentration factor = Metal concentration in the plant tissue/Metal concentration in the soil

Translocation factor (TF) indicates the efficiency of the plant in translocating the accumulated heavy metals from roots to shoots. It is a ratio of the concentration of the heavy metal in shoots (stem or leaves) to that in its roots [29, 30]. Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1, with values greater indicating translocation to the aerial part of the plant [28].

Translocation factor = Metal concentration in aerial parts/Metal concentration in roots

### *18.3.3 Results and Discussion*

Physicochemical characteristics of soil are given in Table 18.3.

#### 18.3.3.1 Nano-phytoremediation of Heavy Metals by *Tradescantia spathacea*

The samples were collected from phyto- and nano-phytoremediation treatments, and the concentration of heavy metals in both roots and shoots of *Tradescantia spathacea* under different treatments was analyzed (Table 18.4). Heavy metal concentration was found to be greater in roots than in shoots. It was found that phytoremediation had a

**Table 18.4** Accumulation of Pb and Cd by *Tradescantia spathacea*

| Plant name | Treatment method | Heavy metal concentration in plant parts (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Pb | | Cd | |
| | | Root | Shoot | Root | Shoot |
| *Tradescantia spathacea* | Phytoremediation | 36.89 | 11.08 | 18.98 | 3.65 |
| | Treatment with nZVI | 71.80 | 12.56 | 19.90 | 12.47 |

**Table 18.5** Translocation factors (TF) of Pb and Cd in *Tradescantia spathacea*

| Treatments | Translocation factor | |
|---|---|---|
| | Pb | Cd |
| Phytoremediation | 0.30 | 0.19 |
| Treatment with nZVI | 0.17 | 0.62 |

**Table 18.6** Bioconcentration factors (BCF) of Pb and Cd in *Tradescantia spathacea*

| Treatments | Bioconcentration factor | |
|---|---|---|
| | Pb | Cd |
| Phytoremediation | 0.48 | 0.45 |
| Treatment with nZVI | 0.84 | 0.64 |

little effect on the heavy metal (Pb and Cd) uptake compared to nano-phytoremediation treatments. Higher concentration of heavy metals in the root samples indicated the immobilization of heavy metals in roots and low mobility from roots to shoots.

Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation purpose. By comparing BCF and TF, we can compare the ability of different plants in taking up metals from soil and translocating them to shoots. Tolerant plants tend to restrict soil-root and root-shoot transfers and therefore have much less accumulation in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass [31]. The translocation factors (TF) of Pb and Cd in *Tradescantia spathacea* are shown in Table 18.5.

BCF for Pb was 0.48 in phytoremediation which was increased to 0.84 in treatment with nZVI. In case of Cd, it was 0.45 in phytoremediation which was increased to 0.64 in treatment with nZVI. It was observed that BCF for Pb and Cd is found to be greater for treatment with nZVI than phytoremediation (Table 18.6).

#### 18.3.3.2 Nano-phytoremediation of Heavy Metals by *Alternanthera dentata*

The samples were collected from two treatments (phyto and nano-phyto), and the concentration of heavy metals in both roots and shoots of *Alternanthera dentata* under different treatments was analyzed (Table 18.7). Analysis of plants after different treatments showed enhanced accumulation of Pb and Cd. In general, the highest concentration and uptake of both the metals were observed in roots compared to

**Table 18.7** Accumulation of Pb and Cd by *Alternanthera dentata*

| Plant name | Treatment method | Heavy metal concentration in plant parts (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Pb | | Cd | |
| | | Root | Shoot | Root | Shoot |
| *Alternanthera dentata* | Phytoremediation | 51.97 | 8.5 | 18.0 | 8.23 |
| | Treatment with nZVI | 59.41 | 14.26 | 20.46 | 15.20 |

**Table 18.8** Translocation factors (TF) of Pb and Cd in *Alternanthera dentata*

| Treatments | Translocation factor | |
|---|---|---|
| | Pb | Cd |
| Phytoremediation | 0.16 | 0.45 |
| Treatment with nZVI | 0.23 | 0.74 |

**Table 18.9** Bioconcentration factors (BCF) of Pb and Cd in *Alternanthera dentata*

| Treatments | Bioconcentration factor | |
|---|---|---|
| | Pb | Cd |
| Phytoremediation | 0.60 | 0.52 |
| Treatment with nZVI | 0.73 | 0.71 |

shoots. It was found that for both the plants, the concentration of metals was found to be greater in nano-phytoremediation than in phytoremediation.

The translocation factors (TF) of heavy metals from roots to shoot for *Alternanthera dentata* are shown in Table 18.8. The magnitude of TF was observed to be higher in treatment with nZVI than phytoremediation. BCF (Table 18.9) also showed a similar trend with higher values for nano-phytoremediation treatment.

#### 18.3.3.3 Heavy Metal Accumulation and Distribution in Plants

##### Mechanisms of Metal Uptake into Roots and Translocation to Shoots

The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing toxicity are found. Plants act both as accumulators and excluders. Accumulators survive despite concentrating contaminants in their aerial tissues as they biodegrade or biotransform the contaminants into inert forms within their tissues. The excluders restrict contaminant uptake into their biomass [32]. The different plant parts contain different quantities of heavy metals with the highest ones being contained in roots and leaves [33].

The mechanism of phytoextraction of heavy metals includes five basic aspects: mobilization of the heavy metals in soil, uptake of the metal ions by plant roots, translocation of the accumulated metals from roots to aerial tissues, sequestration of the metal ions in plant tissues, and metal tolerance. Mechanisms governing heavy metal tolerance in plant cells are cell wall binding, active transport of ions into the vacuole, and chelation through the induction of metal-binding peptides and the formation of metal complexes [34, 35]. Within the plant cell, heavy metal may trigger the production of oligopeptide ligands known as phytochelatins (PCs) and metallothioneins (MTs). These peptides bind and form stable complex with the heavy metal and thus neutralize the toxicity of the metal ion. Phytochelatins (PCs) are synthesized with glutathione as building blocks resulting in a peptide. Appearance of phytochelating ligands has been reported in hundreds of plant species exposed to heavy metals [36].

Metal-tolerant plants with the capacity to keep the metals out of metabolic sites (shoots) are the best candidates for phytostabilization. Although such plants have developed mechanisms to restrict the metals in the rhizosphere or roots, even then concentration of metals in shoots must be monitored. Some plants can detoxify the metals in the rhizosphere by releasing organic acids thus tendering the metals less available. Another process of metal detoxification is immobilization of metals in fine roots through binding with pectins in the cell walls and to the negatively charged cytoplasm-membrane surfaces due to their strong electrochemical potential. Some plants have the ability to reduce the valence of metals by releasing redox enzymes, and thus toxic metals can be converted into less toxic forms. Plants which can survive in metal-contaminated soils without affecting growth and maintain low concentrations of metals in aerial parts, even though concentration of metals is very high in the roots, are known as metal excluder plants. Phytostabilization is considered a very good alternative for those soils which cannot be immediately remediated through phytoextraction. Efficiency of phytostabilization can be enhanced by involving soil amendments like zeolites, beringite, chelates, and nanoparticles.

## 18.4 Summary

Natural degradation processes are involved in the decomposition of organic substances in the environment. The possibility of combining nanoremediation and phytoremediation to remediate soil contaminated with endosulfan was assessed in this study. The unique properties and high surface areas of nZVIs mean that they can improve on many of the advantages offered by traditional iron remediation techniques. The abilities of three plant species *Alpinia calcarata*, *Ocimum sanctum*, and *Cymbopogon citratus* to remove endosulfan from soil in the absence and presence of Nanoscale zero valent iron (nZVIs), i.e., by phytoremediation and nano-phytoremediation, were determined. endosulfan was rapidly and efficiently removed from contaminated soil using nano-phytoremediation method. The removal efficiency decreased in the order *A. calcarata* > *C. citratus* > *O. sanctum*. The terrestrial plant *A. calcarata* and nZVIs completely removed endosulfan from contaminated soil within 1 month. The endosulfan degradation by nZVI mechanism appears to involve hydrogenolysis and sequential dehalogenation. Only small amounts of endosulfan accumulated in the plants because the nZVIs promoted the reductive dechlorination of endosulfan. More work is required to identify the exact mechanisms involved in the nano-phytoremediation process, but it appears that the process involves the reductive dechlorination of endosulfan by the nZVIs and the enzymatic metabolism of endosulfan in the plants.

The use of plants to remove heavy metals from soil (phytoremediation and nano-phytoremediation) has revealed a great potential due to its cost-effectiveness as compared to conventional methods. This study was conducted in *Tradescantia spathacea* and *Alternanthera dentata* to evaluate the efficiency of nanoparticle in enhancing phytoremediation and comparing their effect in phytoremediation process. *Tradescantia spathacea* and *Alternanthera dentata* effectively remediated the selected heavy metals from the metal-contaminated soil by nano-phytoremediation technique. The results showed the highest uptake of metal was observed in roots compared to shoots. In *Tradescantia spathacea*, phytoremediation accumulated about 47% Pb and 45.3% Cd and nano-phytoremediation treatment accumulated 84.4% Pb and 64.8% Cd. In *Alternanthera dentata*, phytoremediation accumulated about 60.5% Pb and 52.5% Cd and nano-phytoremediation treatment accumulated 73.7% Pb and 71.3% Cd. The experimental field trials with nano-phytoremediation technique proved very efficient and confirmed the feasibility of utilizing this plant-based approach for the decontamination of soil polluted with pesticide residues and heavy metals.

## References

1. Campos VM, Merino I, Casado R, PaciosLF GL (2008) Phytoremediation of organic pollutants. Spanish J Agric Res 6:38–47
2. Frazar C (2000) The bioremediation and phytoremediation of pesticide-contaminated sites. Prepared for U.S. Environmental Protection Agency. Office of Solid Waste and Emergency Response, Technology Innovation Office, Washington, DC
3. Harikumar PS, Jesitha K (2016) Nano-phytotechnological remediation of endosulphan using zero valent iron nanoparticles. J Environ Prot 7:734–744
4. US EPA (2007) Nanotechnology white paper. http://www.epa.gov/osa/pdfs/nanotech/epa-nanotechnologywhitepaper-0207.pdf
5. Adeleye AS, Keller AA, Miller RJ, Lenihan HS (2013) Persistence of commercial nanoscaled zerovalent iron (nZVI) and By-Products. J Nano Res 15:1401–1418. https://dx.doi.org/10.1007/s11051-013-1418-7
6. Bootharaju MS, Pradeep T (2012) Understanding the degradation pathway of the pesticide, Chlorpyrifos by noble metal nanoparticles. Langmuir 28: 2671–2679. https://dx.doi.org/10.1021/la2050515
7. Zhao J (2009) Turning to nanotechnology for pollution control: applications of nanoparticles. Dartmouth Undergr J Sci
8. Dayan H, Abrajano T, Sturchio NC, Winsor L (1999) Carbon isotopic fractionation during reductive dehalogenation of chlorinated ethenes by metallic iron. Org Geochem 30:755–763
9. Muller NC, Nowack B (2010) Nano zero valent iron – the solution for water and soil remediation. Observatory NANO focus report 2010. EMPA, Dübendorf
10. Nair AS, Tom RT, Pradeep T (2003) Detection and extraction of endosulphan by metal nanoparticles. J Environ Monit 5:363–365. https://doi.org/10.1039/B300107E

11. Poursaberi T, Konoz E, Mohsen Sarrafi AH, Hassanisadi M, Hajifathli F (2012) Application of nanoscale zero-valent iron in the remediation of DDT from contaminated water. Chemical Science Transactions 1(3):658–668
12. Rahmani AR, Ghaffari HR, Samadi MT (2011) A comparative study on Arsenic (III) removal from aqueous solution using nano and micro sized zerovalent iron. Iran J Environ Health Sci Eng 8(2):175–180
13. Li X, Elliott DW, Zhang W (2006) Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects. Crit Rev Solid State Mater Sci 31:111–122. https://doi.org/10.1080/10408430601057611
14. Madhavi V, Prasad TNVKV, Madhavi G (2013) Synthesis and spectral characterization of iron based micro and nanoparticles. Int J Nanomater Biostruct 3(2):31–34 ISSN 2277-3851
15. Harikumar PS, Jesitha K, Sreechithra M (2013) Remediation of endosulphan by biotic and abiotic methods. J Environ Prot 4:418–425
16. Sun YP, Li XQ, Cao J, Zhang WX, Wang HP (2006) Characterization of zero-valent iron nanoparticles.Adv. Colloid Interface Sci. 120:47–56
17. Tratnyek PG, Johnson RL (2006) Nanotechnologies for environmental cleanup, Nanotoday 1(2):44–48
18. Vodyanitskii N, Mineev VG, Shoba SA (2014) Role of zero-valent iron in the degradation of organochlorine substances in ground water. Moscow Univ Soil Sci Bull 69(4):175–183
19. Shi Z, Fan B, Johnson RL, Tratnyek PG, Nurmi JT, Wu Y, Williams KH (2015) Methods for characterizing the fate and effects of nano zerovalent iron during groundwater remediation. J Contam Hydrol 181:17–35. https://doi.org/10.1016/j.jconhyd.2015.03.004
20. Noubactep Chicgoua (2012) Investigating the Processes of Contaminant Removal in Fe0/$H_2O$ Systems. Kor J Chem Eng 29:1050–1056
21. Agarwal A, Joshi H (2010) Application of nanotechnology in the remediation of contaminated groundwater: a short review. Recent Res Sci Technol 2(6):51–57
22. Aginhotri P, Mahidrakar AB, Gautam SK (2011) Complete dechlorination of endosulphan and lindane using $Mg^0$/Pd(+4) bimetallic system. Water Environ Res 83(9):865–873
23. Giasuddin ABM, Kanel SR, Choi H (2007) Adsorption of humic acid onto nanoscale zerovalent iron and its effect on arsenic removal. Environ Sci Technol *41*(6):2022–2027
24. Prabu D, Parthiban R (2013) Synthesis and characterization of nanoscale zero valent iron (NZVI). Nanoparticles for environmental remediation. Asian J Pharm Tech 3(4):181–184
25. Senila M, Levei E, Miclean M, Senila L, Steganescu L, Marginean S, Alexandru O, Cecilia R (2011) Influence of pollution level on heavy metals mobility in soil from NW Romania. Environ Eng Manag J 10:59–64. https://dx.doi.org/10.30638/eemj.2011.009
26. Zhuang X, Chen J, Shim H, Bai Z (2007) New advances in plant growth-promoting rhizobacteria for bioremediation. Int J Environ Sci Technol 12:353–366
27. Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865
28. Mellem JJ, Baijnath H, Odhav B (2009) Translocation and accumulation of Cr, Hg, As, Pb, Cu and Ni by Amaranthus dubius (Amaranthaceae) from contaminated sites. J Environ Sci Health A Tox Hazard Subst Environ Eng 44(6):568
29. 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
30. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyperaccumulation of metals in plants. Water Air Soil Pollut 184:105–126
31. Fitz WJ, Wenzel WW (2002) Arsenic transformation in the soil–rhizosphere– plant system: fundamentals and potential application of phytoremediation. J Biotechnol 99:259–278
32. Sinha RK, Herat S, Tandon PK (2004) Phytoremediation: role of plants in contaminated site management. In: Environmental bioremediation technologies. Springer, Berlin, pp 315–330
33. Smical AI, Hotea V, Oros V, Juhasz J, Pop E (2008) Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. Environ Eng Manag J 7(5):609–615

34. Mejare M, Bulow L (*2001*) Improving stress tolerance in plants by gene transfer. Trends Biotechnol 19(2):67–73 461
35. Memon AR, Schroder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Environ Sci Pollut Res Int 16(2):162–175. https://doi.org/10.1007/s11356-008-0079-z
36. Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Götz B, Kupper H (2009) Complexation and toxicity of copper in higher plants. II. different mechanisms for copper versus cadmium detoxification in the copper-sensitive cadmium/zinc hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype). Plant Physiol 151(2):715–731. https://doi.org/10.1104/pp.109.144675

# Chapter 19
# Nano-phytoremediation Application for Water Contamination

**Madhulika Bhati and Radhika Rai**

## 19.1 Introduction

Innovation keeps revolutionizing the community by bringing the customized solutions to different fields. Developing technologies at the cross section of two different areas, for instance, nanotechnology is one of the most promising technology applications to phytoremediation an older method and a part of bioremediation. Advanced nanomaterials like zeolites, nanofibers, carbon nanotubes, nanocatalysts, magnetic nanoparticles, and nanosponge-equipped nanofiltration systems can catalytically attack chemical contaminants and toxins to degrade them; magnetic nanoparticles can remove metals due to their interaction capabilities. Nanomaterial applications are not limited to purification; sensing elements like probes have increased sensitivity and selectivity by applying nanomaterial probes in water monitoring devices. Although these applications count for nanotechnology, green technology approach for sustainable development is also important. Nanotechnologies will create immense environmental benefits in terms of water management and treatment by convalescing filtering, decontamination, desalination, conservation, recycling, and sewerage systems and developing sensitive analytics or monitoring systems.

Phytoremediation includes the strategies adopted by plants to decontaminate soil, sludge, sediment, and wastewater. Some of the identified methods of this are phytoextraction; removal of heavy metal/toxic substances from soil/water; phytotransformation/breakdown of organic contaminants; phytovolatilization of contaminants taken up by tissue and then volatilized into the environment; rhizofiltration, a method of filtering water through mass of roots; phytostimulation, stimulating microbial degradation in roots or plant parts; phytostabilization in

---

M. Bhati (✉) · R. Rai
National Institute of Science, Technology and Development Studies [CSIR-NISTADS],
New Delhi, India
e-mail: madhulikabhati@nistads.res.in

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_19

which released compounds increase microbial activity in rhizosphere; and phytoscreening where plant acts like biosensors. Phytoremediation methods are inexpensive, can be applied in situ, and are solar driven.

LeDuc and Terry [1] proved increasing levels of toxic heavy metals and metalloids, such as cadmium, lead, mercury, arsenic, and selenium, into the environment. There is a need to develop low-cost material with improved efficiency and also effective, sustainable methods for contaminant removal or detoxification. For environment remediation in upland ecosystems, plants may be used to accumulate metals/metalloids in their harvestable biomass (phytoextraction). Plants can also convert and release certain metals/metalloids in a volatile form (phytovolatilization). They have mentioned how genetic engineering has been used to develop plants with enhanced efficiencies for phytoextraction and phytovolatilization.

Das [2] has used both of the technologies phytoremediation and nanoremediation application for the treatment of acid mine drainage water. A comparative analysis of these two methods shows that the phytoremediation and nanoremediation technologies are different but can be complementary, where phytoremediation is a method by which remediation of huge amount of metals is possible and it can be employed on a large-scale basis, eco-friendly, cost-effective but the proper selection of plants needed, limitations are, chances of animal consumption of the vegetative parts of the plant, remediation is slower. Whereas nanoremediation is a rapid, effective method due to small size and high surface area but limitations are high cost, chances of accumulation in living organisms, chances of causing eco-toxicity, large-scale implementation is not that feasible. So an interdisciplinary approach to bring these two methods together can be efficient enough to innovative solutions (Fig. 19.1).

The table above provides some nanomaterial sources available for nano-phytoremediation of water.

Therefore, both the technologies are used for water contamination. Developed and developing countries are facing contamination problem. The quantum of problem in India is explained in terms of type of specific contamination in different states of India and population affected. Nano-phytoremediation can provide an effective solution.

### 19.1.1 *Contamination Scenario in Indian Context*

It draws insight about the recent contamination scenario of water resources and challenges ahead. According to the contamination status of drinking water resources by Ministry of Drinking Water and Sanitation, Government of India, data provided by state departments dealing with rural drinking water supply updated on the online Integrated Management Information System (IMIS) of the Ministry, Government of India and reported in Rajya Sabha Parliamentary Answer in 2015. States affected by the type of water contamination are mentioned in the Table 19.1 and pictorially delineated by map of India (Fig. 19.2, Table 19.2).

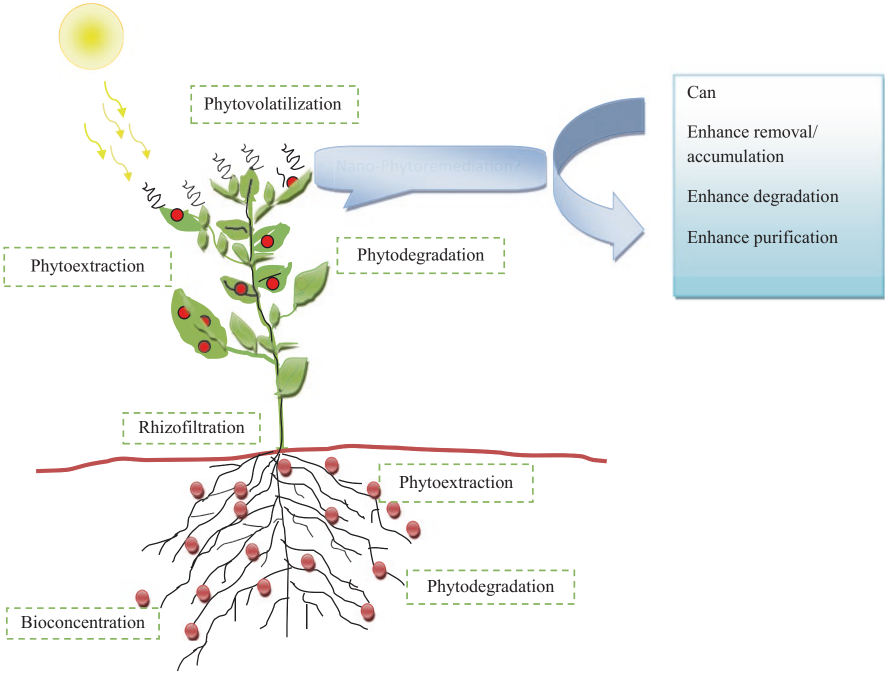


**Fig. 19.1** Phytoremediation methods. Source: Constructed by authors from various sources ([3, 4], and [5])

**Table 19.1** Phytoremediation methods

| | | | |
|---|---|---|---|
| Phytoextraction<br>•Extraction of dangerous elements or compounds by plants from water or soil, usually heavy metals or toxic to the health of living beings even at relatively low concentrations | Phytotransformation<br>• Transformation or breakdown of organic contaminants which are sequestered by plants via metabolic processes, for example, enzymes produced by plants | Phytovolatalization<br>• Contaminants are taken up by plants and changed into volatile substances | Rhizofiltration<br>• Filtration of water by root mass for toxic substances or nutrients |
| Phytostimulation<br>•Microbial degradation stimulation by the activities of plant in the root zone | Phytostabilization<br>•Reduction of mobility of heavy metals, for example, by reducing solubility or bioavailability | Phytoscreening<br>• Plants are used as sensors for contamination level indication on subsurface level and is a simple, fast, noninvasive, and inexpensive method | |

Source: Godheja et al. [6]

**Fig. 19.2** Pictorial representation of contamination status based on the Table 19.3. Source: Bhati and Rai [7]

Bibliometric indicators like publications and patent records using European patent database (Espacenet), World Intellectual Property Organization, Patentscope (WIPO), US Patent and Trademark Office, PatFT, (USPTO), Indian Patent Office, (IPO), and Web of Science have been analyzed to find out the existing S&T technology in this field that are already available, and their technical specification and their working methodology are explained in details.

**Table 19.2** List of plants used to make nanoparticles for contaminants removal

| S. No | Plant | Contaminant | Reference |
|---|---|---|---|
| 1. | *Eucalyptus globulus* | Chromium (Cr (VI)) | Madhavi et al. [8] |
| 2. | *Sapindus emarginatus* | Bacterial<br>*B. subtilis* and *S. aureus* | Swarnavalli et al. [9] |
| 3. | *Cupressus sempervirens* | Dyes | Ebrahiminezhad et al. [10] |
| 4. | *Jatropha curcas* L. | Chemical oxygen demand (COD) and chromium (Cr) | Goutam et al. [4] |
| 5. | *Eucalyptus* | Cr(VI), copper Cu(II), lead Pb(II), and zinc Zn(II) | Weng et al. [11] |
| 6. | *Azadirachta indica* | Antibacterial *E. coli* ATCC 25922 (fecal contamination) | Natarajan and Suuki [12] |

### *19.1.2 Potential of Nano-phytoremediation in Water Purification*

[13] described research in the area of fabrication of phytogenic magnetic nanoparticles (PMNPs) and their applications in the water/wastewater treatment (WWT) where they found that properties like dynamic morphology, desired size, super paramagnetic behavior, and high saturation magnetization value play an advantageous role in their application in water treatment sector. This method of green fabrication of PMNPs is clean, nontoxic, eco-friendly, fast, and cost-effective as compared to other physicochemical technologies. Commercialization aspects of this technology are in the field of wastewater treatment. Limitations of technology are still developing in the area of fabrication mechanism, regeneration, and reusability. This study also provides a model of PMNPs based on zero effluent discharge, and consequently, the WWT process is proposed and shown below in Fig. 19.3. This green technology is technically more feasible and economically sustainable. For this application and development of phytogenic magnetic nanoparticles, optimization of fabrication protocol solution parameters like extract volume, solvent type, pH, strength of precursor, physical parameters like temperature, etc. is required. Improvement in PMNPs' morphology and saturation magnetization will enhance stability of PMNPs after pollutant removal and also the yielding biomass separation. Areas like health impact and sustainability in long-term use are still under study. There is also a promising research option to develop noble PMNPs with wide range of functional groups by manipulating plant metabolites and fabrication protocol as recognized in the article (Fig. 19.4).

***Some other relevant publications described that*** continuous efforts are made for developing nanoparticles from various sources in one finding by [8]; they have synthesized zerovalent iron nanoparticles from *Eucalyptus globulus*, utilized for adsorption of hexavalent chromium (Cr (VI)) with high-order adsorption efficiency.

**Table 19.3** Contamination status in Indian states

| S. No | Contaminants | Affected states | No. of affected population (millions) | Range of contaminant (mg/L) | Health effects |
|---|---|---|---|---|---|
| 1. | Arsenic | West Bengal, Assam, Tripura, Punjab, Bihar, Arunachal Pradesh, Nagaland, Haryana, Manipur, Jharkhand, Uttar Pradesh, Odisha Karnataka, Jammu and Kashmir, Kerala, Maharashtra | 14.338 | 0.02–1.90 | Skin lesions, skin cancer, internal cancers, bladder, kidney, lung, neurological effects, hypertension and cardiovascular disease, pulmonary disease, peripheral vascular disease, and diabetes mellitus |
| 2. | Fluoride | Rajasthan, Telangana, Haryana, Jharkhand, Punjab, Bihar, West Bengal, Kerala, Karnataka, Andhra Pradesh, Assam, Jammu and Kashmir, Maharashtra, Uttar Pradesh, Chhattisgarh, Odisha, Madhya Pradesh, Gujarat | 10.635 | 1.51–12.45 | Chronic kidney disease (CKD)<br>Dental fluorosis and bone fractures/fragility |
| 3. | Iron | Tripura, Assam, West Bengal, Bihar, Jharkhand, Kerala, Nagaland, Odisha, Chhattisgarh, Punjab, Karnataka, Uttarakhand, Arunachal Pradesh, Meghalaya, Telangana, Tamil Nadu, Haryana, Maharashtra, Rajasthan, Madhya Pradesh, Jammu and Kashmir, Uttar Pradesh | 11.271 | 1.02–10.15 | Formation of polycyclic aromatic hydrocarbons PAH (low potency carcinogens) hemochromatosis |

(continued)

**Table 19.3** (continued)

| S. No | Contaminants | Affected states | No. of affected population (millions) | Range of contaminant (mg/L) | Health effects |
|---|---|---|---|---|---|
| 4. | Nitrate | Rajasthan, Telangana, Punjab, Gujarat, Karnataka, Kerala, Maharashtra, Uttarakhand, Jammu and Kashmir, Andhra Pradesh, Tamil Nadu, Jharkhand, Odisha, Bihar, Uttar Pradesh, West Bengal, Chhattisgarh | 2.06032 | 45.65–1227.00 | Methemoglobinemia, retarded bodily growth, and slower reflexes reproductive and developmental toxicity increased risk of cancers of the digestive tract, urinary bladder, and thyroid |
| 5. | Salinity | Rajasthan, Gujarat, Andhra Pradesh, Maharashtra, Uttar Pradesh, West Bengal, Odisha, Haryana, Punjab, Madhya Pradesh, Karnataka, Chhattisgarh, Tamil Nadu, Kerala, Telangana, Bihar | 4.193 | 521.00–7200.00 | Excess of hypertension in pregnancy for women living in coastal areas |
| 6. | Biological | West Bengal, Telangana, Maharashtra, Nagaland, Haryana, Kerala, Madhya Pradesh, Mizoram, Tripura, Rajasthan, Himachal Pradesh, Karnataka, Andhra Pradesh, Assam, Meghalaya, Gujarat, Jammu and Kashmir, Jharkhand, Chhattisgarh, Tamil Nadu, Bihar | 13.463 | – | Gastrointestinal diseases like diarrhea, cholera, and typhoid fever |

Source: Bhati and Rai [7]

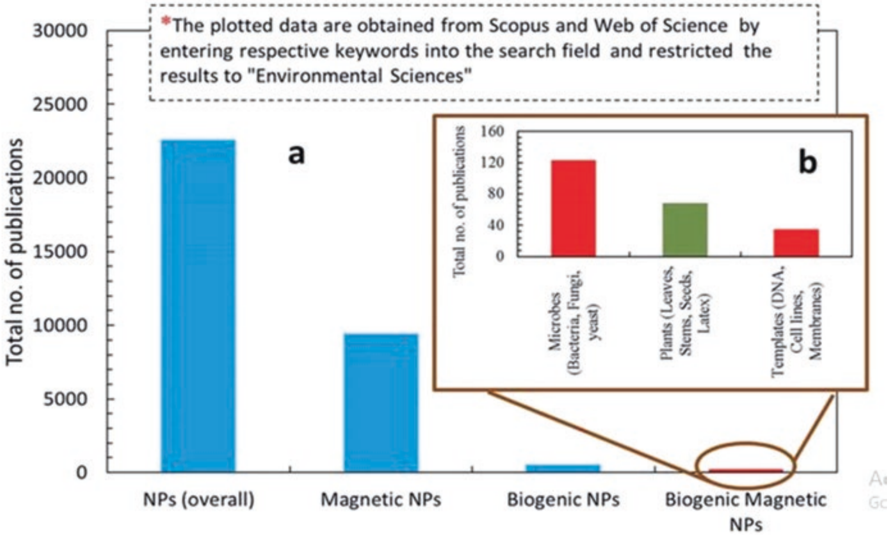


**Fig. 19.3** Publication related to phytogenic magnetic nanoparticle applications in wastewater treatment (WWT). Source: Ali et al. [13]

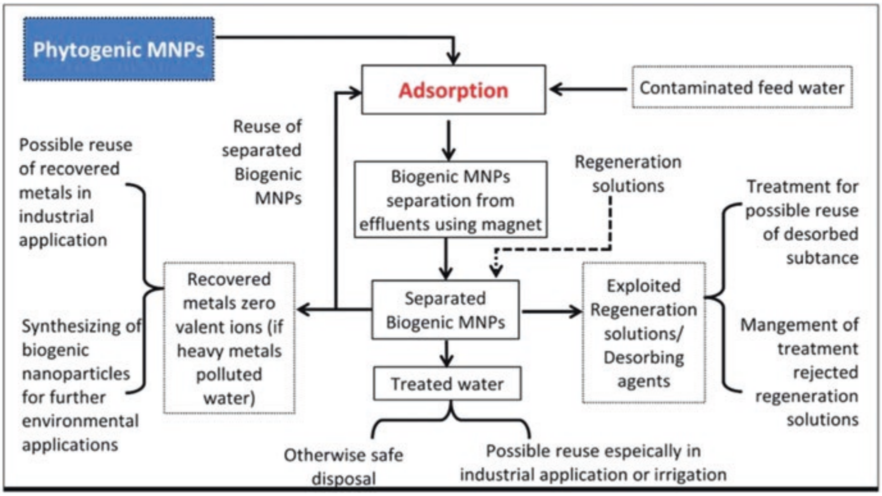


**Fig. 19.4** Schematic of proposed phytogenic magnetic nanoparticle (PMNP)-based zero effluent discharge water/wastewater treatment (WWT) process (using plant metabolites as reducing and capping agents). Source: Ali et al. [13]

Green synthesis of nanoclusters from Mediterranean cypress, *Cupressus sempervirens* with approximately 1.5 nm diameter nanoparticles for effective removal of dyes. These nanoclusters are of ultrasmall zerovalent iron nanoparticles [10].

Some researchers tried developing nanoparticles for photocatalytic degradation like green synthesis of $TiO_2$ nanoparticles using leaf extract of *Jatropha curcas* L. by [4] for remediation of chemical oxygen demand (COD) and chromium (Cr).

Polyurethane foam impregnated with biosynthesized silver nanoparticles made using *Azadirachta indica* for removal of *E. coli ATCC 25922* from water [12].

### *19.1.3 Patents Analysis*

Patents are the valuable asset in analyzing technology know-hows of a country. There are governmental and intergovernmental organizations managing the databases containing legal as well as technical information. Most of the countries have patent offices of their own. Databases of these offices provide a user-friendly method to search for the required patents. Keyword-based and class-based searches are the most favored ones. An international patent classification system is a hierarchical system of language-independent symbols for the classification of patents.

A database of nanotechnology publications was compiled from the different databases using a series of class-based and keyword-based searches. The class strings were derived by the author after going through related literature and in-depth study of the various patents. The string incorporated major cooperative classes like B82, Y02W, and C02F in combination to study patents in phytoremediation and water treatment sector whereas B82 stands for Nanotechnology, Y02W stands for climate change mitigation technology and C02F stands for waste water treatment/ management/ filtration/ purification techniques (Fig. 19.5).

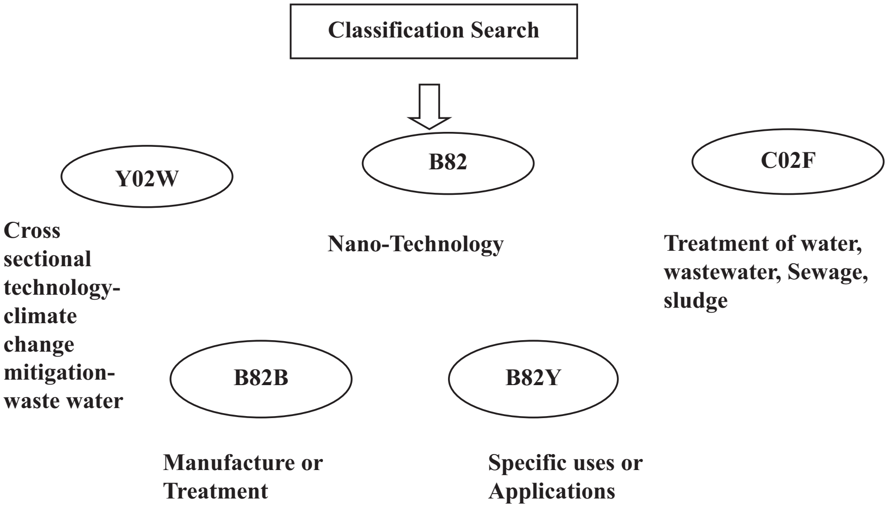


**Fig. 19.5** Pictorial representation of IPC and CPC classes which has been explored in this study. Source: Espacenet classification [14]

#### 19.1.3.1 Patented/Filed Technologies

Bio-rope strengthened phytoremediation system for purifying polluted river the invention discloses by [15] the system is applied for water pollution control methods. According to the system, bio-ropes are adopted to strengthen a phytoremediation technology, so as to form a plant-microorganism strengthened purification repair system. As described in the patent publication, where the upper part of the system is an emergent plant bed, the lower part of the system is a bio-rope fixed bed, and the emergent plant bed and the bio-rope fixed bed are connected by a framework and then are fixed in the river. For this, the emergent plant bed is planted with canna, *Iris tectorum*, calamus, *Cyperus alternifolius*, and the like and is fixed on the framework by adopting a vacuum-plastic water floating bed, for which the bio-rope fixed bed adopts a high-performance contact filtration material, namely, the bio-ropes, the upper and lower ends of each bio-rope are fixed on the framework, and an adsorption layer with the thickness of 2–4 cm is arranged at the bottom of the bio-rope fixed bed and consists of a nano-carbon microsphere layer, a nano-carbon tube layer, and an adsorption mesh from top to bottom. It can be applied in rivers so that catchment river network districts can be solved and pollutants, such as organic matters, nitrogen, and phosphorus, in sewage, are effectively removed (Fig. 19.6).

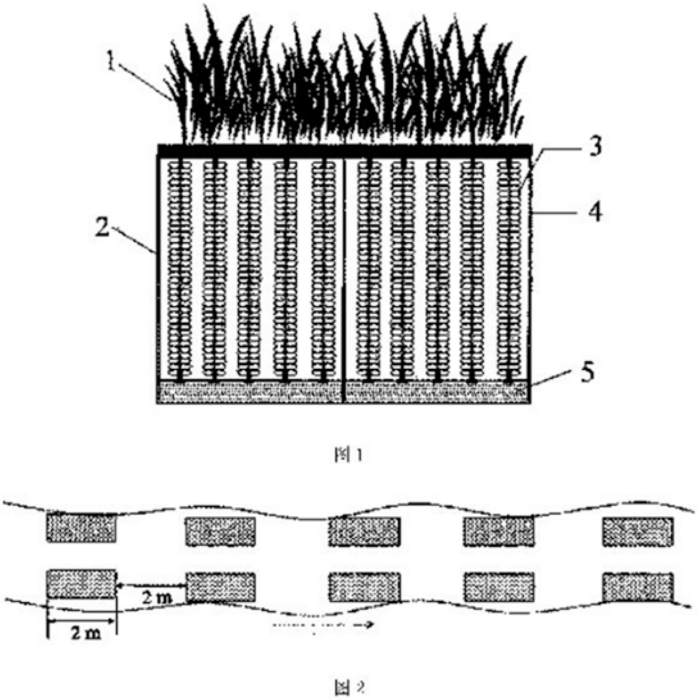


**Fig. 19.6** Bio-rope strengthened phytoremediation system is developed for phytoremediation of polluted river water, as shown in Fig. 19.6. Label 1 represents a water-breathing plant bed, Label 2 represents a biological rope fixed bed, Label 3 represents a biological rope, Label 4 represents a frame, and Label 5 represents an adsorption layer. Source: Zhang et al. [15]

A patent granted for nanoporous brick to Kong M and his team [5]. This patent describes the preparation method and application of nanoporous brick for purifying heavily polluted city watercourse. This brick has a pollutant adsorption capacity and can be used as a carrier for aquatic vegetation in water ecological restoration technology; meanwhile, nanopores of the nanoporous brick can enrich dissolved oxygen in water to promote healthy growth of the aquatic vegetation, and the water purification capacity is enhanced.

One more invention related to current research is filed by [16] in China that relates to an in situ treatment method of a shallow flow rejuvenated water riverway. According to the method, a roundstone bed shallow flow riverway is constructed, and artificial biological filler and *compound nanofiber filler* are arranged in a roundstone bed, so as to perform an in situ advanced treatment on river water which utilizes rejuvenated water as a water supplement source. According to the method, an in situ treatment technology is adopted, the river water is not needed to be guided out of a river water system, and the water body can be directly treated in the waterway, so that the sewage transportation link is omitted and secondary pollution can be prevented from being caused.

## 19.2 Results and Discussion

Nano-phytoremediation technologies were developed at the intersection of the two fields where the one is nanotechnology with advanced approaches and the other well established prior field of phytoremediation. This is an emerging area with the promising approach of sustainable development with improved efficiency of the phytoremediation prior practices and how innovation in current technologies can be brought with the reflection of prior methods to improve capabilities.

**Acknowledgment** Authors would like to thank the Water Technology Initiative (WTI), Department of Science and Technology (DST), Government of India, for their financial assistance for the research project DST/TM/WTI/2K15/135. The authors are grateful to the director of NISTADS for their support.

## References

1. LeDuc DL, Terry N (2005) Phytoremediation of toxic trace elements in soil and water. J Ind Microbiol Biotechnol 32(11-12):514–520 https://link.springer.com/article/10.1007/s10295-005-0227-0
2. Das PK (2018) Phytoremediation and nanoremediation: emerging techniques for treatment of acid mine drainage water. Def Life Sci J 3(2):190–196
3. Chandra R, Dubey NK, Kumar V (2017) Phytoremediation of environmental pollutants. CRC Press, Boca Raton, FL
4. Goutam SP, Saxena G, Singh V, Yadav AK, Bharagava RN, Thapa KB (2018) Green synthesis of TiO2 nanoparticles using leaf extract of Jatropha curcas L. for photocatalytic degradation of tannery wastewater. Chem Eng J 336:386–396

5. Kong M, Zhang Y, Peng F, Wu D, Yang F, Chao J. Nanjing Inst Environmental Sciences Ministry Environment Prot CN104860399. CN. 2015 Aug 26. https://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20150826&CC=CN&NR=104860399A&KC=A
6. Godheja J, Shekhar SK, Siddiqui SA, Modi DR (2016) Xenobiotic compounds present in soil and water: a review on remediation strategies. J Environ Anal Toxicol 6(392):2161–0525 https://www.omicsonline.org/open-access/xenobiotic-compounds-present-in-soil-and-water-a-review-onremediation-strategies-2161-0525-1000392.php?aid=77909
7. Bhati M, Rai R (2017) Nanotechnology and water purification: Indian know-how and challenges. Environ Sci Pollut Res 24(30):23423–23435
8. Madhavi V, Prasad TN, Reddy AV, Reddy BR, Madhavi G (2013) Application of phytogenic zerovalent iron nanoparticles in the adsorption of hexavalent chromium. Spectrochim Acta A Mol Biomol Spectrosc 116:17–25
9. Swarnavalli GC, Dinakaran S, Raman N, Jegadeesh R, Pereira C (2017) Bio inspired synthesis of monodispersed silver nano particles using Sapindus emarginatus pericarp extract–Study of antibacterial efficacy. J Saudi Chem Soc 21(2):172–179
10. Ebrahiminezhad A, Taghizadeh S, Ghasemi Y, Berenjian A (2018) Green synthesized nanoclusters of ultra-small zero valent iron nanoparticles as a novel dye removing material. Sci Total Environ 621:1527
11. Weng X, Jin X, Lin J, Naidu R, Chen Z (2016) Removal of mixed contaminants Cr (VI) and Cu (II) by green synthesized iron based nanoparticles. Ecol Eng 97:32–39
12. Natarajan E, Suuki S (2013) Removal of E. coli ATCC 25922 (indicator of fecal contamination) from drinking water by biosynthesized silver nanoparticles impregnated polyurethane foam. In: 2013 International Conference on Advanced Nanomaterials and Emerging Engineering Technologies (ICANMEET), Jul 24, 2013. IEEE, New York, NY, pp 252–255
13. Ali I, Peng C, Naz I, Khan ZM, Sultan M, Islam T, Abbasi IA (2017) Phytogenic magnetic nanoparticles for wastewater treatment: a review. RSC Adv 7(64):40158–40178 http://pubs.rsc.org/en/content/articlepdf/2017/ra/c7ra04738j
14. Espacenet Classification https://worldwide.espacenet.com/help?locale=en_EP&method=handleHelpTopic&topic=classificationsearch
15. Zhang R, Qian X, Zhu W, Gao H, Wang J, Liu Z. Univ Nanjing CN103819006 (A) 2014 May 28. https://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20140528&CC=CN&NR=103819006A&KC=A
16. Cheng S, Zhong F, Wu J, Xiang D, Yan Y, Zhou Qi. Univ Tongji CN102966071 (A) – CN 2013. https://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20130313&DB=&locale=&CC=CN&NR=102966071A&KC=A&ND=4

# Chapter 20
# Phytoremediation as a Cleansing Tool for Nanoparticles and Pharmaceutical Wastes Toxicity

**Fares K. Khalifa and Maha I. Alkhalf**

## 20.1 General Scope of Phytoremediation

Phytoremediation is described as a natural process carried out by plants and trees in the cleaning up and stabilization of contaminated soils and groundwater. It is actually a generic term for several ways in which plants can be used for these purposes. It is characterized by the use of vegetative species for in situ treatment of land areas polluted by a variety of hazardous substances [1].

Garbisu [2] defined phytoremediation as an emerging cost-effective, nonintrusive, aesthetically pleasing, and low-cost technology using the remarkable ability of plants to metabolize various elements and compounds from the environment in their tissues. Phytoremediation technology is applicable to a broad range of contaminants, including metals and radionuclides, as well as organic compounds like chlorinated solvents, polychloribiphenyls, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives, and surfactants.

According to Macek [3] phytoremediation is the direct use of green plants to degrade, contain, or render harmless various environmental contaminants, including recalcitrant organic compounds or heavy metals. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas.

---

F. K. Khalifa (✉)
Biochemistry Department, Science Faculty, Alsolimania, King Abdulaziz University,
Jeddah, Saudi Arabia

Biochemistry and Nutrition Department, Womens College, Ain Shams University,
Cairo, Egypt
e-mail: Khalifa-fkkhalifa@kau.edu.sa

M. I. Alkhalf
Applied Biochemistry Department, Science Faculty, University of Jeddah, Jeddah, Saudi Arabia

Biochemistry Department, Science Faculty, King Abdulaziz University, Jeddah, Saudi Arabia

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6_20

## 20.2 Mechanisms of Phytoremediation

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil and groundwater. There are several different types of phytoremediation mechanisms [4]. These are:

### *20.2.1 Rhizosphere Biodegradation*

In this process, the plant releases natural substances through its roots, supplying nutrients to microorganisms in the soil. The microorganisms enhance biological degradation.

### *20.2.2 Phyto-stabilization*

In this process, chemical compounds produced by the plant immobilize contaminants rather than degrade them.

### *20.2.3 Phyto-accumulation*

In this process, plant roots absorb the contaminants along with other nutrients and water. The contaminant mass is not destroyed but ends up in the plant shoots and leaves. This method is used primarily for wastes containing metals. At one demonstration site, water-soluble metals are taken up by plant species selected for their ability to take up large quantities of lead (Pb). The metals are stored in the plant's aerial shots, which are harvested and either smelted for potential metal recycling/recovery or are disposed of as a hazardous waste. As a general rule, readily bioavailable metals for plant uptake include cadmium, nickel, zinc, arsenic, selenium, and copper [9].

Moderately bioavailable metals are cobalt, manganese, and iron. Lead, chromium, and uranium are not very bioavailable. Lead can be made much more bioavailable by the addition of chelating agents to soils. Similarly, the availability of uranium and radio-cesium 137 can be enhanced using citric acid and ammonium nitrate, respectively.

### *20.2.4 Rhizofiltration*

Rhizofiltration is similar to phyto-accumulation, but the plants used for cleanup are raised in greenhouses with their roots in water. This system can be used for ex situ groundwater treatment. That is, groundwater is pumped to the surface to irrigate these plants. Typically hydroponic systems utilize an artificial soil medium, such as sand mixed with perlite or vermiculite. As the roots become saturated with contaminants, they are harvested and disposed of.

### *20.2.5 Phyto-volatilization*

In this process, plants take up water containing organic contaminants and release the contaminants into the air through their leaves.

### *20.2.6 Phytoextraction*

Uptake and concentration of substances from the environment into the plant biomass

### *20.2.7 Phyto-degradation*

In this process, plants actually metabolize and destroy contaminants within plant tissues.

### *20.2.8 Hydraulic Control*

In this process, trees indirectly remediate by controlling groundwater trees that act as natural pumps when their roots reach down toward the water table and establish a dense root mass that takes up large quantities of water. A poplar tree, for example, pulls out of the ground 30 gallons of water per day and a cottonwood can absorb up to 350 gallons per day.

## 20.3 Techniques for Phytoremediation

Many techniques and applications are represented under phytoremediation. They differ in the way plants deal with contaminants (removal, immobilization, degradation), as well as in the type of contaminant that the plant species can target (organic or inorganic contaminant) [2].

Research efforts into remediation can be roughly categorized into two sets: exploration of mechanisms and evaluation of claims. Mechanism work has centered on finding theoretical limits and explanations for results observed in the field. Pilot-scale fieldwork has both preceded and followed explanatory laboratory research, and early successes have piqued interest. Long-term, objective field evaluation is critical to understanding how well phytoremediation may work, what the real cost of application will be, and how to build models to predict the interaction between plants and contaminants. Most of the projects are ongoing and thus provide only preliminary data [5].

## 20.4 Using Plants as Phytoremediators

The principal application of phytoremediation is for lightly contaminated soils and waters where the material to be treated is at a shallow or medium depth and the area to be treated is large. This will make agronomic techniques economical and applicable for both planting and harvesting. In addition, the site owner must be prepared to accept a longer remediation period. Plants that are able to decontaminate soils does one or more of the following: (1) plant uptake of contaminant from soil particles or soil liquid into their roots; (2) bind the contaminant into their root tissue, physically or chemically; and (3) transport the contaminant from their roots into growing shoots and prevent or inhibit the contaminant from leaching out of the soil [6].

Moreover, the plants should not only accumulate, degrade, or volatilize the contaminants but should also grow quickly in a range of different conditions and lend themselves to easy harvesting. If the plants are left to die in situ, the contaminants will return to the soil. So, for complete removal of contaminants from an area, the plants must be cut and disposed of elsewhere in a nonpolluting way. Some examples of plants used in phytoremediation practices are the following: water hyacinths (*Eichornia crassipes*); poplar trees (*Populus* spp.); forage kochia (*Kochia* spp.); alfalfa (*Medicago sativa*); Kentucky bluegrass (*Poa pratensis*); *Scirpus* spp., coontail (*Ceratophyllum demersum* L.); American pondweed (*Potamogeton nodosus*); and the emergent common arrowhead (*Sagittaria latifolia*) among others [7].

### *20.4.1 Grasses as Potential Phytoremediators*

#### 20.4.1.1 Vetiver Grass (*Vetiveria zizanioides* L.)

Vetiver (*Vetiveria zizanioides* L.) belongs to the same grass family as maize, sorghum, sugarcane, and lemon grass. It has several unique characteristic as reported by the National Research Council. Vetiver grass is a perennial grass growing 2 m high and 3 m deep in the ground. It has a strong dense and vertical root system. It grows both in hydrophilic and xerophytic conditions [8]. The leaves sprout from the bottom of the clumps, and each blade is narrow, long, and coarse. The leaf is 45–100 cm long and 6–12 cm wide. Vetiver grass is highly suitable for phytoremedial application due to its extraordinary features. These include a massive and deep root system, tolerance to extreme climatic variations such as prolonged drought, flood, submergence, fire, frost, and heat waves. It is also tolerant to a wide range of soil acidity, alkalinity, salinity, elevated levels of Al, Mn, and heavy metals such as As, Cr, Ni, Pb, Zn, Hg, Se, and Cu in soils [9].

Various uses of vetiver grass are known worldwide. In South Africa, it was used effectively to stabilize waste and slime dams from Pt and Au mines. In Australia, vetiver grass was used to stabilize landfill and industrial waste sites contaminated with heavy metals such as As, Cd, Cr, Ni, Cu, Pb, and Hg. In China, vetiver grass was planted in large scale for pollution control and mine tail stabilization. In Thailand, vetiver grass is found widely distributed naturally in all parts of the country. It has been used for erosion control and slope stabilization. Vetiver hedges had an important role in the process of captivity and decontamination of pesticides, preventing them from contaminating and accumulating in crops [9].

#### 20.4.1.2 Carabao Grass (*Paspalum conjugatum* L.)

Carabao grass is a vigorous, creeping perennial grass with long stolons and rooting at nodes. Its culms can ascend to about 40–100 cm tall, branching, solid, and slightly compressed where new shoots can develop at every rooted node. Under a coconut plantation, a yield of about 19,000 kg $ha^{-1}$ of green materials was obtained. It grows from near sea level up to 1700 m altitude in open to moderately shaded places. It is adapted to humid climates and found growing gregariously under plantation crops and also along stream banks, roadsides, and in disturbed areas. This grass can adapt easily to a wide range of soils [10].

## 20.5 Plant Uptake of Organic Pollutants

Plants generally absorb organic pollutants from soil water through their root system and transport into aboveground biomass. Plants including ryegrass, rice, zucchini, maize, cauliflower, poplar radish, pumpkin, and tobacco and nightshade plants can absorb and accumulate organic pollutants in their different organs. Organic pollutants uptake from soil and accumulation in aboveground plant biomass used for feeding fowl and livestock consequently increase the possible exposure of pollutants to humans [11].

## 20.6 Time Scale of Cleanup

Degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase may become the rate-determining step. Therefore, phytoremediation may require more time to achieve cleanup standards than other more costly alternatives such as excavation or ex situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles. In many cases, phytoremediation may serve as a final "polishing step" to close sites after more aggressive cleanup technologies have been used to treat the hot spots [12].

## 20.7 Considerations of Using Phytoremediation in Nanoparticles and Pharmaceutical Wastes Toxicity

### *20.7.1 Technical Considerations*

Several key factors to consider when evaluating whether phytoremediation is a potential site remedy are described below [7, 9].

1. Determine whether evidence of the potential effectiveness of phytoremediation is specific to the site matrix and contaminants. If laboratory studies on the plants and contaminants of interest are the primary evidence used to support the use of phytoremediation at the site, the studies should at least show that the plants to be used at the site are capable of remediating site contaminants.
2. Consider the protectiveness of the remedy during the time it takes the plants associated with phytoremediation to establish themselves at the site to a point where they are containing/degrading the contaminants of interest.
3. Consider whether phytoremediation is likely to clean up the site in an acceptable time frame.
4. An adequate backup or contingency technology should be identified in the event that phytoremediation is attempted and does not succeed.

### *20.7.2 Economic Considerations*

Because phytoremediation is an emerging technology, standard cost information is not readily available. Subsequently, the ability to develop cost comparisons and to estimate project costs will need to be determined on a site-specific basis. Two considerations influence the economics of phytoremediation: the potential for application and the cost comparison to conventional treatments

## 20.8 Application of Phytoremediation in the Uptake of Silver Nanoparticles (AgNPs)

Nanotechnology has grown very quickly in the past few years, and manufactured nanoparticles (NPs) have been broadly used in medicine, health care, biomedical products, pharmaceuticals, children toys, washing machine coatings, electronic devices, engineering materials, wall paints, water purification, textiles, personal care products, renewable energies, transportation, agriculture (fertilizers and plant protection products), fishing, environmental remediation, kitchenware, manufacturing, cosmetics, wound dressing, and food packaging [13].

Silver nanoparticles (AgNPs) are one of the most used NPs with production volumes of 500 tons per year. These NPs can end up in the environment directly, through sewage discharges (released from household and industrial products into wastewaters), runoff, and aerial deposition, and indirectly through organic fertilizers and plant production products (deposition into soils and water), atmospheric emissions, and accidental spills during the manufacturing and transport [14].

The toxicity of AgNPs and their implications in the environment have been intensively studied since these NPs are, among all, the most likely to produce an effect in ecosystems, having a cumulative impact in the environment and in human health [15]. Actually, AgNPs are classified as extremely toxic according to the L(E) C50 values (the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals) for environmentally relevant organisms (L(E)C50 b 0.1 mg $L^{-1}$) and predicted environmental concentrations (PECs) for AgNPs in the environment range between 0.03 and 0.08 mg $L^{-1}$. In addition, a strong antibacterial potential of AgNPs has been reported by several authors [16].

There is a sort of advantages in the application of phytoremediation such as low cost, improvement of soil and water quality, high rhizosphere activity stimulation, and negligible site disruption. However, metal phytoremediation can be negatively affected if the metal takes the NP form [17].

Nanoparticles can suffer several physical-chemical and colloidal transformations being the most important aggregation and disaggregation, sorption onto natural organic matter, and dissolution [18]. These transformations can influence the bioavailability, uptake, accumulation, and stability of metallic NPs, as well as their toxicological effects. In fact, NPs toxicity to plants and associated microorganisms can represent an obstacle to an effective remediation. Rhizosphere microorganisms can be key players in plants phytoremediation potential. But, bacterial communities are sensitive to external pollutants, and shifts in the community composition and productivity can be compromised, affecting the functioning of ecosystems and ecosystem services, since microbial communities have a key role in decomposition and mineralization, inorganic nutrient cycling, disease causation and suppression, and pollutant removal [19].

## 20.9 Application of Phytoremediation in Industrial and Pharmaceutical Pollutants

Various pollutants are released into the environment every year, among which pharmaceuticals are becoming a cause of concern. There are many sources of these pharmaceuticals, such as (1) A surplus of human medicines, like antibiotics, statins, analgesics or cytotoxins used in cancer treatment, are produced and used in range of thousands of tons per year. After administration, they are absorbed and metabolized, after which some amount of chemicals may be excreted into the sewage system [5].

Metabolism of these drugs introduces hydrophilic functionalities into the pharmaceutical molecules that facilitate excretion in urine or feces. These compounds after entering into the wastewater treatment facilities are incompletely removed and can end up in the sewage sludge. Treated sewage sludge can be classified as a biosolid that can be applied to land as a rich source of nutrients for plants. This land application of biosolids further raises concerns about the environmental release of pharmaceuticals, which may be present in the biosolid [20].

Antibiotics form the third largest group among all the pharmaceuticals in the human medicine. Mostly prescribed antibiotics are tetracyclines, β-lactams, and macrolides; (2) large amounts of veterinary medicines, like antibacterials, antifungals, and parasiticides, may also contribute to the stress on the environment by being released from aquaculture and agriculture. They are secreted to soils or surface waters by pasture animals. They can also indirectly enter the environment through the application of slurry and manure as fertilizers [11].

Antibiotics for the treatment of fish or shrimp in aquaculture directly find their way into surface waters. In this category, 70% of the medicines are antibiotic agents. Tetracyclines, sulfonamides, aminoglycosides, β-lactams, and macrolides are majorly used in veterinary medicines [21]; (3) during the production of pharmaceuticals, the residues could be released into the surface waters; (4) disposal of unused medicines and containers make up other minor routes of entry into the environment [22].

There are many routes through which pharmaceuticals can enter the environment, but wastewater treatment plants form a major source. Municipal wastewater treatment plants are designed to remove pharmaceuticals from the wastewater, but their residues remain in the output of these treatment plants. A conventional wastewater treatment plant generally employs two attenuation mechanisms: (1) sorption to particles and (2) biotransformation [23]. Some extensively use activated sludge processes, reverse osmosis, advanced oxidative processes, activated carbon adsorption, membrane filtration, and membrane bioreactors. Excavation, photolysis, incineration, and pump-and-treat systems are some of the established methods of remediation [24].

Plants required for phytoremediation have general properties such as fast growth rate, high biomass yielding capacity, large and dense root systems, high levels of degrading enzymes, and ability to accumulate large amounts of pollutants in harvestable tissues and are hardy, competitive, and tolerant to pollution. Phytoremediation has gained popularity in the past 10 years, among government and industries due to advantages like relatively low cost, easy implementation, ability to conjugate with other methods, and in situ processing [25].

## 20.10 Phytoremediation of Heavy Metals, Antibiotics, Hormones, and Analgesics

Increased levels of heavy-metal contaminants above nutritional needs can cause extreme imbalance in the ecological system. Moreover, they also cause toxic effects on plants, microbes, and humans like neurotoxicity, carcinogenicity, inhibition of plant growth, biochemical and physiological changes, alterations in structures, and microbial toxicity via food chain, soil, or water sources. On the other hand, antibiotics, hormonal waste, and analgesics constitute the pharmaceutical waste which gets exposed in the environment in the form of improperly disposed medicines, dumping of unused or expired tablets along with domestic waste, and unmonitored dosage of pills without proper prescription. It poses health issues through entry into food chains via plants as well as uptake through consumption of contaminated water [26].

### *20.10.1 Heavy Metals: Associated Health Concerns and Toxic Effects*

Heavy metals are a class of toxic metals which are basically transition metals. Heavy metals are not named so on the basis of their molecular weights but rather on the basis of their specific density which is more than 5 g $cm^{-3}$ [27]. Some of the heavy metals are transition species that cause oxidative stresses to the plant and lead to the production of ROS, i.e., reactive-oxygen species. ROS form free radicals that hamper with the protein structures and cause peroxidation of lipids which can lead to alteration in genetic constitution of an organism leading to severe malfunctioning. For example, cadmium toxicity leads to the dysfunctioning of the kidney; likewise nickel can cause allergic reactions [28].

### *20.10.2 Pharmaceutical Wastes*

Most commonly found pharmaceutical wastes like antibiotics, hormones, steroidal medicines, analgesics, and therapeutic agents are an outcome of the improper disposal of pharmaceuticals that are being used to treat humans and animals. These contaminants usually come through irrigation, sewage wastes, and throwing of expired and unused medicine into domestic drains. Eventually they enter into the groundwater and become a part of the food chain [29].

### *20.10.3 Antibiotic Waste*

Certain antibiotics which remain in wastewater even after treatment are tetracycline, oxytetracycline, ibuprofen, ciprofloxacin, and norfloxacin. Technology for their cleanup from the environment has to be explored, and certain plants have been reported to perform remediation for it such as *Lupinus albus* is found to remediate acetaminophen under hydroponic arrangements [30].

### *20.10.4 Hormonal Waste*

It has been reported that wastewater from municipal wastes and urinary waste of humans are the sources of hormones especially steroids in them. These hormones are basically used as contraceptives and therapies. With excreta and household drains, animal waste, they become a part of hormonal waste in contaminated water streams. They are known to dismantle endocrine systems in aquatic organisms such as fishes [31]. The most novel way of separation of these contaminants from water resources are ELISA and gas chromatography (GC)/tandem mass spectrometry (MS) [22]. Only very few literatures suggest the role of plants in the removal of hormone contaminants from wastewater. Plants called as *Salix exigua* and *Arabidopsis thaliana* have been reported to extract synthetic estrogen and other derivatives from the solutions, thus indicative of phytoremediation properties in them [32, 33].

### *20.10.5 Improper Disposal of Analgesics*

Analgesics or painkillers are basically NSAIDs, i.e., nonsteroidal anti-inflammatory drugs or paracetamol. These bioactive compounds and their metabolic products become a part of wastewater streams and river waters even after treatment processes. The unused day-to-day medicines such as aspirin, paracetamol, etc. are disposed off just as domestic waste which causes undesirable effects such as renal toxicity, hepatotoxicity, etc., when they enter into the water table and food chains. Some plants perform remediation for their removal from the environment such as *Phragmites australis* is known for the removal of ibuprofen contamination [34].

## References

1. Ho YN, Shih CH, Hsiao SC, Huang CC (2009) A novel endophytic bacterium, Achromobacter xylosoxidans, helps plants against pollutant stress and improves phytoremediation. J Biosci Bioeng 108:S94

2. Garbisu C, Allica JH, Barrutia O, Alkorta I, Becerril JM (2002) Phytoremediation: a technology using green plants to remove contaminants from polluted areas. Rev Environ Health 17(3):173–188
3. Macek T, Francova K, Kochánková L, Lovecká P, Ryslava E, Rezek J, Sura M, Triska J, Demnerova K, Mackova M (2004) Phytoremediation—biological cleaning of a polluted environment. Rev Environ Health 19(1):63–82
4. Cortez PC (2005) Assessment and phytoremediation of heavy metals in the Panlasian creek. An unpublished high school thesis, University Science High School, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
5. Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254
6. Lee WY, Iannucci-Berger WA, Eitzer BD, White JC, Mattina MI (2003) Plant uptake and translocation of air-borne chlordane and comparison with the soil-to-plant route. Chemosphere 53(2):111–121
7. Pereira RC, Camps-Arbestain M, Garrido BR, Macías F, Monterroso C (2006) Behaviour of α-, β-, γ-, and δ-hexachlorocyclohexane in the soil–plant system of a contaminated site. Environ Pollut 144(1):210–217
8. Inui H, Wakai T, Gion K, Kim YS, Eun H (2008) Differential uptake for dioxin-like compounds by zucchini subspecies. Chemosphere 73(10):1602–1607
9. Havelcová M, Melegy A, Rapant S (2014) Geochemical distribution of polycyclic aromatic hydrocarbons in soils and sediments of El-Tabbin, Egypt. Chemosphere 95:63–74
10. 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(3):455–465
11. Zhu X, Ni X, Liu J, Gao Y (2014) Application of endophytic bacteria to reduce persistent organic pollutants contamination in plants. Clean Soil Air Water 42(3):306–310
12. Andria V, Reichenauer TG, Sessitsch A (2009) Expression of alkane monooxygenase (alkB) genes by plant-associated bacteria in the rhizosphere and endosphere of Italian ryegrass (Lolium multiflorum L.) grown in diesel contaminated soil. Environ Pollut 157(12):3347–3350
13. Gambardella C, Costa E, Piazza V, Fabbrocini A, Magi E, Faimali M, Garaventa F (2015) Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar Environ Res 111:41–49
14. Nam DH, Lee BC, Eom IC, Kim P, Yeo MK (2014) Uptake and bioaccumulation of titanium- and silver-nanoparticles in aquatic ecosystems. Mol Cell Toxicol 10(1):9–17
15. Klitzke S, Metreveli G, Peters A, Schaumann GE, Lang F (2015) The fate of silver nanoparticles in soil solution—sorption of solutes and aggregation. Sci Total Environ 535:54–60
16. Metreveli G, Philippe A, Schaumann GE (2015) Disaggregation of silver nanoparticle homoaggregates in a river water matrix. Sci Total Environ 535:35–44
17. Andreotti F, Mucha AP, Caetano C, Rodrigues P, Gomes CR, Almeida CM (2015) Interactions between salt marsh plants and Cu nanoparticles–effects on metal uptake and phytoremediation processes. Ecotoxicol Environ Saf 120:303–309
18. Schaumann GE, Philippe A, Bundschuh M, Metreveli G, Klitzke S, Rakcheev D, Grün A, Kumahor SK, Kühn M, Baumann T, Lang F (2015) Understanding the fate and biological effects of Ag-and TiO 2-nanoparticles in the environment: the quest for advanced analytics and interdisciplinary concepts. Sci Total Environ 535:3–19
19. Bissett A, Brown MV, Siciliano SD, Thrall PH (2013) Microbial community responses to anthropogenically induced environmental change: towards a systems approach. Ecol Lett 16(s1):128–139
20. Deblonde T, Hartemann P (2013) Environmental impact of medical prescriptions: assessing the risks and hazards of persistence, bioaccumulation and toxicity of pharmaceuticals. Public Health 127(4):312–317
21. Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils—a review. J Plant Nutr Soil Sci 166(2):145–167

22. Herklotz PA, Gurung P, Heuvel BV, Kinney CA (2010) Uptake of human pharmaceuticals by plants grown under hydroponic conditions. Chemosphere 78(11):1416–1421
23. Sedlak DL, Pinkston KE (2011) Factors affecting the concentrations of pharmaceuticals released to the aquatic environment. J Contemp Water Res Educ 120(1):7
24. Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39
25. Onesios KM, Jim TY, Bouwer EJ (2009) Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 20(4):441–466
26. Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manage 92(3):407–418
27. Denton BP (2007) Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi. MMG 445 Basic Biotechnol J 3(1):1–5
28. Das KK, Das SN, Dhundasi SA (2008) Nickel, its adverse health effects & oxidative stress. Indian J Med Res 128(4):412
29. Iori V, Pietrini F, Zacchini M (2012) Assessment of ibuprofen tolerance and removal capability in Populus nigra L. by in vitro culture. J Hazard Mater 229:217–223
30. Kotyza J, Soudek P, Kafka Z, Vaněk T (2010) Phytoremediation of pharmaceuticals—preliminary study. Int J Phytoremediation 12(3):306–316
31. Cartinella JL, Cath TY, Flynn MT, Miller GC, Hunter KW, Childress AE (2006) Removal of natural steroid hormones from wastewater using membrane contactor processes. Environ Sci Technol 40(23):7381–7386
32. Khan S, Afzal M, Iqbal S, Mirza MS, Khan QM (2013) Inoculum pretreatment affects bacterial survival, activity and catabolic gene expression during phytoremediation of diesel contaminated soil. Chemosphere 91(5):663–668
33. Bound JP, Voulvoulis N (2005) Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environ Health Perspect 13(12):1705
34. Oliveira V, Gomes N, Almeida A, Silva A, Simões MM, Smalla K, Cunha Â (2014) Hydrocarbon contamination and plant species determine the phylogenetic and functional diversity of endophytic degrading bacteria. Mol Ecol 23(6):1392–1404

# Index

A. A. Ansari et al. (eds.), *Phytoremediation*,
https://doi.org/10.1007/978-3-319-99651-6