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

Phytoremediation

Management of Environmental Contaminants, Volume 5

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Preface

"Obscurity knows Nature will light the lamps"

Dahomean Proverb

The editors of *Phytoremediation: Management of Environmental Contaminants* originally planned a two-volume book to provide a broad global perspective on the development and use of phytoremediation to repair and restore contaminated terrestrial and aquatic habitats. The success and acceptance of Volumes 1 and 2 led to the production of three additional volumes that provide a wide diversity of phytoremediation laboratory studies and case histories completed in many parts of the world. Volume 5 contains the final chapter contributions in the series and adds new information on the application of soil microorganisms as inoculants or enhancement agents in contaminated terrestrial habitats including petroleum-contaminated sites. Other chapters describe the use of both woody and herbaceous plants for the biomonitoring and treatment of contaminants and provide new information on the trace element and toxic metals present in medicinal plants.

In the area of aquatic ecosystems, Volume 5 offers chapters that describe important new approaches to applying phytoremediation to increase the efficiency of aquaculture systems and the management of pharmaceutical and personal care products using constructed wetlands. Other chapters describe the general use of aquatic plants and floating wetlands to treat polluted water.

Several chapters in Volume 5 offer special applications of phytoremediation in terrestrial and aquatic habitats and include information on the genetic control of metal sequestration in hyperaccumulating plants, the use of engineered nanomaterials to remove metals/metalloids and their implications on plant physiology, applying plant biosorbents to extract metals from soils and water, and the phytomining of rare and valuable metals. Nutrient management strategies for coping with climate change in irrigated smallholder cropping systems and the phytoremediation of landfill leachates are covered in two chapters, and a chapter on the modeling of phytoremediation and another on the phytoremediation of contaminated air complete Volume 5.

The complete five-volume series of *Phytoremediation: Management of Environmental Contaminants* is designed to share a diversified sample of the current laboratory research and field applications of phytoremediation in a global context. As editors, we hope that the series will be both useful and informative to academics, government officials, and private sector managers and consultants interested in the potential for cost-effective and sustainable approaches to improving the environmental quality of terrestrial and aquatic ecosystems.

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Part I

Phytoremediation Using Soil Microorganisms

Chapter 1
Microbial Inoculants-Assisted Phytoremediation for Sustainable Soil Management

Elizabeth Temitope Alori and Oluyemisi Bolajoko Fawole

Abstract Agricultural soil pollution refers to its accumulation of heavy metals and related compounds which could be from natural or anthropogenic sources. This threatens food quality, food security, and environmental health. The traditional physico-chemical technologies soil washing used for soil remediation render the land useless as a medium for plant growth, as they remove all biological activities. Others are labor-intensive and have high maintenance cost. Phytoremediation, sustainable and cheaper in situ remediation techniques was therefore considered. However, plants do not have the capability to degrade many soil pollutants especially the organic pollutant. It is therefore imperative to take advantage of the degrading ability of soil microorganisms. This chapter therefore focuses on phytoremediation techniques augmented by microbial inoculants.

Keywords Inoculants • Microbes • Phytodegradation • Phytoremediation • Soil pollution • Soil management • Sustainable

1.1 Introduction

Pollution of agricultural soils refers to its accumulation of heavy metals and related compounds which could be from natural or anthropogenic sources. This threatens food quality, food security, and environmental health [1]. Soil pollution produces change in the diversity and abundance of biological soil populations [2]. This is critical because of the role of soil organisms in plant establishment and survival. Such elimination of soil organisms can lead to problems with plant establishment and survival. Crops raised on polluted soil may contain harmful levels of pollutants that can be passed on to the animals and human that eat them [3]. Inhaling dust

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blown from polluted soil can be injurious to one that inhales it. More also, polluted soil cannot be used for commercial development, parks or recreation [4]. Soil pollutants alter plant physiology. It can cause cell membrane disruption, damage to photosynthetic apparatus, and can also alter the physical and chemical properties of the soil where plants are growing [5].

Cleaning of polluted soil may be very difficult because both soil pollutants and soil minerals carry small electric charges that cause each to bond with each other. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized [6]. Traditionally, remediation of heavy metal-contaminated soils is either on-site management or excavation, and subsequent disposal to a landfill site [7]. However, this method of disposal merely shifts the contamination problem elsewhere. Soil washing for removing contaminated soil is an alternative to excavation and disposal to landfill. This method is however costly and produces a residue rich in heavy metals, which will require further treatment or burial. Moreover, these physico-chemical technologies used for soil remediation render the land useless as a medium for plant growth, as they remove all biological activities. Other technologies such as vitrification, leaching, electrokinetics soil vapor extraction, thermal desorption, chemical processing, etc., are labor-intensive and have high maintenance cost [8, 9]. It is therefore imperative to develop a sustainable on-site technique for remediation of heavy metal contaminated sites.

For better soil management, an increase in use of biological potential is important. Phytoremediation is one of the sustainable and cheaper in situ remediation techniques to be considered. Phytoremediation is a novel green technology that uses specialized plants and associated soil microbes to remove, destroy, sequester, or reduce the concentrations or toxic effects of contaminant in polluted soil and water [4]. The plant root-colonizing microbes or the plants themselves absorb, accumulate, translocate, sequester, and detoxify toxic compounds to non-toxic metabolites. Five important approaches can be considered in the use of plants to clean up polluted soil. (1) Phytostabilization, a process in which pollutants are immobilized by plant activity resulting in attenuation of the wind and soil erosion and runoff processes into the ground water or air. (2) Hydraulic control, plants act like a pump, draws the groundwater up through their roots to keep it from moving. This reduces the movement of contaminated groundwater toward clean areas off-site. (3) Phytovolatization involves use of plants to take up certain contaminants and then converts them into gaseous forms that vaporize into the atmosphere. (4) Phytofiltration refers torhizofiltration where contaminants such as metals are precipitated within the rhizosphere. (5) Phytoextraction (Phytoaccumulation) which involves metal hyperaccumulating plants which can contain more than 1% of metals in harvestable tissues [10, 11] (Fig. 1.1).

However, plants do not have the capability to degrade many soil pollutants. It is therefore imperative to take advantage of the degrading ability of soil organisms. Organic toxins containing carbon such as the hydrocarbons found in gasoline and other fuels can only be broken down by microbial processes [12]. Symbiotic root

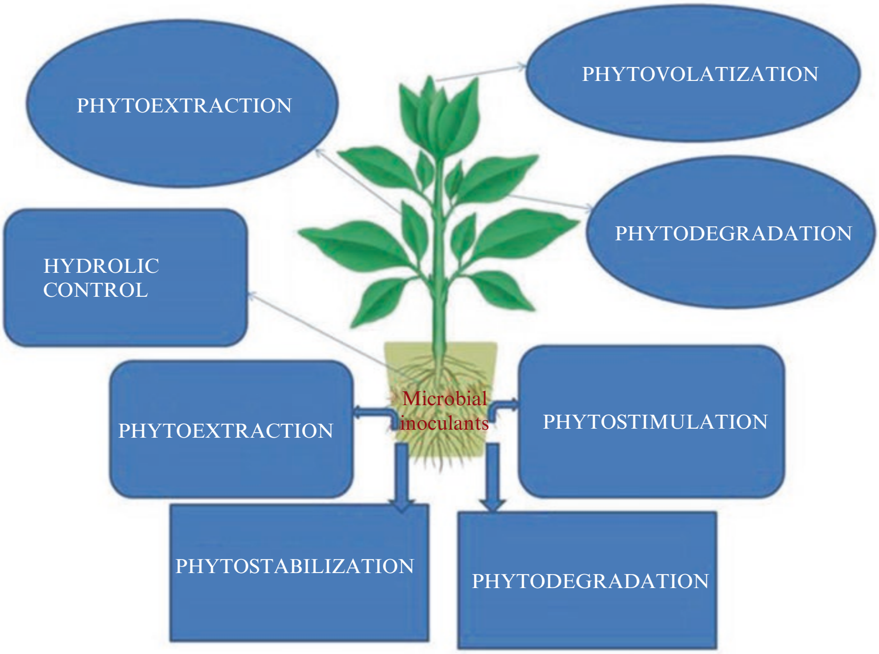

Fig. 1.1 Mechanisms of microbial-assisted phytoremediation

colonizing microorganism through metal sequestration increases metal tolerance in plants. The remediation by plant using the degrading ability of soil organisms is called phytodegradation. This helps us to understand integrated activity patterns between plants and microbes [13]. Some soil microbes such as the arbuscular mycorrhizal fungi (AMF) secret glycoprotein called glomalin. This can form complexes with metals. Microbial organisms within the rhizoplane can take part in phytoremediation by protecting the plants from the toxic effect of the contaminants while the plants in return provide the microbial processes the boost they need to remove organic pollution from the soil more quickly. Plants excrete organic materials that serve as food for microbes thus playing a key role in determining the size and health of soil microbial population. Bioaugmentation enables an increase of biodegradation of contaminated sites by the introduction of single strains or assemblages of microorganisms with the desired catalytic capabilities [14]. Microbial assemblages are found to be efficient since each partner can accomplish different parts of the catabolic degradation [15]. In this chapter, our focus is mainly on phytoremediation augmented by microbial inoculants. We begin with the contribution of plants and microbial inoculants in phytoremediation process. Then the methods of inoculating plants with microbial inoculants, the various mechanisms used by the microbial inoculants to assist plant in remediation, and the limitations of microbial inoculants-assisted phytoremediation are summarized and discussed.

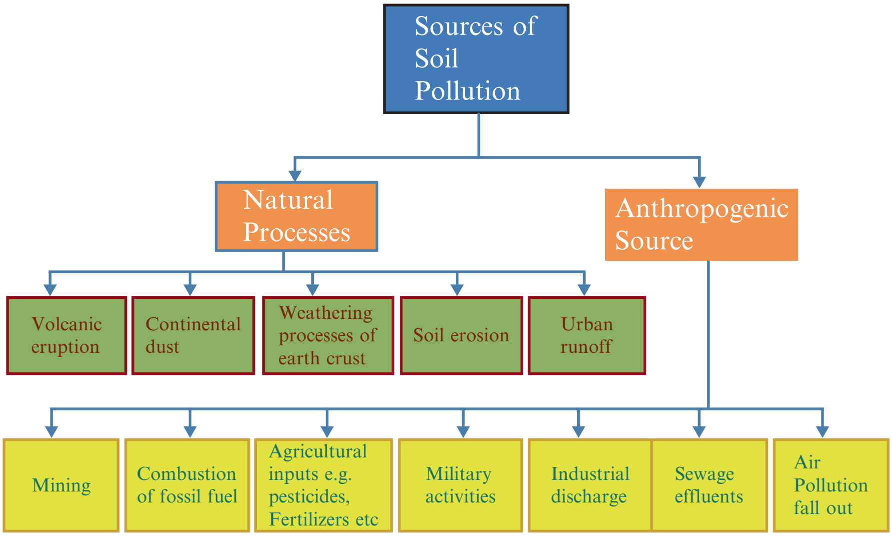

Fig. 1.2 Sources of soil pollutants

1.2 Sources of Soil Pollution

Soil pollutants get introduced to the soil from various sources ranging from natural (Lithogenic) to anthropogenic activities (Fig. 1.2). Heavy metals commonly get introduced via human activities that are related to energy and mineral consumption [5], while petroleum hydrocarbons usually come from accidental spills of petroleum-based products commonly used. Various industrial processes and anthropogenic activities in urban areas induce the release of metals and metalloids (MM) (toxic and genotoxic compounds) in natural environments.

Agricultural inputs such as chemical fertilizers, herbicides, and pesticides leaves the soil polluted with heavy metals [16]. According to Pietrzak and Uren [17], excessive use of fungicides and herbicides that are rich in heavy metal results in soil pollution. Copper for instance is used as a broad-spectrum bacterial and fungicidal agricultural pesticide and as fertilizer component because of its antimicrobial properties, but Cu is a common soil pollutant that persists in the soil providing a chronic, long-term stress on the soil microbial community [18]. Industrial activities such as chemical works, service stations, metal fabrication shops, paper mills, tanneries, textile plants, waste disposal sites, and intensive agriculture equally brings about the appearance of serious environmental problems such as soil pollution [19]. Indiscriminate waste disposal practices have led to significant build upon a wide range of metal(loid)s, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), selenium (Se), and zinc (Zn) in soils [20]. Kierczak et al. [21] found that soils in the areas around historic smelters are highly polluted with metal(loids)s (up to 4000 mg/kg Cu, 1500 mg/kg Zn, 300 mg/kg As, and 200 mg/kg Pb). Fossil fuel combustion is another source of soil pollution reported by Krgović et al. [22]. Vehicle emissions, industrial processes, or waste incineration plants were revealed to introduce some pollutant such as heavy to what should have been valuable soil [23]. Soil pollutants could originate from the mining and smelting of metal ores [24], runoff of urban soils, fertilizer application, or effluents discharged [25].

1.3 Contributions of Plants and Microbial Inoculants in Phytoremediation

Microbial-assisted phytoextraction optimizes the synergistic effect of plants and microorganisms and has been used for the cleaning-up of soils contaminated by metals [2].

Plant translocates and sequesters pollutions such as heavy metals while microbes degrade organic contaminants. Plants can store many contaminants in biomass that can later be harvested, while microbial assemblages can also convert contaminants such as heavy metals to stable and/or less toxic form. They can facilitate the uptake of pollutants such as heavy metals by plant roots. Microorganisms that reside on or within aerial plants tissue can help to stabilize and/or transform contaminants that have been translated which may limit the extent of volatization [13]. Plant root exudates such as enzymes, amino acids, aromatics, simple sugars, and aliphatics stimulate the growth of root-associated microorganisms; on the other hand, microbes can reduce the phytotoxicity of the contaminants in the soil or augments the capacity of the plant to degrade contaminant [3]. Ability of plant root to extend deeper into soil, allowing access to water and air and therefore changing the concentration of carbon dioxide, the pH, osmotic potential, redox potential, oxygen concentration, and moisture content of the soil, could lead to an environment that will better able to support high micro-biomass [26]. This enhanced trace element uptake by plants can be ascribed to an increase in root absorption ability and/or to an enhancement of trace metal bioavailability in the rhizosphere, mediated by microorganisms.

Plants can increase biodegradation through the transfer of oxygen to the rhizosphere and the release of soluble exudates that provide nutrient sources for microorganisms [27]. Thus, plants enhance microbial growth and hence the associated contaminant-degradation processes. Microorganism contribution in immobilizing elements or facilitating plant absorption plants may significantly contribute to MM removal through uptake in biomass [28]. Microbial assemblages improve plant health and growth, suppress disease-causing microbes, and increase nutrient availability and assimilation [29].

1.4 Methods of Inoculating Plants with Microbial Inoculants

Plants to be used as phytoremediator to clean polluted soils could be inoculated with microbial assemblages via quite a number of techniques. These methods could include: (1) Seedinoculation, (2) Soaking plant roots with microbial suspension, when the root of ryegrass was soaked with a suspension of an endophytic *Massilia* sp. (Pn2) the same was found to have been translocated to the plant shoots [30]. (3) Painting plant leaves with microbial suspension [31–33]. Afzal et al. [34] discovered the cells of *Burkholderiaphytofirmans*PsJN in the internal tissue of the shoot and root when the plant was inoculated via leaf painting. Root colonization strategy was found to be the optimal colonization method for circumventing the risk of plant organic contamination [32].

1.5 Types of Soil Pollutants

Soil pollutant could be organic or inorganic present in the hydrosoluble fraction (complexed, adsorbed onto particles or dissolved). The most common inorganic contaminants are heavy metals and mineral oils such as Cd, Cr, Pb, Cu, Hg, NiSe, As, and Zn [35]. Industrial effluents release organic pollutants like hydrocarbons, polycyclic aromatic hydrocarbons, and anionic detergent. Other soil pollutants include plant organic materials, petroleum hydrocarbons, and organochlorines [36]. Table 1.1 reveals some examples of soil pollutants that could be removed from soil via a microbial-assisted phytoremediation technique.

1.6 Mechanisms of Microbial Inoculants in Phytoremediation of Polluted Soil

Microbial inoculants can improve pollutant removal through various mechanisms. Some has the potential to produce metal chelating siderophores, which could improve metal bioavailability [37]. Moreover, they produce biosurfactants (rhamnolipids) that can enhance the solubility of poor water-soluble organic compounds and the mobility of heavy metals [38]. Formation of biofilm is another mechanism by which microbial inoculants assist plants in remediation of polluted soils [39]. In addition, these microbes can transform metals into bioavailable and soluble forms through the action of organic acids, biomethylation, and redox processes [39]. Diverse soil microbes have the ability to secrete plant hormones such as indole-3-acetic acid (IAA), cytokinins, gibberellins (GAs), and certain volatiles which promote plant growth by altering root architecture [16]. The microbial plant growth stimulatory actions result from the manipulation of the complex and balanced network of plant hormones that directly are responsible for growth and root formation. For example, IAA produced by soil microbes has been demonstrated to enhance

Table 1.1 Some examples of soil pollutants that could be removed from soil via microbial-assisted phytoremediation technique

Plant	Microorganism	Pollutants	References
Helianthus annus	*Micrococcus* sp. MU1 and *Klebsiella* sp. BAM1	Cd	Prapagdee et al. [50]
Polygonum pubescens	*Enterobacter* sp. JYX7 and *Klebsiella* sp. JYX10	Cd	Jing et al. [51]
Zea mays L	*Azotobacter chroococum* and *Rhizobium leguminosarum*	Pb	Hadi and Bano [52]
Solanum melongena	*Pseudomonas* sp.	NaCl	Fu et al. [53]
Vigna unguiculata	*Scutelospore reticulate, Glomus phaseous*	Al, Mn	Alori and Fawole [2]
Solanum nigrum	*Pseudomonas* sp. LK9	Cd	Chen et al. [54]
Brassica napus	*Pantoea agglomerans* Jp3–3, and *Pseudomonas thivervalensis* Y1–3-9	Cu	Zhang et al. [55]
Brassica juncea	*Paenibacillus macerans NBRFT5, Bacillus endophyticus NBRFT4, B. pumilus NBRFT9*	Cu	Tiwari et al. [56]
Loliummultiflorum Lam	*Staphylococcus* sp. strain BJ06	Pyrene	Sun et al. [57]
Brassica oxyrrhina	*Pseudomonas* sp. *SRI2, Psychrobacter* sp. SRS8 and *Bacillus* sp. *SN9*	Ni	Ma et al. [58]
Brassica napus	*Acinetobacter* sp. Q2BJ2 and *Bacillus* sp. Q2BG1	Pb	Zhang et al. [55]
Cytisus striatus	*Rhodococcus erythropolis* ET54b *Sphingomonas* sp. D4	Hexachlorocyclohexane (HCH)-	Becerra-Castro et al. [59]
Cichorium intybus	*Rhizophagus irregularis*	Diesel	Driai et al. [60]
Medicago sativa	*Pseudomonas aeruginosa*	(Cu, Pb and Zn and petroleum hydrocarbons)	Agnello et al. [35]

(continued)

Table 1.1 (continued)

Plant	Microorganism	Pollutants	References
Orychophragmus violaceus	*Bacillus subtilis, B. cereus, B. megaterium,* and *Pseudomonas aeruginosa*	Cd	Liang et al. [61]
Cytisusstriatus (Hill) Rothm	*Rhodococcus erythropolis* E T 54b and *Sphingomonas* sp. D4		Becerra-Castro et al. [62]
Arabidopsis thaliana	*Achromobacter xylosoxidans*	Phenolic	Ho et al. [63]
Solanum lycopersicum	*Penicillium janthinellum* LK5	Al	Khan et al. [64]
Brassica napus	*Rahnella* sp. JN6	Cd	He et al. [65]
Triticum aestivum	*Pseudomonas putida KT2440*	Cd, Hg, Ag	Yong et al. [66]
Brassica juncea	*Bacillus subtilis SJ-101*	Ni	Zaidi et al. [67]
Sedum plumbizincicola	*Bacillus pumilus* E2S2 and *Bacillus* sp. E1S2	Cd	Ma et al. [68]
Brassica napus	*Pseudomonas fluorescens G10* and *Microbacterium* sp. *G16*	Pb	Sheng et al. [69]
Trifolium repens	Arbuscular mycorrhizal fungi and *Bacillus cereus*	Heavy metals	Azcón et al. [70]
Iris pseudacorus	Arbuscular mycorrhiza fungi	Pb, Fe, Zn, and Cd	Wężowicz et al. [71]
Brassica juncea	*Rhizobium leguminozarum*	Zn	Adediran et al. [72]
Rahnella sp.	*Amaranthus hypochondriacus, A. Mangostanus* and *S. nigrum*	Cd	Yuan et al. [73]
Brassica juncea	*Staphylococcus arlettae* NBRIEAG-6	As	Srivastava et al. [74]
Orycoprhagmus violaceus	*Bacilus subtilis, B. cereus, Flavobacterium* sp. and *Pseudomonas aeroginosa* (Zhang et al. [55])	Zn	He et al. [75]
Lupinus luteus	*Burkholderia cepacia* VM1468	Ni and trichloroethylene (TCE)	Weyens et al. [76]
Alnus firma	*Bacillus thuringiensis* GDB-1	As	Babu et al. [77]

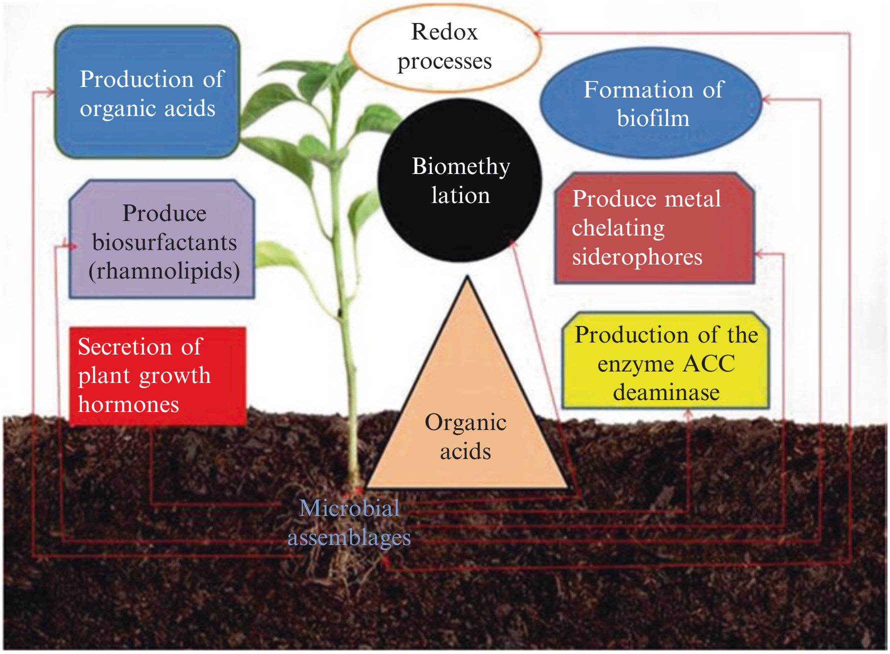

Fig. 1.3 Strategies of phytoremediation through microbial assemblages

root proliferation [40]. In addition, soil microbes possess growth- promoting traits, including phosphorus solubilization, nitrogen fixation, iron sequestration, and phytohormone, which improve plant growth and increase plant biomass [39].

In addition to degrading soil pollutants microbial assemblages, also partake in phytoremediation by producing hormones, fixing atmospheric nitrogen, or solubilizing P [41]. One of the most important mechanisms by which microbial assemblages respond to stress condition such as from soil pollutant is by increasing ethylene levels that result to an increase in cell and plant damage [42]. Many microbes that augment phytoremediation destroy a precursor of the ethylene (1-aminocyclopropane-1-carboxylate (ACC)) that by producing the enzyme ACC deaminase, that in turn facilitates plant growth and development by decreasing plant ethylene levels [39]. Figure 1.3 depicts strategies of phytoremediation through microbial assemblages.

1.7 Challenges of Microbial Inoculants-Assisted Phytoremediation

The success of microbial inoculation-assisted phytoremediation encounters some set back due to the following reasons: (1) The number of degrading microbes available regarding the pollutant to be degraded may be low or non-detectable, (2).

The physical and chemical properties of pollutants. The various types of soil pollutants vary in their mobility, solubility, degradability, and bioavailability. These properties play very important role in the removal of the pollutants from the soil. Pollutant or mixtures of pollutants sometimes require several metabolic pathways operates simultaneously with sometimes metabolic intermediates whose toxicity toward indigenous microbes may be high, and (3) Some polluted areas requiring long microbial adaptation period of time justifying soil bioaugmentation [14, 43]. Other abiotic factors that also affect the success of microbial inoculation-assisted phytoremediation include; temperature, aeration, soil pH, cation exchange capacity (CEC), soil organic matter content, sorptive capacity of soil, and redox potential. According to Diels and Lookman [44], microbial inoculation-assisted phytoremediation is influenced by temperature in the range 5–30 °C. It therefore means that the success of microbial inoculation-assisted phytoremediation will depend largely on season as this will be ineffective during winter in temperate countries. Grundmann et al. [45] reported that the efficiency of microbial inoculation-assisted phytoremediation depends on pH in the range 5–8. Many metal cations like Cd, Cu, Hg, Pb, and Zn are reported to be more soluble and available in the soil solution at low pH (below 5.5) [46]. However, Phytoremediation of atrazine by two microbial consortia was seriously affected by pH and soil organic matter content. At pH 6.1 only one consortium degraded atrazine but at pH >7 atrazine was effectively degraded by the consortia, the microbial inoculants were ineffective at pH 5.7 because of their interaction with organic matter [47]. pH for the degradation of phenol and TCE was observed to vary from 6.7 to 10 depending on whether the microbial inoculant cells are free or immobilized [48]. As revealed by Bhargava et al. [46] higher CEC of soil permits greater sorption and immobilization of the metals. Depending on contaminant characteristics, different microbial-assisted phytoremediation mechanisms require different final electron acceptors. For example because of the highly reduced state of petroleum hydrocarbons, the preferred and most thermodynamically relevant terminal electron acceptor for microbial process is O_2 while the degradation of chlorinated solvents, depending on the degree of halogenation, is different from that of petroleum hydrocarbons and other oxidized chemicals, and the preferred redox condition is anaerobiosis [44].

1.8 Characteristics to Consider in the Choice of a Plant for Microbial-Assisted Phytoremediation

A key aspect in biological remediation methods is the selection of appropriate plant–bacteria partnerships for the remediation of polluted soils [3]. Some of plant properties to be considered include: exceptional contaminant tolerance, ability to quickly grow on degraded land, and rapid biomass production. For instance alfalfa (*Medicago sativa* L.) that is often used in phytoremediation of contaminated soil is a fast growing species. Another critical characteristic to be considered is the composition of plant-recruited microbial communities. Plants that develop extensive tap root system favor the establishment of rhizosphere microorganisms. Plants ideal for phytoremediation should possess the ability to grow outside their area of collection, to produce high biomass, easy harvesting and accumulation of a range of heavy metals in their harvestable parts [49]. Poplar and willow possess deep root systems, produce great biomass, can be grown in a wide range of climatic conditions and these explain why they are effective phytoremediator of polluted soil [46].

1.9 Conclusions

Soil pollutant could be organic or inorganic present in the hydrosoluble fraction adsorbed onto particles or dissolved. Microbial-assisted phytoremediation remove, destroy, sequester, or reduce the concentrations or toxic effects of contaminant in polluted soils. Production of siderophores, biosurfactants, formation of biofilms, organic acids production, biomethylation, and redox processes and plant growth hormones stimulation are mechanisms employed by microbial inoculants in phytoremediation. The number of available degrading microbes and the physical and chemical properties of pollutants determine the success of microbial inoculants-assisted phytoremediation. Exceptional contaminant tolerance, ability to quickly grow on degraded land, ability to grow outside their area of collection, and rapid biomass production are important plant characteristics to be considered in the choice of plant for phytoremediation.

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Chapter 2
Phytoremediation of Salt-Impacted Soils and Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Enhance Phytoremediation

Karen E. Gerhardt, Gregory J. MacNeill, Perry D. Gerwing, and Bruce M. Greenberg

Abstract Soil salinization negatively impacts plant growth and soil structure, which leads to environmental stress and agricultural/economic losses. Improved plant growth during salt-induced ionic and osmotic plant stress is the key to successful phytoremediation of salt-impacted sites. Using plant growth-promoting rhizobacteria (PGPR) in PGPR-Enhanced Phytoremediation Systems (PEPS), positive effects of PGPR on plant biomass and health have been observed in greenhouse and field experiments. Revegetation is arguably the most important aspect of salt phytoremediation and substantial biomass increases occur in PGPR-treated plants in both sodic and saline soils. PGPR protect against inhibition of photosynthesis and plant membrane damage, which suggests that they confer tolerance to plants under salt stress. Using PEPS, decreases in soil salinity are observed due to uptake of sodium and chloride from the soil into foliar plant tissue. Although rates of uptake do not change due to PGPR inoculation, higher plant biomass due to PGPR enhancement of plant performance leads to greater salt uptake on a per area basis relative to that of untreated plants. Significant improvements in plant growth and commensurate

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sodium chloride uptake, and the results of mass balance studies used to assess the direct impact of ion uptake on actual observed changes in soil salinity, provide evidence that phytoremediation of salt-impacted soil is feasible within acceptable time frames using PEPS.

Keywords Field trials • NaCl • PGPR-Enhanced Phytoremediation System(s) (PEPS) • Polyamines • Reactive oxygen species (ROS) • Revegetation • Salt remediation

Abbreviations

ABA	abscisic acid.
ACC	1-aminocyclopropane-1-carboxylic acid.
ACCD	ACC deaminase.
Ca^{2+}	calcium ion.
Chl *a*	chlorophyll *a.*
Cl^-	chloride ion.
CT	composite tailings.
EC_e	electrical conductivity of a soil-saturated paste extract.
F_0	minimal fluorescence.
F_m	maximal fluorescence.
$F_{m'}$	maximal fluorescence in light-adapted tissue.
F_s	steady-state fluorescence.
F_v/F_m	maximum quantum yield.
IAA	indole acetic acid.
K^+	potassium ion.
Mg^{2+}	magnesium ion.
NaCl	sodium chloride.
PAH	polycyclic aromatic hydrocarbon.
PAM	pulse amplitude modulated.
PEPS	PGPR-Enhanced Phytoremediation Systems.
PGPR	plant growth-promoting rhizobacteria.
PHC	petroleum hydrocarbon(s).
PSII	photosystem II.
qN	non-photochemical quenching of fluorescence.
qP	photochemical quenching of fluorescence.
ROS	reactive oxygen species.
SAR	sodium adsorption ratio.
SOS	salt overly sensitive.

2.1 Introduction

2.1.1 Overview of Phytoremediation

Phytoremediation is a strategy whereby plants are used to extract, immobilize, contain and/or degrade soil contaminants. Although the term "phytoremediation" was not coined until the 1980s, the strategy has been employed for removing soil contaminants for at least 300 years [1, 2]. Rapid expansion occurred in this field in the 1990s, and phytoremediation has now become a useful strategy for on site and/or in situ removal of many contaminants, including petroleum hydrocarbons (PHC), metals, radionucleotides, munitions waste (e.g., trinitrotoluene) and salt [1, 3–5]. Microbe-assisted phytoremediation, especially when used in conjunction with contaminant-tolerant plant species and high-level agronomic practices, can be a particularly effective green strategy for remediation and revegetation of impacted soils [6–13].

Plants have extensive rooting systems that can explore large volumes of soil to allow for effective remediation of various contaminants within different soil types. Typically, four types of phytoremediation processes for impacted soils are discussed in the literature [1, 4, 8]. During phytoremediation, contaminants can be broken down in the soil (e.g., rhizodegradation of PHC, also referred to as rhizoremediation) or taken up by the roots and stored in plant tissue (typically in the foliage, as in phytoextraction of metals and salt). Some small molecules can be taken up by the roots, and the unmodified or modified forms are then transported via the transpiration stream to leaves, where they are released to the atmosphere via transpiration (e.g., phytovolatilization of trichloroethylene). Various contaminants can be bound within the rhizosphere (area immediately surrounding plant roots), making them less bioavailable (phytostabilization), and therefore less harmful to biota. In addition to these four main processes, plant roots can also alter soil chemistry via pH changes, which can further aid phytoremediation (e.g., breakdown of calcium carbonate in sodic soils provides calcium ions that can replace sodium ions at binding sites in the soil and allows for leaching or uptake of sodium) [14].

2.1.2 Prevalence and Sources of Salt-Impacted Soils

Soil salts can occur naturally (e.g., weathering of geologic formations, encroachment of seawater) or they can be released into the environment as a result of anthropogenic activities (e.g., irrigation, upstream oil and gas exploration/production, application of road salts) [15, 16]. Various environmental impacts associated with excess salt in soil include degradation of chemical and physical properties of the soil, diminished groundwater quality, and impaired plant growth. This results in substantial global agricultural and economic losses, sustenance issues for subsistence farmers, and ecosystem imbalances [17].

A significant buildup of salt often occurs in soils due to crop irrigation, and this has been suggested as "the first man-made environmental problem" [18, 19]. Irrigation waters tend to have high concentrations of calcium, magnesium, and sodium ions [16]. Use of this brackish water, particularly without adequate drainage management, results in the accumulation of salts in the rooting zone of plants due to evapotranspiration [17]. Calcium and magnesium tend to precipitate into carbonates, leaving sodium as the most prevalent ion in the soil, and this negatively impacts both plant growth and soil structure. Soil salinization affects 20% of irrigated land worldwide, which equates to an area approximately the size of France (62 million ha) [20, 21]. The resulting annual crop value losses have been estimated to be $27 billion (US) [17, 21]. Salinization, which occurs in virtually all geographic regions, has been a problem for millenia and continues to be a global concern of paramount importance: Soil salinity due to irrigation is thought to be a contributing factor to the downfall of the Sumerian civilization more than 4000 years ago, and irrigated land continues to be degraded by salt at a rate of 2000 ha/day [16, 22, 23].

Elevated salt levels in soils are as much of a problem for the upstream oil and gas industry as petroleum-impacted soils [24–26]. Most petroleum was formed from the remains of marine life that existed in ancient shallow seas. Consequently, oil deposits often occur in reservoirs that contain water with dissolved salts (brine), and the brine (which usually contains sulfates, bicarbonates, and chlorides of sodium, calcium, and magnesium) is frequently co-extracted with the oil [24, 27]. Any leakage into, or on, soils around the oil well will result in not only petroleum impacts, but also salt impacts. Furthermore, salt may be used during oil extraction. For example, sodium is often introduced during the extraction of bitumen from oil sands ore, and then winds up in the tailings. This is a major concern in the Athabasca oil sands region of Alberta, Canada where large volumes of fluid fine tailings are produced and stored in tailings ponds [25]. It was estimated that by the end of current upstream heavy oil operations, more than one billion cubic meters of fine tailings will be stored in these ponds. To reduce the stored volume, the composite tailings (CT) process is used, which involves the addition of gypsum or alum as a coagulant [28]. During this process, water containing high levels of salt is released from the CT, and this saline CT water makes reclamation of the CT deposit areas difficult. Efforts to revegetate the CT are hindered if salt from the CT water accumulates in the rooting zone.

Application of road salts (sodium chloride [NaCl], calcium chloride, potassium chloride and magnesium chloride), particularly in large urban areas, also leads to elevated soil salt levels in ecosystems adjacent to roads, snow removal dump sites, and some salt storage facilities [29, 30]. An average of 5×10^6 tonnes of road salts (primarily NaCl) are applied annually to Canadian roadways [29, 30]. This negatively impacts physical and chemical properties of surrounding soils, which consequently impacts associated biota. This problem was deemed critically important in the Canadian Environmental Protection Act, 1999, which categorized road salts as toxicants [30], and implemented new guidelines for their use (Code of Practice for Environmental Management of Road Salts) [29].

2.1.3 Soil Salt Chemistry

Based on a system developed by the US Salinity Laboratory [31], salt-impacted soils can be broadly classified as either saline (high concentration of soluble salts), sodic (high concentration of sodium), or saline-sodic (high concentrations of both soluble salts and sodium). More recently, the USDA Natural Resources Conservation Service classified salt-impacted soils into seven types that incorporate soil characteristics that are observable in the field as well as chemical analyses [32]. In this chapter, the US Salinity Laboratory classifications will be used when discussing soil salt impacts. Because NaCl is the most prevalent salt contaminant in the environment [33], the term "salt" refers to NaCl in subsequent sections of this chapter, unless specified otherwise.

One of the most common ways to measure total soluble soil salt concentration is electrical conductivity of a saturated soil-water paste extract (EC_e, measured as dS/m) [31]. Soil sodicity can be calculated using the sodium adsorption ratio (SAR). It is based on the ratio of sodium ions (Na^+) to calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}) in the soil, and takes into account the difference in adsorption strengths of the ions to clay particles:

$$SAR = \frac{\left[Na^{+}\right]}{\sqrt{\dfrac{\left[Ca^{2+}\right]+\left[Mg^{2+}\right]}{2}}}$$

where the ionic concentrations are expressed in milliequivalents per liter in soil extract solution in equilibrium [31, 32]. Saline soils have an $EC_e > 4$ dS/m and $SAR < 13$ in their saturation extract. Sodic soils have an $EC_e < 4$ dS/m and $SAR > 13$. Saline-sodic soils have an $EC_e > 4$ dS/m and $SAR > 13$.

Saline soils tend to have white crusts formed from crystallized salts that have precipitated at the soil surface. Sodic soils tend to have poor physical structure, low permeability (i.e., restricted movement of water and air through the soil), and high pH (7.8–8.5), all of which are detrimental to plant growth [32]. Poor structure is, in part, because Na^+ displaces Ca^{2+} and Mg^{2+}, which are important for holding clay lattices/particles together [34, 35]. In weakly aggregated soils, dispersion of soil particles can fill soil pores and impermeable surface crusts can form after repeated wet/dry cycles, which inhibit root penetration and growth. High pH limits the availability of some key plant nutrients and micronutrients such as phosphates, cobalt, copper, iron, manganese, and zinc, which are all more bioavailable at pH < 7. For more detailed descriptions of characteristics and chemistry of sodic and saline-sodic soils, please refer to Qadir et al. [36].

2.1.4 Responses of Plants to Salt Impacts

Plants are perhaps the most vulnerable sector of the biosphere to salt. Rapidly manifesting drought stress symptoms can occur in salt-impacted soils despite the presence of adequate water, because the resulting increase in osmotic pressure diminishes water uptake by plants [37, 38]. Over time, uptake of salt ions can lead to toxicity in plant tissues (particularly accumulation of Na^+; as well, chloride ions [Cl^-] can reach toxic levels in some sensitive species), and the presence of excess ions in the soil can interfere with nutrient availability (e.g., high concentrations of Na^+ in the rhizosphere interfere with K^+ uptake, due to the similar chemical nature of the ions, and this leads to K^+ deficiency and growth inhibition in plants) [33, 37–40]. Salt stress negatively impacts germination, plant growth, and reproduction by affecting physiological processes such as photosynthesis, respiration, transpiration, and enzyme function; membrane properties are affected, upregulation of the stress-responsive hormones abscisic acid (ABA) and ethylene occurs, and reactive oxygen species (ROS) are generated [41–45].

2.1.4.1 Uptake and Transport of Na^+, K^+, and Cl^-

During phytoextraction of salt, ions are taken up from the soil into plant tissues. Na^+ and Cl^- are taken up by plants primarily through passive symplastic pathways driven by concentration gradients and transpiration fluxes [46, 47]. Ions are transported from the root cells to the leaves via the transpirational stream of the xylem (Fig. 2.1) [33]. These ions are typically stored in the leaves, and little ion flow occurs via the phloem down to the roots (Fig. 2.1) [38, 47, 48]. Ion homeostasis, involving primarily Na^+, K^+, Ca^{2+} and Cl^- is extremely complex, both at the cellular and whole plant level [38, 40, 48, 49]. Various ion channels and pumps in plant cells, many of them tissue-specific, regulate the flow of ions from the soil into roots, translocation from roots to foliar tissue, and storage within the cells or excretion from them [40, 50–54] (Fig. 2.2a). Phytoextracted salt can be removed from a site by harvesting the foliar tissues with accumulated salt ions.

2.1.4.2 Salt Stress and ROS Damage

Salt stress (both osmotic and ionic) frequently results in an increase in ROS, including hydrogen peroxide, superoxide anion radicals, hydroxyl radicals, and singlet oxygen [55–57]. Formation of ROS occurs primarily in chloroplasts; however, it also occurs in mitochondria and peroxisomes [55, 56, 58]. During salt stress, cytosolic polyamines are exported to the apoplast, where they are oxidized to ROS [59]. Excessive formation of ROS leads to oxidative damage of many cellular components, including proteins, DNA, and lipids (e.g., membrane lipid peroxidation), ultimately leading to growth inhibition or capitulation of plants [15, 60].

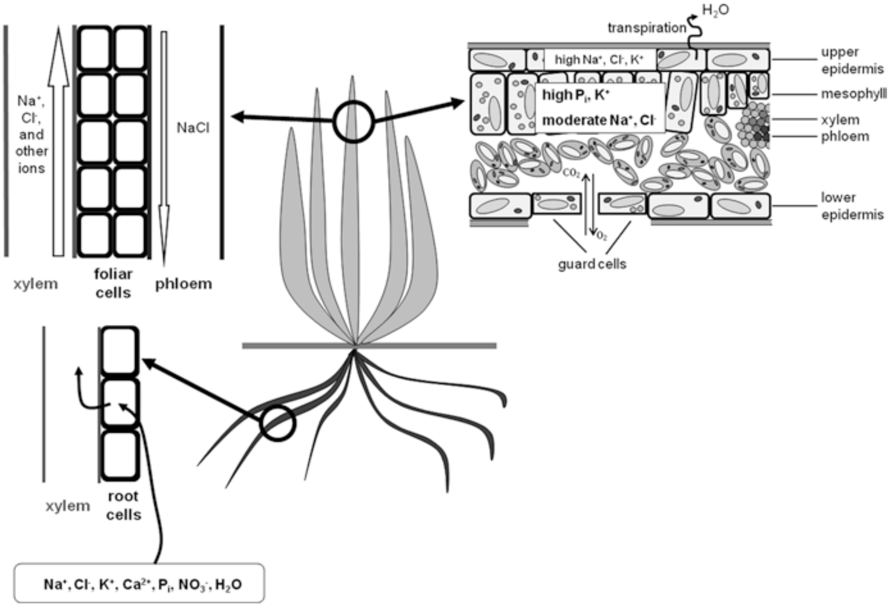


Fig. 2.1 Phytoextraction of salt. Uptake of ions from the soil to root epidermal cells occurs first. Ions are translocated via the root symplast to the xylem. Na^+, Cl^-, and other ions extracted from the soil are transported through the xylem to leaf tissue, and are stored in vacuoles. There is minimal flow of Na^+ and Cl^- back down the phloem

2.1.4.3 Salt Stress and Acclimation Signaling Pathways

Although excessive salt-induced generation of ROS can impair metabolic processes, leading to oxidative stress and cellular damage, ROS can also signal responses to mitigate salt stress damage [56, 61–63]. Increases in antioxidant enzyme activities have been correlated with salt tolerance [57]. For example, the antioxidant enzymes catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase are activated in response to salinity stress in the European olive [64]. The ROS signaling pathways that result in acclimation to salt stress are integrated with numerous other signaling pathways related to salt tolerance. These include calcium, hormone and protein phosphorylation pathways, as well as complex interactions with polyamine pathways [56, 63].

Accumulation of polyamines is a key factor in achieving plant tolerance to salt stress [63]. Polyamines such as spermine, spermidine, and putrescine play a significant dual role in ROS homeostasis by acting both as ROS scavengers, and as substrates for amine oxidases in the apoplast that catalyze formation of ROS involved in stress response signaling [59, 63, 65]. Some of the ways by which polyamines influence ion transport during salt stress via complex signaling pathways are shown in Fig. 2.2b. For example, polyamines exported from the cytosol to the apoplast can block non-specific cation channels in the plasma membrane to limit Na^+ influx and

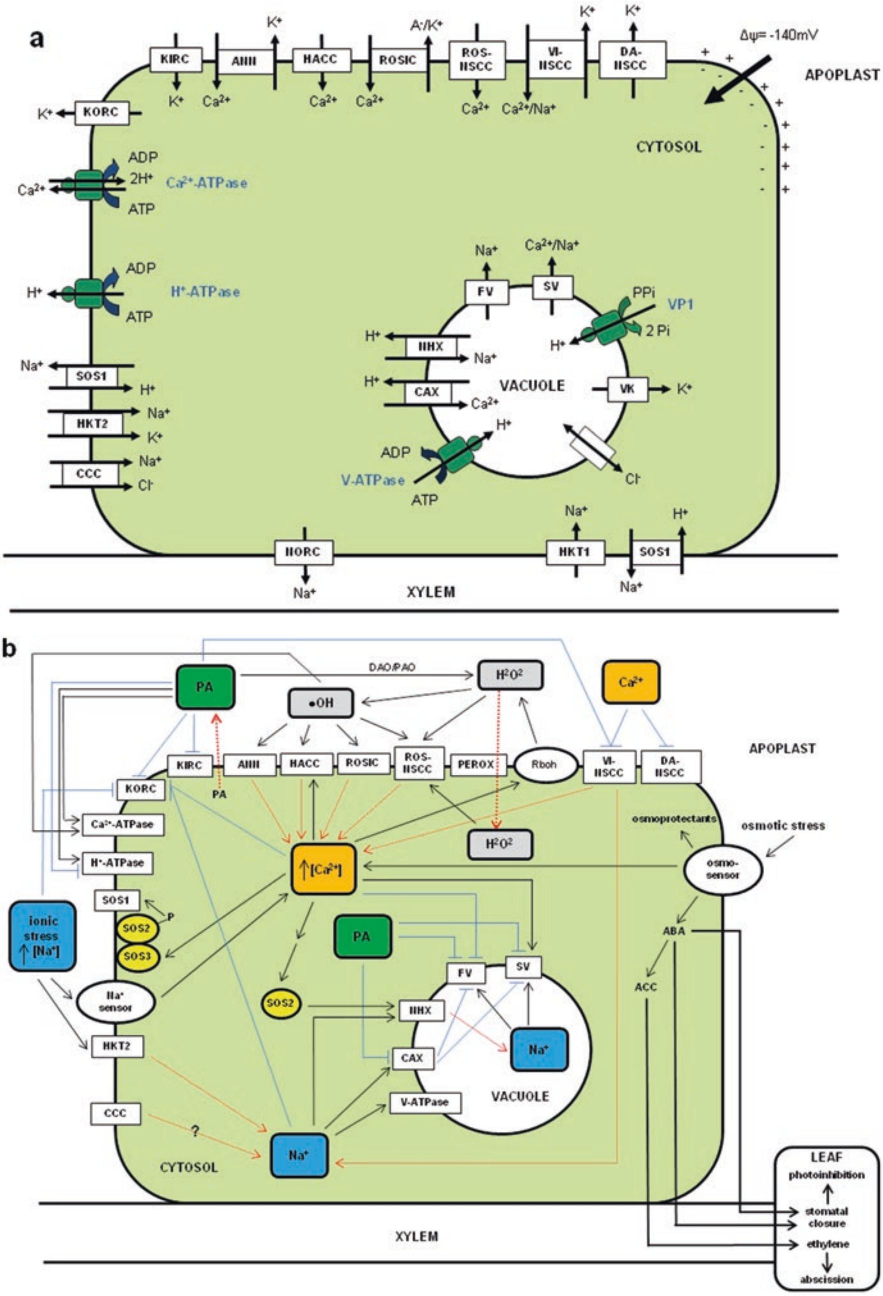


Fig. 2.2 Ion pumps, channels, and signaling in plant salt stress and adaptation. (**a**) Numerous ion pumps and channels involved in salt stress and tolerance are shown. Not all of them are found in all species, or in all cells, and the specifics of ion conductance depend on a variety of conditions. *ABA* abscisic acid, *ACC* 1-aminocyclopropane-1-carboxylic acid, *ANN* annexin-formed channel, *CAX* cation/H^+ exchanger, *CCC* cation-chloride-cotransporter, *DA-NSCC* depolarization-activated non-selective cation channel (NSCC), *DAO* diamine oxidase, *FV* fast vacuolar channel, H_2O_2 hydrogen peroxide, *HACC* hyperpolarization-activated Ca^{2+} influx channel, *HKT1* low-affinity Na^+ histidine kinase transporter, *HKT2* Na^+/K^+ histidine kinase symporter, *KIRC* K^+ inward-rectifying,

K^+ efflux, and cytoplasmic polyamines can inhibit cation channels in the tonoplast to limit Na^+ efflux to the cytoplasm, thereby helping to maintain proper cellular K^+/Na^+ ratios: this may be crucial for achieving salt stress tolerance [49, 63].

One well-researched signaling pathway for Na^+ exclusion from cells was discovered using the salt overly sensitive (SOS) line in *Arabidopsis* [16, 48, 66, 67] (see Fig. 2.2). Following exposure to salt, an unidentified salt sensor in the root plasma membrane perceives the stress and a Ca^{2+} spike is generated in the cytoplasm. This activates a signal transduction cascade involving the SOS proteins: SOS3, a calcium binding protein, activates SOS2, a kinase that phosphorylates the plasma membrane antiporter, SOS1. Cytoplasmic Na^+ is then transported out of root cells, either from the cytosol to the apoplast (epidermal cells), or from the cytosol to the xylem (parenchyma cells) [68]. In leaves, a similar pathway exists, but SOS3 is replaced by SCaBP8) [67]. SOS3 and SOS2 have been shown to play regulatory roles in salt tolerance [69].

Salt stress can result in increased levels of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), resulting in stress ethylene production [39, 70]. This response is mediated by ABA, and ultimately leads to leaf abscission, ridding the plant of tissue that contains toxic levels of Na^+ [56]. Upregulation of ABA also promotes stomatal closure to avoid water loss during osmotic stress, but may cause a shortage of CO_2 for carbon fixation, which leads to a decline in photosynthesis [44, 55–57].

Signaling pathways involved in salt stress and subsequent acclimation are very complex (some of the signaling pathways are illustrated in Fig. 2.2b). The linear SOS pathway is the best understood, but it is not the only signaling pathway for adaptation to salt stress [67, 69]. There is good evidence that crosstalk between SOS and ABA signaling pathways occurs, and a complex signaling network with crosstalk between polyamine, ROS, and ABA pathways has also been shown [63, 67]. Plant growth-promoting rhizobacteria (PGPR) have been shown to positively influence many of these pathways and processes to mitigate salinity stress (see Sect. 2.1.5.3).

Fig. 2.2 (continued) *KORC* K^+ outward rectifying channel, *ROSIC* non-selective voltage-independent conductance, *NHX* Na^+/H^+ antiporter, *NORC*, *•OH* hydroxyl radical, *PA* polyamine, *PAO* polyamine oxidase, *PEROX* peroxiporin, *Rboh* respiratory burst oxidase homolog (an NADPH oxidase), *ROS-NSCC* ROS activated non-selective cation channel, *SOS1* Na^+/H^+ antiporter, *SOS2* protein kinase, *SOS3* Ca^{2+} sensor, *SV* slow vacuolar channel, *V-ATPase* vacuolar H^+-ATPase, *VI-NSCC* voltage-independent NSCC, *VK* K^+-selective channel, *VP1* vacuolar H^+ pyrophosphatase. Hyperpolarization of the plasma membrane activates KIRC (more influx of K^+ than N^+). At the onset of salinity stress, KORC is activated by membrane depolarization, allowing the influx of Na^+ and efflux of K^+. Details for other ion channels and pumps can be found in Sects. 2.1.4.1 and 2.1.4.3, and references therein. (**b**) Some of the signaling pathways involved in salt stress and adaptation are outlined, with emphasis on PA/ROS-related pathways. Dotted lines with arrows indicate some relevant sources of the ionic and molecular pools, solid lines with arrows indicate positive regulatory actions, and lines with bars indicate negative regulatory actions. Salt stress leads to a Ca^{2+} burst that activates the SOS pathway. Increased Ca^{2+} is perceived by SOS3, which interacts with the kinase SOS2. This complex phosphorylates the SOS1 antiporter at the plasma membrane, which leads to diminished accumulation of Na^+ in the cytosol. It also leads to increased activity of NHX at the tonoplast, which results in sequestration of excess Na^+ in vacuoles. Further details for signaling pathways can be found in Sects. 2.1.4.2 and 2.1.4.3, and references therein

Detailed descriptions of the numerous interconnected signaling pathways, and the salt ion channels, pumps and molecules involved in toxicity and tolerance are beyond the scope of this review. For further details, please see Blumwald [71], Gao et al. [72], Kronzucker and Britto [49], Kumar et al. [73], Kurusu et al. [74], Miller et al. [56], Pottosin and Shabala [65], Saha et al. [63], Uozumi and Schroeder [75], and Zhu [33].

2.1.4.4 Physiology of Salt Tolerance in Halophytes and Glycophytes

There are many different parameters that have been used to define halophytes in the literature [15, 40]. Generally, they can be defined as plants that grow well in salt-impacted soils. Plants that are not halophytes are frequently classified as glycophytes. A more realistic view is not a division into two broad categories of plants, but rather a continuum of salt tolerance ranging from extremely tolerant to extremely sensitive plants [76]. Many major agricultural crops are sensitive to salt stress [57]. Salt tolerance can be assessed in terms of survival (more meaningful for perennials than for annuals) and/or biomass production [77].

Halophytes can be obligate (absolute requirement for elevated salt habitats), facultative (can grow in salt-impacted soils, but optimum growth and health occurs in soils with low or no salt) or habitat-indifferent (can flourish in soil with or without salt) [15]. Glycophytes have varying sensitivities to salt, ranging from tolerant to completely intolerant. Depending on the circumstances, a given plant might be described as a facultative halophyte or a salt-tolerant glycophyte.

Halophytes have evolved different mechanisms that allow them to survive and thrive in salt-impacted soils: salt exclusion (minimizing uptake), salt accumulation, and salt excretion [78]. Some of these strategies are also employed by salt-tolerant glycophytes. Salt exclusion mechanisms are varied and complex; however, the main contributing factors are low permeability of root epidermal cell membranes, low net uptake of Na^+ by root cortex cells, and tight control of xylem loading via the pericycle [15, 40, 51]. In salt accumulators, Na^+ is taken up, transferred to leaf tissue and sequestered in vacuoles to minimize damage to cytoplasmic components (Figs. 2.1 and 2.2). Small organic osmolytes (compatible solutes), such as proline, betaine, and mannitol, accumulate in the cytoplasm to maintain osmotic balance within the cells, and some act as osmoprotectants to scavenge/quench ROS and prevent damage to membrane structure, enzymes, and proteins [33, 38, 55]. Salt excretion, prevalent in halophytes, is accomplished using leaf epidermal salt glands and hairs that remove salt from mesophyll cells via secretion at the leaf surface [14, 79].

2.1.5 Remediation and Phytoremediation of Salt

Remediation of salt-impacted soils has proven difficult and costly due to the absence of a versatile in situ technology [24]. Often the impacted soil must be removed to landfill and replaced with clean soil. In addition to the physical challenges encountered with ex situ remediation (soil excavation and soil replacement), these methods are also costly and unsightly. Various in situ treatments have also been employed to remediate salt-impacted soils. Three widely used methods are leaching, chemical amendments followed by leaching, and organic amendments [14, 24, 36]. For leaching, excess water is applied to the soil to move soluble salts from the surface soil to lower horizons. This can lower EC_e values in surface and rooting zone soils, but not SAR, and is therefore effective primarily for saline soils [14]. Leaching results in valuable water resources being wasted, and diminishes soil stability and quality [14]. For sodic soils, numerous chemicals can be applied to the soil to promote ion exchange, often replacing Na^+ with Ca^{2+} at the cation binding sites on clay particles. The amendments can be very costly, however, and leaching is required afterward to remove the Na^+ to lower soil horizons [14]. Organic amendments can be used to increase dissolution of soil calcite and improve soil structure, however these amendments are also costly and dissolution is a slow process [14].

Several mechanisms are involved in salt remediation when using plants (phytoremediation). Uptake of salt ions into plant tissue results in a decrease in soil EC_e and, possibly, SAR. Lowering soil pH via root exudates can increase the dissolution of soil calcium carbonate (calcite), thereby providing Ca^{2+} to displace the adsorbed Na^+ in the soil. Displaced Na^+ leads to improved soil structure with the resultant uptake and removal of Na^+ from soil by plants. Root growth and the associated organic matter additions to the rhizosphere within impacted areas will increase hydraulic conductivity of the soil, which increases the potential for natural leaching of salt from upper to lower soil horizons [14, 36, 80, 81].

2.1.5.1 Advantages of Phytoremediation of Salt

Clearly, technologies are needed that can remediate salt-impacted soils in an environmentally responsible and cost-effective way. Phytoremediation has numerous advantages over conventional techniques for salt remediation. Some of the advantages are greater environmental stewardship (e.g., soil is treated and reused, not hauled to landfill for disposal), ease of application, and lower cost. Using plants, co-contaminants such as salt, PHC, and metals can be remediated simultaneously [82]. As an added benefit, some crops that are grown for phytoremediation can be sold for bioenergy sources, cellulose production, or livestock feed [14, 81].

2.1.5.2 Choosing Plants for Phytoremediation of Salt

Numerous plant species have been shown to effectively decrease EC_e and SAR in salt-impacted soils [14, 81, 83, 84]. Plants chosen for phytoremediation must be sufficiently salt-tolerant to survive and grow in impacted soils. Some of the most salt-tolerant halophytes are very slow growing, and consequently these plants do not attain sufficient biomass to achieve phytoremediation in an acceptable time frame. Many halophytes excrete salt ions through specialized leaf glands, and others drop older organs that have accumulated toxic levels of Na^+ and other ions: neither of these tolerance mechanisms leads to phytoremediation because the salt essentially returns to the soil near the plant. Similarly, plants that exclude Na^+ and Cl^- from roots are not efficient remediators of NaCl because the salt remains in the soil, although it might be more easily leached due to plant-related improvements in soil hydraulic conductivity.

A salt tolerance mechanism that is desirable for phytoremediation is uptake and storage of Na^+ and Cl^- into above-ground tissues. Maintaining low concentrations of cytoplasmic Na^+ is a key factor in salt tolerance. As noted in Sect. 2.1.4.4, many halophytes and salt-tolerant glycophytes sequester Na^+ to leaf vacuoles to achieve this [85, 86]. This prevents damage to cytosolic enzymes, and also counteracts the low extracellular osmotic potential resulting from salt stress [33]. Ideally, if soils are highly sodic, the chosen plant will have high Na^+ uptake, but lower uptake of Ca^{2+} and Mg^{2+}, which will lower SAR values [14]. Also, for effective phytoremediation, the plants chosen should be suitable for repeated harvesting of the foliar tissues containing phytoextracted salt.

2.1.5.3 PGPR-Enhanced Phytoremediation

One criterion that is essential for successful phytoremediation is substantial plant biomass production. Unfortunately, as discussed in Sect. 1.4, plants growing in salt-impacted soils are prone to the combined detrimental effects of water stress, ion toxicity, and nutritional deficiencies, which result in substandard plant growth. Traditional plant breeding programs and genetic engineering have been employed in attempts to improve salt tolerance in plants; however, the suite of genes and multiple pathways involved in salt tolerance, as well as the time involved to successfully breed or engineer salt tolerant plants, make this a daunting task [55, 87, 88].

One strategy that has been utilized to overcome the challenges of abiotic stressors is to employ plant growth-promoting rhizobacteria (PGPR); these soil microbes can promote growth and health in plants during stress conditions [8, 10, 89–92]. PGPR accelerate plant growth under stress conditions by increasing plant tolerance to elevated salt, PHC and/or trace metal levels, as well as other environmental stressors such as saturated soil or drought conditions. This leads to rapid growth of plants, including their roots. The vigorous plant growth that ensues leads to greater proliferation of naturally existing microbes in the soil, resulting in a very active rhizosphere that is typical of soils with normal plant growth. The substantial root biomass that accumulates in the soil provides a sink which allows for rapid partitioning of salt ions out of the soil, and their subsequent accumulation in the foliar tissues of some plants.

PGPR have been shown to confer salt tolerance in a variety of plants, by ameliorating both the osmotic and ion toxicity effects of salt stress [6, 58, 89, 93–95]. Some PGPR confer salt tolerance via tissue-specific regulation of HKT1, a plasma membrane Na^+ uniporter [95] (see Fig. 2.2a). When plant growth inhibition is the result of stress ethylene production, PGPR with ACC deaminase (ACCD) can be employed [70]. ACCD metabolizes ACC, a precursor of ethylene in the biosynthetic pathway, thereby limiting the amount of stress ethylene that can be produced [6, 90]. Polyamines produced by PGPR have also been shown to lower stress ethylene levels and mitigate osmotic stress [96, 97]. PGPR have been shown to promote synthesis of antioxidants (including polyamines), and indole acetic acid (an auxin) which can promote root growth [6, 58, 89]. Recently, PGPR were shown to regulate a ROS-triggered caspase-like activity in rice; there was a concomitant decrease in programmed cell death, a phenomenon previously linked to caspase-like activity and salt-induced oxidative stress [58, 98]. Other mechanisms linking PGPR to salt tolerance in plants include altered mineral uptake, which results in a beneficial increase in the cellular K^+/Na^+ ratio; and elevated production of quorum sensing molecules, which can lead to alterations in the rhizosphere [92, 99–101].

2.1.5.4 Successful Remediation of Salt-Impacted Soils

Numerous studies have been conducted to assess phytoremediation of salt-impacted soils. For example, beet and millet were grown for 70 days in the greenhouse, in saline calcareous soil from Southern Ghor in Jordan [81]. Substantial amounts of Na^+, K^+, and Cl^- were taken up into above-ground tissues, decreases in $EC_{1:1}$ of 54–69% occurred, and better soil hydraulic conductivity was observed. Purslane has been shown to remove considerable amounts of NaCl from saline soils, and was recommended as an intercrop for salt removal in salt-sensitive fruit orchards based on pot experiments [102]. Hue et al. [82] used material dredged from Pearl Harbor, Hawaii. This material was amended with a soil from Oahu, Hawaii that was high in calcium and magnesium, to achieve a final EC_e of ~18 dS/m. After growing a combination of two salt-tolerant grasses and a legume for 3 months in a greenhouse, soil EC_e decreased by ~50%. This was attributed primarily to Na^+ uptake by the legume and one of the grasses. *Atriplex halimus* plants were grown for 90 days in pot experiments using saline and saline-sodic soils from the Ninavah province of Iraq [103]. Decreases in EC were observed in both saline and saline-sodic soils (21 and 32%, respectively). Decreases in SAR were also observed for both saline and saline-sodic soils (29 and 50%, respectively).

Field experiments were performed in the Khorezm Region of Uzbekistan [104], where *Chenopodium album* and *Apocynum lancifolium* were grown in soil with EC_e values of ~10.5 and 13, respectively (top 15 cm). Uptake of Cl^- and Na^+ in *C. album* (105 and 34 mg/g dry weight, respectively) was substantially higher than that of *A. lancifolium* (49 and 12 mg/g dry weight, respectively). Despite a root depth that was less than half that of *A. lancifolium*, *C. album* had much greater salt uptake from the soil (570 kg/ha, compared to 130 kg/ha for *A. lancifolium*). Thus, *C. album* was deemed a good choice for remediation of salt-impacted soils, and was recommended for integration into crop rotation programs, whereas *A. lancifolium* was deemed a salt-tolerant species not suitable for salt remediation because, relative to *C. album*, the rate of salt removal was deemed too low. For more results of phytoremediation experiments in the greenhouse and field, please see the following reviews of the literature [8, 14, 81, 85].

2.1.5.5 Obstacles Affecting Phytoremediation of Salt-Impacted Soils in the Field

Efforts to translate phytoremediation research from successful laboratory and greenhouse experiments to the field have proven challenging [9]. Although there have been many successful trials, there have also been numerous inconclusive and unsuccessful attempts at phytoremediation of salt in the field. A few general problems have emerged: different experimental conditions between the laboratory and the field, difficulty in accurately assessing salt remediation, and length of time required for salt remediation.

Numerous biotic and abiotic plant stress factors not present in laboratory and greenhouse studies can result in significant problems in field applications of phytoremediation. These include, but are not limited to, variations in temperature, nutrients, and precipitation; herbivory (insects and/or animals); plant pathogens; and competition by weed species that are native to the area [105]. Further, in the greenhouse, soils are generally homogeneous; in the field, contaminant concentrations vary across any given site, resulting in “hot spots”. Factors such as root structure, soil structure, organic composition of the soil, soil pH, moisture content, and microbial activity also exhibit spatial variability at a given site, and can change over time [105, 106].

For salt, conventional means of assessing phytoremediation (e.g., decrease in EC_e in soil over time) may not be adequate to show that salt impacts are actually decreasing, although in many cases active remediation may be occurring. Salt readily migrates from lower soil horizons into the rooting zone of plants (i.e., the area where phytoremediation takes place) due to evaporation and transpiration [107]. Thus, it can be difficult to assess remediation exclusively by measuring soil salt levels in upper horizons. Assessing ion uptake into plant tissues and calculating estimates of total salt uptake at a given site can provide an estimate of actual salt removal and remediation over time.

Another challenge to phytoremediation of salt in the field is the length of time required to fully remediate the impacted soils [36]. Although this cannot be considered a failure of the technology, it is a disadvantage compared with traditional methods such as excavation and soil removal. It has also been suggested that salt remediation rates decrease over time, because in terms of mass balance, fewer salt ions are removed from the soil when salt gets diluted in leaching water (natural or applied) [14]. If salt uptake is the predominant removal mechanism, this should not be a factor. In fact, the reverse should be true: as soil quality improves with each successive growing season, plant root and shoot biomass should increase, providing a greater sink for salt ions.

2.1.5.6 Revegetation as a Measure of Successful Phytoremediation of Salt

As noted in Sect. 2.1.5.5, salt readily migrates from lower horizons to upper horizons in the soil, and moves with water flow in general. When plants grow in soil, this upward migration of water and salt is enhanced. Thus, it can be problematic to accurately assess remediation based on soil salt levels. Unlike heavy metals, which can be highly toxic to humans and other animals at levels found in soils, NaCl is generally not considered hazardous. Therefore, in the case of salt, the essential goal of phytoremediation is to overcome plant salt stress. Ideally, plants that grow rapidly with high rates of salt uptake and accumulation (e.g., kallar grass and oats) can be used to achieve both revegetation and salt removal from the soil [80]; however, generic regulatory criteria that depend solely on diminishing soil EC_e levels and SAR in impacted soils may be too stringent and unnecessary in some cases. Because soil salts (including NaCl) are generally not hazardous to humans and other animals, and plants are the most sensitive part of the biosphere, we propose that, rather than achieving mandated levels of EC_e and SAR, sustained revegetation of an impacted site should be the goal of salt phytoremediation. In this case, achieving 75% sustainable plant productivity compared to reference sites should qualify as successful phytoremediation of salt. This is in accordance with the reclamation objectives of some Canadian and American regulatory bodies that seek to ensure a self-sustaining ecosystem devoid of long-term toxicity, and to establish equivalent land capability that existed prior to industrial activities [26, 108].

If revegetation is the goal, selection of plant species is important. Many halophytes that accumulate large quantities of salt on a per mass basis grow too slowly to provide sufficient biomass for revegetation within an acceptable timeframe. Also, they may not be native to the site being remediated. Plants that exclude salt by limiting uptake into the root, or plants that excrete the salt from aerial tissues, cannot effectively remove salt from the soil. However, if the goal is only revegetation, these species could be considered for use. In general, for revegetation, the goal should be to achieve aggressive plant growth with species native to the impacted site.

2.2 PGPR-Enhanced Phytoremediation Systems (PEPS)

To fill the need for a versatile, green, in situ technology for remediation of contaminated soils, PGPR-Enhanced Phytoremediation Systems (PEPS) have been developed [9, 10, 83, 84, 91, 109–114]. To achieve successful PGPR-enhanced phytoremediation, a skill set beyond being able to plant seeds is required. A fundamental understanding of soil science, contaminant chemistry, plant biology, soil microbiology, agriculture, forestry, and regulatory guidelines is crucial for application of this green technology. The key to successful remediation within an acceptable time frame is to achieve vigorous plant growth because large amounts of biomass are necessary for phytoremediation; however, this is generally difficult due to suboptimal soil conditions (e.g., low organic content and poor soil structure) at impacted sites. Phytoremediation is therefore facilitated by preparing high-quality seed beds and utilizing other agronomic practices. After plant growth is established, contaminant chemistry, including degradation and/or uptake of the contaminants from soil, must be monitored. The standard PEPS protocol includes inoculation of seeds with PGPR to accelerate plant growth under stress conditions (see Sect. 1.5.3), soil pre-treatment (tilling soils to achieve homogeneity, as well as fertilizing and adding other required amendments to the rooting zone of plants), and adequate monitoring of the site (including contaminant assessments).

Mixtures of grass species, including cereals, are most commonly used in PEPS. Most *Poaceae* species (grass family) are facultative halophytes (also described in the literature as salt-tolerant glycophytes): they can grow on salt-impacted soils, but plant growth and health will be negatively impacted relative to growth in soils with low or normal salt levels [15]. Specific grass species have been used because they have been shown to be salt-tolerant, they accumulate salt in foliar tissue which can be removed easily from impacted sites, and they produce substantial amounts of root biomass. Using more than one plant species (co-cropping) can enhance overall microbe-assisted phytoremediation because the unique characteristics and properties of each plant species may support different microbial communities in the rhizosphere, differentially penetrate the soil matrix, and have different temperature and moisture optima, which increase the overall odds of success in the field; co-cropping has also been shown to limit weed proliferation and herbivory [115].

The PGPR used in PEPS are non-pathogenic, non-genetically modified soil bacteria (usually pseudomonads) that are present in the soils under remediation [9, 112]. These strains are naturally occurring, and express ACCD. They also synthesize indoleacetic acid (IAA), which promotes root cell growth of host plants [116]. They are sensitive to common antibiotics, do not grow at 37 °C (i.e., they cannot proliferate in the human body), and are all classified as Biosafety Level 1 (the safest possible designation). They are ubiquitous in nature, common to soils around the world, and pose no threat to humans, wildlife, or the environment. With PEPS, the PGPR are used only via a seed treatment, whereby the seeds are treated in a controlled environment. The plant roots of the treated seeds are thus inoculated with PGPR as they pass through the seed coat during germination. Notably, PGPR, including those used in PEPS, increase the number of root hairs in grass seedlings under stress, relative to plants without PGPR ([100], Greenberg et al. unpublished data). Root hairs contribute substantially to the surface area of roots, and most of the ion uptake (including Na^+) occurs across the plasma membrane of the root hair epidermal cells [51]. Thus, PGPR-treated PEPS plants have a greater capacity for Na^+ uptake from the soil than untreated plants, which generally corresponds to decreases in both soil salinity and sodicity.

A mixture of PGPR can be used if the right combination of mixed microbial strains can be found (e.g., [10, 58]). The rationale is that taxonomically different PGPR have different optimum pH, temperature, and moisture requirements for colonizing rhizospheres/roots; and different PGPR may have different modes of action for promoting plant growth that could be additive or synergistic in a microbial mix, further increasing the odds of successful phytoremediation in the field. Sometimes, however, it is preferable to use a single strain of PGPR to avoid antagonistic effects. Greenhouse experiments have been performed to ascertain whether or not different PGPR strains should be used independently (e.g., CMH3) or in combination (e.g., UW3 + UW4) [83, 117].

When possible, PEPS plant species are chosen that are native to the area in which phytoremediation is being undertaken. This eliminates the ecological risk associated with introducing a non-native species to an ecosystem and facilitates native habitat reconstruction/reclamation following remediation. Native PGPR are also used, whenever possible. For instance, PGPR that have been isolated from the site being remediated can be used. This provides multiple benefits: PGPR isolated from salt-contaminated soils are salt-tolerant, acclimated to the soil conditions in that area, and may be more competitive in situ than non-native bacteria [118].

2.2.1 *Development, Proof, and Full-Scale Application of PEPS*

In the initial stages of PEPS development, remediation of PHC (including large recalcitrant polycyclic aromatic hydrocarbons [PAHs]), heavy metals (lead, copper, and cadmium) and a pesticide (DDT) were the focus of the research [91, 113, 114, 119, 120]. The original process involved proven agronomic techniques, and plant growth with PGPR [91, 113, 114], with both laboratory and small-scale field trials [9, 112]. A variety of monocot and dicot species were used in the initial plant growth and phytoremediation experiments. Seeds were treated with various naturally occurring, non-pathogenic *Pseudomonas* strains, both individually and in microbial mixes. Although phytoremediation was observed in the absence of PGPR treatments, enhanced remediation rates were observed with PGPR seed treatments. A summary of the development, proof, and full-scale application of PEPS for PHC remediation was published recently [10].

2.2.2 *Adapting PEPS for Salt Remediation*

As discussed in Sect. 2.1.2, soil salinization is as much of an environmental issue as soil contaminated with compounds such as PHC (including PAHs) and metals [24–26]. For this reason, PEPS research was expanded to include phytoremediation of salt. Laboratory, greenhouse, and field experiments were conducted, resulting in the adaptation of PEPS for salt remediation [83, 84, 109–111, 117, 121, 122].

2.2.2.1 Lab/Greenhouse Experiments

The effects of salt stress on plant growth, photosynthesis, and membrane integrity were assessed in a series of greenhouse and laboratory experiments [109, 117, 121, 122]. Soils with a range of salinity (EC_e) and sodicity (SAR) values were obtained from sites in Saskatchewan, Canada. Three strains of PGPR, *Pseudomonas* sp. UW3 (GenBank Accession Number KF145175), *Pseudomonas* sp. UW4 (GenBank Accession Number CP003880), and *Pseudomonas corrugata* CMH3 (GenBank

Accession Number KF041156), were used for seed treatment prior to phytoremediation to promote plant growth and increase tolerance to salt [83, 123, 124]. All these strains are naturally occurring, produce IAA and express ACCD, the enzyme that consumes the precursor to ethylene, a plant stress hormone. UW3 and UW4 were isolated from unimpacted Ontario soils during a previous research project. CMH3 was isolated from the rhizosphere of grasses grown on a highly saline soil (EC_e value of 20–50 dS/m) at an upstream petroleum site in Saskatchewan. Details of PGPR isolation, analysis, and identification; ACCD and IAA assays; bacterial inoculation of seeds; greenhouse trials; fluorescence assays; and electrolyte leakage assays can be found in Chang et al. [83] and Greenberg et al. [109].

Effects of Salinity and PGPR on Plant Growth

Previously, a decrease in biomass for wheatgrass grown without PGPR for 90 days in saline soils (EC_e = 30 dS/m) was reported [109]. Biomass decreases for barley and oats grown for 45 days in saline soils (EC_e = 9 dS/m) were also reported previously [83]. Data for oats grown on saline (EC_e = 14 dS/m) and sodic (SAR = 24) soils are provided here as other examples. A decrease in oat biomass due to salt stress was observed in the absence of PGPR seed treatments. Oats without PGPR had 40% lower shoot biomass (Fig. 2.3) and 50% lower root biomass [117] than control plants grown on unimpacted soils (ProMix™). PGPR (UW3 + UW4) completely alleviated the root and shoot growth inhibition caused by salinity.

In fact, the shoot biomass of plants treated with PGPR exceeded that of the controls. UW3 + UW4 improved the fresh weight of oat shoots (Fig. 2.3) and roots [117] by ~100%, relative to untreated (−PGPR) plants after 20 days in sodic soil (EC_e, 3.2 dS/m; SAR, 24) and 45 days in saline soil (EC_e, 14 dS/m; SAR, 11). Under the more saline conditions in wheatgrass experiments, PGPR (UW3 + UW4, CMH3) ameliorated salt stress, but did not bring biomass levels back to those of control plants grown under non-saline conditions [109]. Notably, the growth promotion effect was much greater using a mix of UW3 and UW4 than using either UW3 or UW4 independently (Fig. 2.3a). When the kinetics of oat growth is examined, with and without PGPR, it can be seen that PGPR protected the seedlings, especially during emergence and early growth phases (Fig. 2.4). This allows the plants to become established in impacted soils. It has been suggested that protection of young leaves is crucial for salt tolerance, due to the dearth of vacuoles available for Na^+ sequestration in these leaves, and the detrimental effects of Na^+ on protein synthesis and other processes crucial to plant growth [32, 79].

The studies described in this section, and those described in Sect. 1.5.3, show that PGPR can improve plant growth on salt-impacted soils. This indicates that crops and other plants not considered salt-tolerant can grow on saline soils with PGPR inoculation. This also supports the concept that equivalent land use can be achieved with PEPS and that phytoremediation of salt-impacted land via revegetation is feasible.

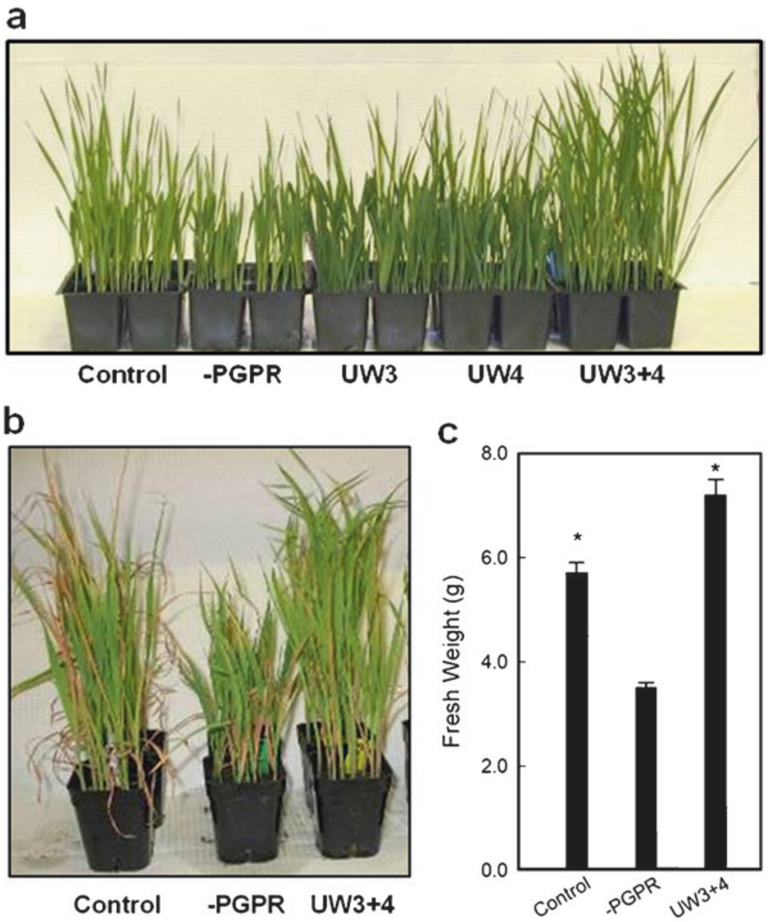
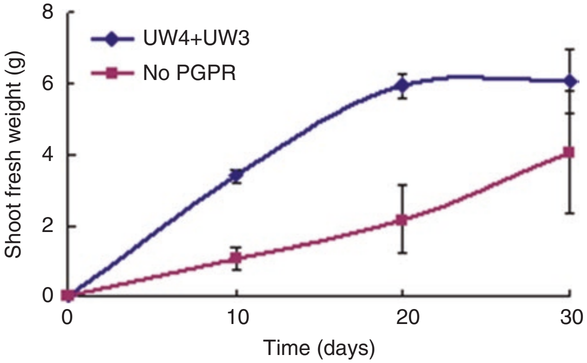


Fig. 2.3 Effects of PGPR treatment on plant growth in salt-impacted soil. (**a**) A representative photograph of oat growth after 20 days in sodic field soil from a site in Saskatchewan, Canada with low EC_e (3.2 dS/m) and high SAR [24]. “Control” shows the baseline normal plant growth in ProMix™ (EC_e < 2 dS/m) growth medium, “-PGPR” shows plants grown in saline soil without PGPR treatment, “UW3” shows plants that were grown from oat seeds treated with UW3 PGPR, “UW4” were treated with UW4 PGPR and “UW3 + 4” were treated with a mix of UW3 and UW4 PGPR. (**b**) A representative photograph of oat growth after 45 days in saline field soil with moderate EC_e (14 dS/m) and SAR [11]. (**c**) Fresh weight (g) of oat shoot biomass after 45 days growth in moderately saline field soil (EC_e = 14 dS/m, SAR = 11). The results are expressed as means ± SEM of four independent replicates ($n = 4$). Data were analyzed by one-way analysis of variance (ANOVA) and Dunnett’s test. * indicates statistical differences ($P < 0.01$) in biomass relative to untreated oats grown in saline soil

Fig. 2.4 Effects of PGPR on kinetics of plant growth in salt-impacted soil. Shoot biomass (FW) of oats ± PGPR in sodic field soil (EC_e, 3.2 dS/m; SAR, 24) at 10, 20, and 30 days



Alleviation of Salt Inhibition of Photosynthesis in PGPR-Treated Plants

Numerous abiotic environmental stresses, including salinization, result in deleterious effects on photosynthesis in plants [16, 57, 91, 109, 125–127]. Inhibition of photosynthesis is a good measure of the physiological state of the plant; therefore, measurement of various photosynthetic parameters can be used as an indication of the extent to which plants are salt-stressed. Indeed, negative impacts on plant growth due to salt stress are often associated with a decrease in photosynthetic rate, possibly the result of a decrease in stomatal conductance and the ensuing decrease in CO_2 uptake [44, 128–131]. Osmotic stress, which occurs rapidly following plant exposure to salt, results in a decrease in chloroplast volume and an increase in Na^+ concentration in the cytosol and chloroplasts. This can lead to inhibition of the photosynthetic electron transport chain [57, 125].

Chlorophyll *a* (Chl *a*) fluorescence is a useful technique for assessing photosynthetic activity [125, 132]. Chl *a* fluorescence parameters obtained using pulse amplitude-modulated (PAM) fluorometry (e.g., F_v/F_m, yield, qP, and qN) can be used to assess the efficiency of photochemistry in plants and to study the effect of salinity on photosynthetic electron transport [133]. F_m (maximal fluorescence of dark-adapted tissue) and F_0 (minimal fluorescence [background fluorescence]) can be used to calculate F_v/F_m ($[F_m - F_0]/F_m$) which indicates the maximum quantum yield of photosystem II (PSII) [133]. Optimal F_v/F_m values range from 0.79 to 0.83 for most plant species [134, 135]. Lower values indicate damage to the photosynthetic apparatus, and resultant plant stress. Yield of steady-state photosynthesis $[(F_m' - F_s)/F_m']$ can be calculated from the maximal fluorescence in light-adapted tissue (F_m') and steady-state fluorescence (F_s). Yield is a measurement of continuous photosynthesis (i.e., the amount of light absorbed by PSII chlorophyll that gets used in photochemical reactions) [136]. The parameter qP ($[F_m' - F_s]/[F_m' - F_0']$) is a measure of photochemical quenching, which indicates the proportion of open (or functional) PSII reaction centers [137–140]. Non-photochemical quenching of fluorescence, qN ($1 - [F_m' - F_0]/[F_m - F_0]$), is related to the dissipation of energy as heat and indicates the extent of photoinhibition [133, 139, 141].

Table 2.1 Effects of salinity and PGPR on chlorophyll *a* fluorescence of oats

Chlorophyll *a* fluorescence parameters	Control (ProMix™)	No PGPR (salt soil)	UW3 + UW4 (salt soil)	CMH3 (salt soil)
F_v/F_m	0.806 ± 0.002***	0.752 ± 0.013	0.801 ± 0.002***	0.803 ± 0.002***
Yield	0.690 ± 0.005***	0.488 ± 0.030	0.711 ± 0.020***	0.706 ± 0.022***
qP	0.910 ± 0.004***	0.729 ± 0.030	0.865 ± 0.010***	0.877 ± 0.006***
qN	0.301 ± 0.016**	0.429 ± 0.042	0.358 ± 0.032	0.317 ± 0.019*

PAM measurements were obtained after 20 days growth on ProMix™ (ECe < 2 dS/m) or salt-impacted soil (ECe = 30 dS/m) ± PGPR (UW3 + UW4 or CMH3)
F_v/F_m (maximal PSII activity), Yield (steady-state PSII activity), qP (photochemical quenching; indicates net energy storage), qN (non-photochemical quenching; indicates energy loss)
Results are expressed as means ± SEM of 12 independent replicates ($n = 12$). Data were analyzed by one-way analysis of variance (ANOVA) and post-hoc Dunnett's tests. * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) indicate significant differences between values for "No PGPR (salt soil)" relative to the other treatments

An example of salt effects on photosynthesis is given in Table 2.1. Oats, with and without PGPR treatment, planted on salt-impacted soil ($EC_e = 30$ dS/m) and control soil (ProMix, $EC_e < 2$ dS/m), were grown for 20 days [122]. Various photosynthetic parameters were measured using a PAM fluorometer using methods published previously [109, 142]. For plants without PGPR, all Chl *a* fluorescence parameters (F_v/F_m, yield, qP and qN) showed significant negative impacts due to growth on saline soil (note: qN rises under stress conditions, while the other parameters fall during stress) (Table 2.1). This suggests that the photosynthetic apparatus was damaged and photosynthesis was impaired [136, 143, 144]. These data are in agreement with our previously published results, and with those of numerous other researchers using oats and other plant species [61, 91, 109, 145, 146].

The negative impacts of salinity on overall photosynthesis were largely alleviated by PGPR treatment of plants (both UW3 + UW4 and CMH3): most Chl *a* fluorescence parameters (F_v/F_m, yield and qP) of the PGPR-treated plants had values that were similar to plants grown in control soil (Table 2.1). These results are consistent with the improvements in plant growth on salt-impacted soils that were observed in greenhouse experiments (Figs. 2.3 and 2.4). Similar relationships between growth promotion and photosynthetic capacity were reported in *Brassica* [130] and lettuce [44].

Oats are considered to have low tolerance to salt [147]. Despite the sensitivity of this species, treatment with PGPR alleviated photosynthetic stress. Thus, PGPR seed treatment can result in salt-sensitive species becoming more tolerant, thereby making them candidates for phytoremediation of salt and/or revegetation of salt-impacted soil.

Effects of Salinity and PGPR on Cell Membrane Integrity

Plant cell membranes play an important role in the maintenance of the micro-environment and metabolism of plant cells, and are often the first targets of abiotic plant stressors [41]. ROS-mediated membrane damage is a major cause of the

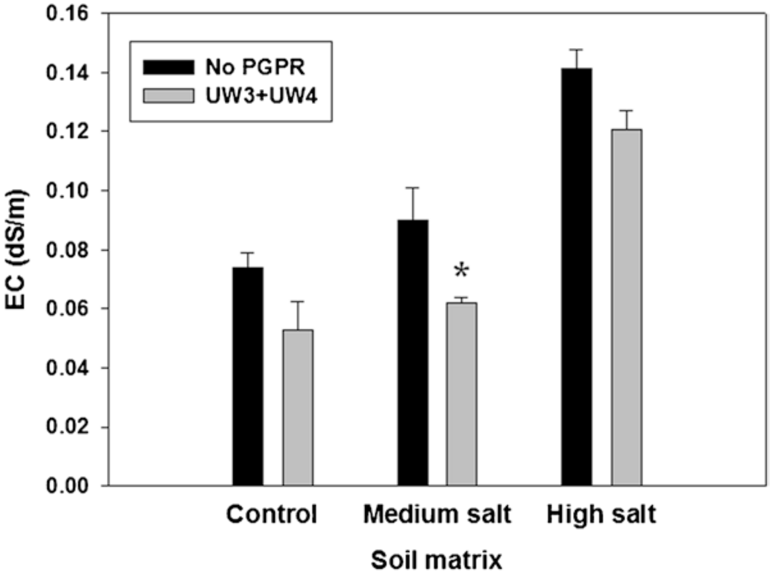


Fig. 2.5 Effects of salinity and PGPR on membrane damage in oats. Electrolyte leakage assays were performed on excised oat leaves from plants grown for 12 days on ProMix™ (ECe < 2 dS/m), moderately impacted saline soil (medium salt, $EC_e = 12$ dS/m), or highly impacted saline soil (high salt, $EC_e = 18$ dS/m) ± PGPR (UW3 + UW4). Results are expressed as means ± SEM of six independent replicates ($n = 6$). Data were analyzed by one-way analysis of variance (ANOVA) and post-hoc Bonferroni tests. * ($P < 0.05$) indicates a significant difference between values for "No PGPR" relative to PGPR-treated plants

cellular toxicity induced by salt stress in a variety of plants (see Sect. 2.1.4.2). Salt-induced ROS lead to damage to plant cell membranes and increase their permeability, allowing electrolytes that are contained within the membrane to leak into surrounding tissues [148]. Therefore, maintaining cell membrane stability and integrity is important for salt tolerance. The degree of damage to cell membranes can be estimated by measuring electrolyte leakage from cells, by comparing the electrical conductivity of the leaked contents (into water) from salt-stressed plant tissues to that of control plant tissues [109, 148, 149].

As an example, data on electrolyte leakage in oat leaves is shown in Fig. 2.5. The objective of the experiment was to assess cell membrane integrity following salt stress, and to determine whether PGPR could ameliorate the damage. Oat seeds, with and without PGPR treatment, were planted on moderately and highly impacted saline soils ($EC_e = 12$ and 18 dS/m, respectively) and control soil (ProMix™, $EC_e < 2$ dS/m) [122]. Shoots were removed from plants for electrolyte leakage analysis after 12 days of growth. Electrolyte leakage was measured as electrical conductivity (dS/m) of solutions containing ions that escaped from oat cells, presumably via damaged plasma membranes [109] (Fig. 2.5). The higher the EC (dS/m) value of the receiving water, the greater the damage to plant membranes.

Electrolyte leakage from plant tissues increased as soil salinity increased (Fig. 2.5), indicating that plant membrane damage increased with salinity level. The amount of electrolyte leakage was greatly diminished in PGPR-treated oats, indicating less damage to plasma membranes (Fig. 2.5). Similarly, Kang et al. [42] observed a protective effect of PGPR following induction of high salt stress: leaves of PGPR-treated cucumber had 21% less electrolyte leakage than control plants. The electrolyte leakage results indicate membrane damage due to salt stress, and are consistent with the photosynthesis results in the previous section (Alleviation of Salt Inhibition of Photosynthesis in PGPR-treated Plants). For instance, the lower yield and higher qN values in the absence of PGPR inoculation indicate loss of thylakoid membrane integrity relative to that in PGPR-treated plants.

2.2.2.2 Field Trials

The effects of salinity and PGPR on plant growth and salt uptake in field experiments were reported previously [83, 84, 110, 111, 117, 122]. Field trials were performed at upstream oil and gas sites with poor quality soils of varying soil salinities (EC_e, 2–40 dS/m) and sodicities (SAR, 1–45) in Manitoba, Saskatchewan, Alberta, and the Northwest Territories, Canada. Three strains of PGPR (UW3 + UW4, CMH3) were used to treat various grass species (tall fescue, tall wheatgrass, ryegrass, barley, oats) prior to phytoremediation to promote plant growth and increase tolerance to salt. Field trials were conducted over a period of two or three consecutive growing seasons. Details of bacterial inoculation of seeds, field trials, and analyses for Na^+ and Cl^- in plant tissues can be found in previously published work [83, 84].

Effects of Salinity and PGPR on Plant Growth

Results for the effects of salinity and PGPR on plant growth in the field have been published previously [83, 84]. Effects on plant growth were similar to those observed in the greenhouse: increases in soil salinity led to decreased plant biomass production in the absence of PGPR treatment. Treatment of seeds with PGPR alleviated the plant stress such that root and shoot biomass and ground cover were comparable to control plants grown in non-saline/sodic soils. In general, on saline soils, shoot biomass increases of 100–200% were observed in PGPR-treated plants, relative to untreated plants.

Uptake of NaCl from Soil

The NaCl concentrations in above-ground tissue of barley and oats from nine upstream oil and gas sites in Saskatchewan were measured following a single growing season [83, 84]. On a per mass basis, above-ground plant NaCl concentrations ranged from 22 to 97 g/kg (DW). Generally, on a per mass basis, about 2–3 times more Cl^- than Na^+ was stored in above-ground plant tissues. Notably, NaCl accumulation in plant foliage was accompanied by decreases in soil salinity (10–20%) at the field sites. Results from the field were in agreement with results previously obtained in greenhouse experiments [83, 109]. Salt removal (kg/ha) by barley and oats was comparable to literature values for various glycophytes, and Na^+ uptake was comparable to that of millet, another grass species [81].

Data from the Saskatchewan sites where PEPS was applied were compiled to obtain average values for typical salt remediation using this remedial strategy [84]. Standard PEPS experimental protocols were similar to those detailed in Chang et al. [83]. NaCl uptake into foliage averaged 29 g/kg (DW), with three times more Cl^- than Na^+ accumulation by weight in the foliage. An average of 150 kg/ha of NaCl was removed from the sites per harvest of above-ground biomass. A concomitant average annual decrease in EC_e of 15% was observed when the salt was only in the top 30 cm of soil (i.e., the rooting zone).

PGPR treatment did not result in increased NaCl uptake on a plant biomass basis [83]. That is, the concentrations of salt in the foliage were similar with and without PGPR treatment. However, the increases in plant biomass due to PGPR treatment were substantial (generally 100–200%). This is in agreement with our results from greenhouse experiments [109] and the findings of other researchers. For instance, Jesus et al. [14] indicated that a search of the literature showed biomass increases as a result of PGPR inoculation, but there was not a reference that showed an increase in salt phytoextraction on a biomass basis in any plant as a result of PGPR treatment. Similarly, treatment of a perennial rhizome grass with a plant growth-promoting mycorrhizal fungus did not increase uptake of Na^+ and Cl^- from salt-impacted soils [150]. Because of the increases in biomass due to PGPR or fungal treatments, the net effect was a much higher rate of NaCl removal from the soil for inoculated plants than that of untreated plants [14, 150]. These results are in contrast with those of Ozawa et al. [86]. They found that inoculation of a glasswort (a halophyte from the Chenopodiaceae family that sequesters Na^+ in valcuoles) with *Pseudomonas pseudoalcaligenes* did not increase fresh or dry weight of the glasswort shoots, but did increase Na^+ accumulation relative to uninoculated plants. This difference may be due to dissimilar plant growth conditions in general, the plant species used (a succulent marine halophyte), or the PGPR (an endophytic nitrogen-fixing bacteria).

Little research has been done to determine the connection of ion uptake by plants to actual observed changes in soil salinity in full-scale phytoremediation trials of salt-impacted soils. This was investigated when PEPS was employed on a salt-impacted (EC_e = 5.97 dS/m) upstream petroleum site in Saskatchewan, Canada [121]. Data from this field trial were used to conduct mass balance studies, to determine the efficacy of PEPS on saline soils. Plant tissue collected over two successive growing seasons was assayed for ionic content and these data were compared to measured changes in soil salinity (EC_e) for each field season. Based on the amount of five predominant ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-) in the plant tissue samples, removal of these ions from soil was measured, and the expected change in soil EC was calculated. These values were used to determine how much of the observed change in soil salinity could be attributed to ion uptake by PEPS plants during a given field season.

Soil EC_e decreased by 0.96 and 0.45 dS/m in the first and second year, respectively. The mass of salt ion uptake into plant biomass and total annual biomass were compared to the measured changes in soil salinity over the two field seasons. Taking into account the effect of each salt ion on the ionic strength of the soil solution, uptake of soil salt ions into foliar plant tissue accounted for 60.5 and 76.8% of the change in salinity in the first and second year, respectively. Notably, only five salt ions were included in the mass balance calculations; therefore, the change in soil salinity that was attributed directly to phytoremediation using PEPS was likely underestimated. This research provided evidence that, for PEPS field trials, the uptake of ions from the soil into plant biomass plays a predominant role in soil salinity decreases, and is not the result of water flux through the soil and movement of ions into deeper soil horizons.

In general, phytoremediation research in the greenhouse and the field has shown that salt concentrations in the foliage tend to be fairly similar on a per mass basis, independent of PGPR or fungal treatment, plant type, soil EC_e, and SAR. However, the increases in plant growth due to PGPR or fungal treatment tend to be large (average shoot biomass increase of 150%), particularly in poor soils and those with moderate to severe salt impacts. The extra biomass due to PGPR treatment will translate to greater salt removal from the soil. We suggest that the key to salt phytoremediation is to maximize growth with PGPR treatment or other means. Greater plant biomass should result in higher rates of salt remediation each growing season when PEPS are employed.

2.2.3 Feasibility of Salt Phytoremediation Using PEPS

For salt remediation, PEPS are effective for several reasons: (1) The PGPR alleviate plant stress and promote growth by conferring salt tolerance to the plants, as well as conferring tolerance to potential co-contaminants such as PHC and metals. (2) The PGPR protect plants against other potential abiotic stressors (e.g., cold) that result in the production of stress ethylene and decreased rates of plant growth. (3) The large amount of root biomass produced in the soil allows for effective partitioning of NaCl out of the soil into the biosphere. (4) Foliar tissues of PEPS plants can be harvested, thereby removing accumulated salt from the site. Harvested vegetation will not have sufficiently high levels of salt ions to be considered high-salt waste. (5) PEPS are adapted to site-specific conditions (i.e., from the site in question), which increases the chance of successful remediation.

Since 2009, PEPS has been deployed for full-scale remediation of several salt-impacted sites in Manitoba, Saskatchewan, Alberta, and the Northwest Territories, Canada [111]. Remediation goals were met at eight of these sites, either by lowering soil salinity (EC_e) levels to generic regulatory criteria, or by restoring plant growth and productivity to equivalent land use (i.e., equivalent growth and productivity to areas surrounding the site). We have observed that the EC_e drops at a rate of approximately 15% per year when the salt is present only in the rooting zone [84].

The average amount of NaCl taken up into the leaves of PEPS grass plants is 29 g/kg (DW). An average of 150 kg/ha NaCl is removed from a field per crop harvest. Thus, soils with an EC_e of 10–15 dS/m, spread to a depth of 0.5 m (approximate rooting zone of grasses used in PEPS) can be remediated in about 5 years [111]. We note that as remediation proceeds, and soil salt levels drop and the soils improve, the plants will grow better, which should lead to accelerated rates of revegetation and remediation. More biomass will be produced per growing season, and the levels of NaCl taken up by plants does not drop as the EC_e decreases (see the section entitled "Uptake of NaCl from Soil"). Given that research has shown that we can successfully establish plant growth using PEPS on salt-impacted sites before soil salt levels drop to generic regulatory criteria, phytoremediation based on revegetation and equivalent land use will occur sooner than the 5 year estimate based on salt uptake and biomass calculations.

2.3 Conclusions

Research described in this chapter indicates that salt phytoremediation is feasible using PEPS and other systems. Rapid plant growth leads to revegetation of salt-impacted sites, typically in less than 5 years. The calculations for the estimated time required to remove NaCl from salt-impacted soils suggest that salt ions can be phytoextracted from soil at an acceptable rate, which will lead to unimpacted soil in the long term. Revegetation and removal of salt from impacted soils should accelerate in successive years of PEPS treatment as the soil improves, because this will facilitate increased plant growth over time, which in turn will provide a larger sink for soil salts. Finally, revegetation may be the most important aspect of salt phytoremediation, and in many cases can be considered a key measure of successful salt phytoremediation.

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Chapter 3
Successful Integrated Bioremediation System of Hydrocarbon-Contaminated Soil at a Former Oil Refinery Using Autochthonous Bacteria and Rhizo-Microbiota

Valentina Spada, Pietro Iavazzo, Rosaria Sciarrillo, and Carmine Guarino

Abstract The development of industrialized global economy have produced a strong contamination by the petroleum-based products resulting from the activities related to the petrochemical industry; in the last years, the hydrocarbons become one of the major environmental problems. Bioremediation is a new approach based on the use of microorganisms (bacteria and fungi) and plants, and it has been researched extensively for possible applications related to hydrocarbon degradation in the petroleum industry.

The scope of the application of this technology on soil of a former oil refinery is the production in situ of strong and diverse enzymatic activity such as to attack the hydrocarbon molecules through various routes of enzymatic degradation. The application of a remediation based on the biological degradation process by means of a strategy of action based on in situ degradation principles of aerobic bacteria, fungi, and plants either through biostimulation actions of the indigenous microbial population, both by increasing the content of the same flora through further introduction of native bacteria, fungi, and plants has the advantage of reducing the risks of residual contaminants and/or inverse transformation.

Keywords Bioremediation • Total petroleum hydrocarbons • Autochthonous bacteria • Land farming • Biostimulation • Phytoremediation • Bioaugmentation • In situ treatments

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3.1 Introduction

In the last years, there is a widespread knowledge that the soil is an important component of the environment, and it is not an inexhaustible resource. An improper use of extracted organic substances from the ground can lead to a depletion of resources and a possible loss into the environment. One of the major global problems is contamination by the petroleum-based products, resulting from the activities related to the petrochemical industry; especially in the past, when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today [1]. Petroleum products are principal components of our society, and increasing number of sites contaminated by hazardous organic contaminants are detected. The industrialized countries have regulated the emission of toxic substances into the environment and stated the need to reclaim the now-contaminated environments because the pollution with petroleum and petrochemical products has been recognized as a significant and serious problem. Most components of petroleum oil are toxic and hazardous to the health of plants, animals, and human, and it is easy to incorporate into the food chain; these dangerous aspects have increased scientific interest in examining the distribution, fate, and behavior of oil and long-term damage to aquatic and soil ecosystems and natural resources. At first, conventional technologies were used for the soil remediation, for instance, chemical oxidation, thermal desorption, and excavation with *off-site* disposal in landfill [2] but these technologies have shown many disadvantages as more expensive with high energy consumption and can also lead to incomplete decomposition of contaminants. Later, biological methods were applied in contrast to traditional soil remediation technologies; they are environmentally friendly approaches and cost-effective having a positive impact on public opinion and can often be carried out in situ. With bioremediation, we identify a set of eco-friendly techniques that use biological agents, such as bacteria, fungi, and green plants to remove or neutralize hazardous substances in polluted site, known green technology like land farming, biostimulation, phytoremediation, and bioaugmentation [3, 4]. Bioremediation offers many advantages over traditional remediation technologies also because it can be applied in situ without the need to remove and transport the contaminated soil and is usually less expensive and less labor intensive [5]. Land farming is both ex situ technique in which contaminated soil is excavated and periodically tilled until pollutants are degraded and *on-site* method because it is spread the contaminated soil in a thin layer on the surface to be decontaminated in order to stimulate aerobic autochthonous microbial activity and to facilitate degradation of pollutants. Phyto- and bioaugmentation are a set of processes: fungi and bacteria can detoxify and remove by breaking down pollutants such as hydrocarbons into less harmful substances through their difference metabolic capabilities (enzymes with biodegradative activity). Green plants (hyperaccumulators or metallophytes), instead, can aerate polluted soil or stimulate enzymatic microbial activity, with petroleum contaminants, and they can absorb heavy metals into their tops, which are then harvested. The plants are able to tolerate phytotoxic level of heavy metals, and they can survive and reproduce in polluted soil developing systems to survive and to adapt themselves at extreme environmental condition (increased the tolerance to heavy metal ions or restricted the entry or root-to-shoot translocation) [6–11]. Through biostimulation we can add nutrients, oxygen, and electron acceptors or donors to increase the population and the activity of naturally bacteria that are able to degrade pollutants [12, 13] or to stimulate plants ability to adsorb inorganic compound by environment respecting environment characteristics. Finally, into some bioremediation strategy with a mix of pollutants, plant growth-promoting bacteria (PGPB) are used because they can interact with roots of many different plants and some of them colonize the interior of the plant as well, showing advantages for each other [5, 14–17]. Each technique shows the ability to remediate a specific pollutant but the removal of toxic compounds from the sites is further complicated when the pollution is multiple and involves numerous classes of compounds, as heavy metals or total petroleum hydrocarbons (TPHs), divided into aliphatic ($C < 18$ and $C > 18$) and aromatic hydrocarbons. Bioremediation techniques are rapidly increasing because it is a good alternative to conventional cleanup methods and has been used in sites worldwide with success. It requires highly qualified staff, engineers, and chemists who cooperate to improve remediation of polluted sites with knowledge, great potential, and experiences into innovative technologies. Some researchers demonstrated reducing of the contaminant concentration and ecotoxicity in the soil via bioremediation processes made by autochthonous microorganisms [3, 18, 19]. Under the selective pressure of environmental pollution, only some microorganisms are able to resist and degrade pollutants as TPHs. Contaminant are transformed and breaking down by living organisms through their enzymatic metabolic processes, but the biologic degradation is often a result of the action of consortium of microorganisms (autochthonous bacteria of soil and rhizobacteria). Recent studies have isolated and identified a large number of species of microorganisms that are able to degrade a wide range of natural and xenobiotic compounds like hydrocarbonic or aromatic molecules (*Bacillus, Pseudomonas, Comamonas, Acinetobacter, Alcaligenes, Streptomyces, Sphingomonas*) and different fungi (*Aspergillus*, nonligninolytic or ligninolytic fungi) [7, 20–22]. Each of them is able to use contaminants as sole carbon source and to develop common biochemical pathways for degradation (protein pattern or specific catabolic genes).

Until now, many works of soil bioremediation have been carried out in laboratory, instead the field experiments are scarce [23, 24]. This biotechnological approach has received a great attention in the recent years.

Below, we have shown our case study of an integrated bioremediation system of hydrocarbon-contaminated soil from a decommissioned refinery in Italy using autochthonous bacteria and rhizo-microbiota. A total biological remediation process allowed to overcome many of the restrictions linked to the application of individual techniques achieving successful results with an in situ combined strategy of different technologies, according to the site-specific features previously detected in laboratory-scale assay. The main principle of selected strategy is represented by stimulation of the aerobic degradation of autochthonous bacteria activity and their emphasizing, previously isolated in laboratory. Our Multi-Process System has allowed to reduce the concentration of contaminants in short time respect to other single method.

3.2 Integrated Bioremediation System of Hydrocarbon-Contaminated Soil: Case Study

In order to close this section for readers and to explain the possibility of in situ application of this multi-process system, we describe the aim of our work: it is the remediation of a TPHs-contaminated soil in a former oil refinery in northern Italy by using an in situ application of an integrated bioremediation system with autochthonous bacteria, rhizo-microbiota, and plants. The basic principle of the aerobic biodegradation performed by autochthonous bacteria associated to a bioaugmentation step with the indigenous bacteria consortium previously isolated and characterized in laboratory.

According to the site-specific features previously detected in laboratory scale assay, a total biological remediation allowed to overcome many of the restrictions linked to the application of individual techniques achieving successful results with an in situ combined strategy of different green technologies: land farming, biostimulation, phytoremediation, and bioaugmentation as previously described. Experimental design consists of different and specific steps that follow one another. After identifying the polluted area, we carried out:

- detection of pollutant area and sampling
- laboratory activities split into isolation and identification of autochthonous biodegrading bacteria, quantization and characterization of TPHs, and mesocosm trials in order to show the best combined technologies to remediate polluted soils;
- application of bioremediation integrated method in situ (trial area). The third step was made from: land farming, biostimulation, bioaugmentation, phytoremediation, and biosparging and all performed based on the laboratory results and monitoring.

Below we will analyze individually steps of our pilot work and they are summarized in Table 3.1.

3.2.1 Detection of Pollutant Area and Sampling

Our study was carried out with the soil from a decommissioned refinery (about 400.000 m^2) located in Italy, contaminated by TPHs. On the basis of previous analysis, we selected six points with different levels of contaminants at two different depths.

Table 3.1 Activities performed into bioremediation integrated method (in laboratory and in situ techniques)

Activities		Actions	Targets
Laboratory activities		Quantization of TPHs	In order to determinate hazardous organic contaminants detected into soil
		Isolation and characterization autochthonous microorganisms from polluted soil	To identify indigenous biodegrading bacteria to inoculate in mesocosm and in situ field
In situ application			
Preliminary operations	To the surface	Removing spontaneous vegetation, stones, and inert materials	Prepare experimental in situ plane
		Levelling of soil	
		Water drainage system	
Land farming + biostimulation	0–1.5 m	Soil plowed deeply and added of fertilized	To promote aeration and stimulate and enhance metabolism and the oxidation of contaminants
Phytoremediation		Planting of selected species	Vigorous root system that are able to create luxurious rhizosphere ideal for microbial growth
Bioaugmentation (PGPB)		Introduction of previously selected indigenous bacteria and PGPB	To allows the increase of microbial biomass
Biosparging	1.5–2.5 m	Introduce air and nutrients	

Fig. 3.1 Core drill used to collected soil

Fig. 3.2 Example of collected soil for laboratory analysis

These samples were used for laboratory analysis to detect the amount of total petroleum hydrocarbons (especially the more recalcitrant fraction, C > 12) and cultivable aerobic microbial population was evaluated (Figs. 3.1 and 3.2). Environmental investigations carried out at different depths of pollutant soil highlighted values higher than Italian regulatory limits provided by D. Lgs. 152/06.

3.2.2 Laboratory Activities

3.2.2.1 Analytical Analyses

Soil samples were collected using a core drill (Fig. 3.1) in order to arrive at two different depths (max 3 m) and arriving in laboratory the samples were stored at −20 °C until specific analysis. To determine the amounts of TPHs and their molecular characterization, soil samples were evaporated and analyzed with analytical method that combines gas chromatography and mass spectrometry (GC-MS). We have identified following groups, considered even for in situ experiments as: low molecular weight hydrocarbons ($C \leq 12$), aliphatics (C_{13}–C_{18} and C_{19}–C_{36}), aromatics (C_{11}–C_{22}), and high molecular weight hydrocarbons ($C > 12$). In all pollutant-collected samples, the values of hydrocarbons are higher than Italian regulatory limits provided by D. Lgs. 152/06.

3.2.2.2 Characterization of Autochthonous Biodegrading Bacteria

Before starting with the isolation of hydrocarbon-degrading bacteria, we have made a total viable count of cultivable aerobic native bacteria (for all points, we have about 10^6 colony forming units, CFU); after opportunity dilutions and planting on LB agar (incubated at 26 °C for a week), the colonies were counted and values were expressed as colony forming units. Enrichment cultures performed with contaminated soil and diesel as sole carbonic source (5%) allowed the selection and genomic DNA was extracted from every isolates using specific DNA purification Kit (Wizard® Genomic DNA purification Kit, Promega). 16S rRNA gene was used as template with universal primers, F27 and R1492. Polymerase chain reaction (PCR) was performed according to supplier's instructions and the PCR-amplified DNA was sequenced using an automated DNA sequencer (ABI 3500 Genetic Analyzer). The partial 16S rRNA gene sequences from the isolates were deposited in the GeneBank database in order to obtain the characterization of isolated strains.

A total of about 30 different bacterial strains were isolated and identified from TPHs-contaminated soils (Table 3.2). The isolated strains were evaluated also for the tolerance to the heavy metals and the capacity to produce auxine indoleacetic acid (IAA), one of the most important plant growth-promoting molecules. Phylogenetic analysis (with BLAST analysis) showed high identity to strains belonging to the phylum of *Proteobacteria* (10% *α-proteobacteria*, 30% *β-proteobacteri*a, and 60% *γ-proteobacteria*).

Having characterized indigenous bacteria, the consortium was lyophilized and stored until the next application in situ step.

Often, as in our study, soil bacteria owning hydrocarbons-degradation pathways, also show plant growth-promoting features and actually the use of plants in conjunction with hydrocarbons-degrading and plant growth-promoting bacteria (PGPB) offers much more potential for the remediation of hydrocarbons-contaminated soils [17]. Additionally, PGPB mitigate plant stress responses thus enhancing plant

Table 3.2 Microorganisms isolated from the hydrocarbon-contaminated site; tolerances to heavy metals, capacity to production auxine as idoleacetic acid (IAA) and capacity to grow on Diesel oil a sole C source; microorganism with same genera and specie names show genetic sequence difference, and it is highlighted even by different responses at pollutants

Bacterial isolates/closest described relative	$CuCl_2$ (150 mg/L)	$NiCl_2$ (25 mg/L)	$HgCl_2$ (10 mg/L)	AsIII (10 mM)	AsV (100 mM)	Growth on DIESEL oil	Production of the IAA (mg/mL)
	Tolerance to metals						
Acetobacter pasterianus	+	+	–	+	+	–	–
Achromobacter marplatensis	+	+	–	–	–/+	–	–
Achromobacter spanius	+	+	–	–	–/+	–	–
Achromobacter marplatensis	+	+	–	+	–/+	–	–
Comamonas koreensis	+	+	–	–	+	–	–
Comamonas testosteroni	+	+	–	–	+	–	–
Comamonas testosteroni	+	+	+	–	+	–	–
Comamonas aquatica	+	+	–	+	+	–	53.54
Delftia acidovorans	+	+	–	+	+	–	–
Delftia acidovorans	+	+	–	–	+	–	–
Ochrobactrum anthropi	+	+	–	–	+	–	–
Pseudomonas stuzeri	+	+	–	–	+	–	–
Pseudomonas brassicacearum	+	+	–	+	+	+	–
Pseudomonas brassicacearum	+	+	–	+	+	+	13.44
Pseudomonas migulae	+	+	+	–/+	+	+	–
Pseudomonas mandelii	+	+	+	+	+	+	10.95
Pseudomonas frederiksbergensis	+	+	+	–	–	+	7.21
Pseudomonas mandelii	+	+	–	–	–/+	+	17.90
Pseudomonas chloraphis	+	+	–	–	–	–	–

Pseudomonas chloritidismutans	+	+	–	–	–/+	–	–
Pseudomonas putida	+	+	–	+	+	+	6.41
Pseudomonas putida	+	+	+	–	–	+	6.63
Pseudomonas resinovorans	+	+	+	–	+	–	–
Pseudomonas resinovorans	–/+	+	–	–	–/+	–	–
Pseudomonas alcaliphila	–/+	+	–	–	–/+	–	19.73
Pseudomonas corrugata	+	+	–	–	–/+	–	–
Pseudoxanthomonas mexicana	+	+	–	–	–/+	–	–
Sphingobium abikonense	–/+	+	+	–/+	–/+	+	4.19
Stenotrophomonas rhizophila	+	+	–	–	+	–	–
Pseudomonas frederiksbergensis	+	+	+	–	–	+	63.95

growth and development [25]. Some of these bacteria are able to degrade and survive alone into TPHs-contaminated soil (as *P. putida* or *P. mandelii*). Many scientists have studied the expression of specific pathway enzymes of several *Pseudomonas* or *Sphingomonas* strains that are able to degrade a wide range of natural or xenobiotic compounds or pesticides in no-toxic-substances [20].

3.2.2.3 Mesocosm Trials

After identification of indigenous biodegrading bacteria, we have designed a mesocosm trial in order to test different remediation methods using polypropylene trays containing sieved soil and monitoring all environmental parameters (as temperature and aeration).

Only eight of 12 samples were used for mesocosms and represented different contamination levels. We have applied three different experimental conditions for 90 days to verify the best bioremediation methods in order to use for in situ experiments: first, we have simulated site conditions and the soil is dampened by the rain, called Natural Attenuation (NA); in the second set of trays, we have added N-P-K mixture as nutrients only one time and irrigated twice a week with demineralized water. So we have plowed soil to promote aeration and biodegrading bacteria activities, and this treatment is called Land farming (L). In the third set, we have treated the soil with the same manner as the second one and after 60 days we have added indigenous bacteria strains that we have isolated and identified (LB). At the end of 90 days, we have collected one sample for each tray, and we have quantified the amounts of TPHs after treatments in order to define the optimum method. Analytical analyses were made at T_0, $T_{60,}$ and T_{90} days for all trays and the positive effects of major variation and decrease of hydrocarbons were highlighted after LB treatment.

3.2.3 In Situ Activities

3.2.3.1 Pilot Field

According to site-specific features and data obtained from mesocosm trials, a total biological remediation process with an innovative and sustainable strategy based on the combination of mechanical, microbial, and plant growth processes was applied; this is an integrated bioremediation system.

For in situ experiments, we have used only a trial area of about 700 m^2 at two different soil depths (Fig. 3.3), and we have applied integrated bioremediation system for a period of 5 months, started after laboratory experiments, as mesocosm trials with positive results which have shown different enzymatic degradation pathways by aerobic bacteria. Specifically, we have worked in two depths (0–1.5 m and 1.5–2.5 m), in which we have applied simultaneously different biological remediation

Fig. 3.3 Pilot area

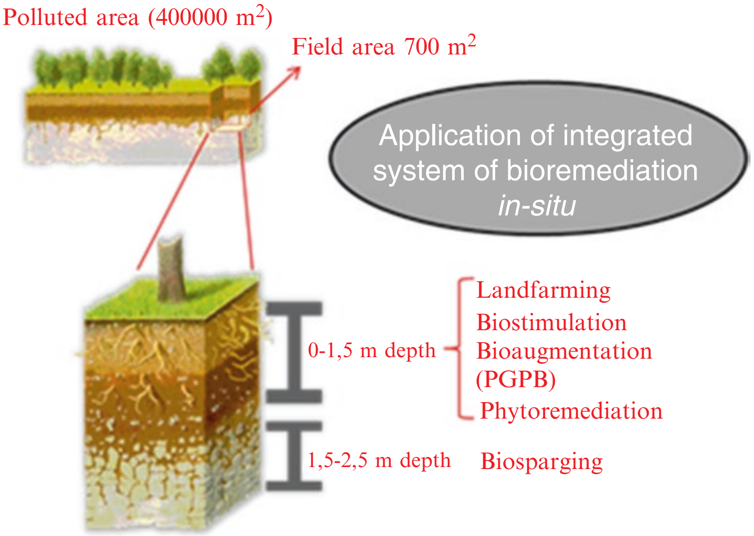


Fig. 3.4 Experimental design of in situ application of integrated bioremediation system

techniques. The main applied methods are: cleanup of soil, land farming, biostimulation, phytoremediation, and bioaugmentation with the injection of specific commercial mix of PGPB and finally biosparging (Fig. 3.4).

Before starting with all activities of bioremediation set, we have applied some operations that are necessary to clean up and prepare the field: mowing of spontaneous vegetation, removal of stones or inertial materials; these operations are inserted into total bio-approach.

Fig. 3.5 Moving top-soil with excavator

3.2.3.2 Land Farming

At first, land farming method was applied in order to promote aeration and to biostimulate the oxidation of contaminants by indigenous bacteria. Soil was plowed deeply, weekly and for 40 days, up to about 1.5 m, using a mechanical excavator (Fig. 3.5). Then, levelled field for the following activities and to facilitate the processing of the hydraulic-agricultural settlement of soil, we have created system for the outflow of excess water and draining surface one; all these operations were developed into a period of 2 months.

3.2.3.3 Biostimulation

After the preparation of soil steps, nutrients were added to the soil at different time and the selection of type and amount were made according to biological and agronomic conditions. Biostimulation method was performed in order to increase the degrading activities of indigenous community and to ensure rapid root development. The mycorrhization was developed in two steps: first, before planting, in order to create the best condition for future plant growth (about 100 g/m^2 and at 20 cm of depth); second, after planting, directly applying mix to the roots manually and wetting the surface to promote the germination of fungal spores (Fig. 3.6). Finally, we have fertilized with slow-release ternary fertilizer containing Nitrogen-Phosphorus-Potassium (N-P-K) about 100 g/m^2 of fertilizer were uniformly distributed by hand in order to promote degrading and energy metabolisms of autochthonous bacteria and create optimal condition for following plants.

Fig. 3.6 In situ field application of fertilizer and mycorrhizae manually

3.2.3.4 Phytoremediation

In phytoremediation step, we have selected in reference to literature and preliminary studies of the soil (chemical characteristics and amount of pollutants) and planted: *Festuca arundinacea* Schreb., *Phragmites australis* (Cav.) Trin. ex Steud. (autochthonous), and *Populus nigra* L. (autochthonous) (Table 3.3). All selected plants have a vigorous root system that is able to create an optimal structural function for microbial growth. Phytoremediation has been recognized an effective and eco-sustainable method to remove inorganic and some organic molecules by employing a variety of mechanisms often to support microbial degradation [26].

After doing pump system, we have planted the vegetable species into pilot area split in two parts: parcel A *P. australis* + *F. arundinacea* and parcel B *P. nigra* + *F. arundinacea* (Fig. 3.7). With a frequency of two times a week, we have irrigated the plants during the dry period with a frequency of two times a week.

3.2.3.5 Bioaugmentation

Bioaugmentation method was divided into two steps: preparation of inoculum to inject starting by isolated and characterized biodegrading consortium bacteria plus selection of commercial mixture of PGPB and then the injection of each mixture.

We have used similar growth condition and enrichment steps of laboratory step for preparation of bacteria inoculum for in situ application, starting from consortium prior lyophilized. The bacterial consortium was grown using bioreactors

Table 3.3 Plants used for phytoremediation and their main feature

	Application in Phytoremediation	Structural functions	Planting technique	Distribution in situ
Festuca arundinacea	Change the composition of the rhizospheric microbial population promoting the degradation capacity of organic compounds	High coverage density and deep root system (up to 80 cm)	Hand seeding	Uniform distribution
Phragmites australis	Mostly used for constructed wetland	Deep root system (60–80 cm): the long rhizomes allow the creation of oxidized micro-zones easily colonized by aerobic bacteria	Planting of autochthonous rhizomes	70 × 70 cm
Populus nigra	Widely used in rhizodegradation for the intensive phytoremediation performed by roots	Preservation of aquifers made by large root system	Transplant of autochthonous trees	1 × 1 m

Fig. 3.7 Pilot field after planting and pump system

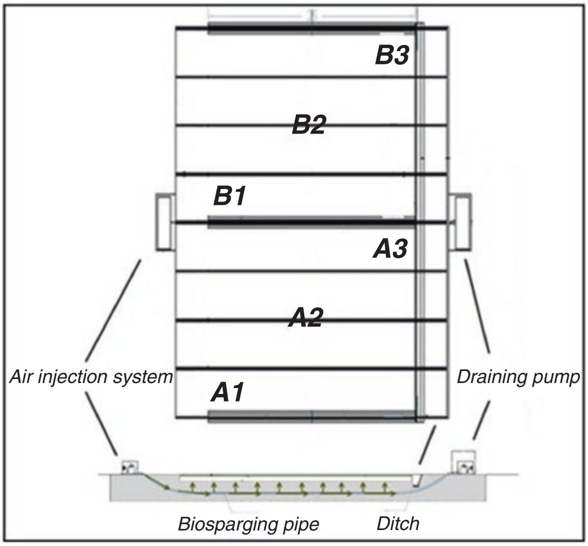
Fig. 3.8 Bacterial consortium

(BIOSTAT® Cplus, Sartorius) that are able to control growth condition and to keep constant for all time (28 °C, pH 6.8, flow air 10 (middle) and we added 5% diesel as sole carbonic source). We have achieved a final concentration of 10^9 CFU/mL (Fig. 3.8). The injection was realized using a flow-controlled injector pole which allowed precise distribution at low pressure and the pole was pointed in proximity to the root system and the pump was necessary to assure homogenous air distribution. The injector pole was pointed in proximity to the root system (20–30 cm of depth) and about 220 mL/m^2 of consortium were inoculated in the whole pilot field (Fig. 3.9). By adding bacterial consortium, we increase the microbial concentration and stimulate autochthonous bacteria distributing air flow in order to enhance the pollutants biodegradation. It is advantageous to increase both the tolerance and the resistance to variations in natural environment.

In addition to autochthonous consortium, we have selected even a commercial microgranular consortium in according to site-specific features and added into rhizosphere with action like plant growth promoting. Commercial inoculum was made from mycelium and vital spores of arbuscolar micorrhizal fungi of *Glomus* sp. enriched by natural microorganisms (*Trichoderma* sp., *Bacillus* spp., *Streptomyces* sp., *Beauveria* sp., *Metharhizium* sp) that are able to stimulate and emphasize microbial degradation of TPHs.

Fig. 3.9 Direct inject into field with injector pole

Fig. 3.10 Layout (name of samples analyzed) and area divided with biosparging system



3.2.3.6 Biosparging

The biosparging system is the same used to biostimulate (Fig. 3.10) and add the microbial consortium; we have stimulated the previously detected activity of autochthonous bacteria in saturated zone distributing an air flow in order to enhance the biodegradation. All over the pilot field, we have realized nine dug-out (every 25 m). Along each dugout, a horizontal piping formed by two coaxial tubes was laid at about 1.80–2 m b.g.s. The external tube (ϕ = 160 mm) was provided with

Fig. 3.11 Structure of biosparging system into pilot field

microslots along the buried length. The inner microslotted tube ($\phi = 63$ mm) was connected to a low pressure air compressor at one side and to a draining pump at the opposite side in order to purge out the excess water that was then stocked in a tank container for further dump. The air compressor worked in continuum at about 120 mbar of pressure for 80 days. Pump pressure was estimated to overcome hydrostatical pressure and to assure homogenous air bubble distribution along the whole piping length in the overheading soil layer (Fig. 3.11). At the same time, contaminants volatilization due to air sparging was taken into account and evaluated as negligible. Finally, to increase oxygen distribution process into saturated zone, we have injected a commercial product, with a high quality calcium peroxide powered, and widely used to enhance aerobic bioremediation processes due to the slow release of oxygen and heat when in contact with water.

3.3 Integrated Process Monitoring and In Situ Results

Periodically, the efficiency of applied remediation methods were evaluated monitoring the concentration of pollutants into soil samples (at 50 cm of depth) at different times: T_0, before remediation process, T_1 (after land farming and biostimulation,

Fig. 3.12 Soil sampling points (in *red* the points corresponding to T_0, T_1, T_2, T_f; in *blue* only T_f)

before phytoremediation and bioaugmentation, day 50), T_2 (after phytoremediation and bioaugmentation, day 100), T_f (at the end of the trial, day 150). Besides, the plant growth was conducted with visual monitoring at the end of the process (T_f) and determinated the total microbial biomass (CFU) (Fig. 3.12).

The goal of our study was to show mainly the efficiency of different integrated technologies applied in situ by decreasing TPHs amount followed by an increase of biodegradation activity of autochthonous bacteria, in a short time (150 days). Moreover, we have used plants with potential role into phytoremediation approach [27–29].

At first, we have evaluated the plants growth and their activity, even if the short range time of experiments did not allow to develop all parts of them. At T_f (the end of trial), the suitable rooting and the first plants growth signs were detected: *Festuca arundinacea* covered homogenously the whole pilot field; little trees of *Populus nigra* had all responded well to the transplant and began to form the first dormant buds, and first shoots of *Phragmites australis* began to rise up. These parameters did not yet show effect of plants into remediation process because probably the root system of plants was still at the early stage of growth but they are able to grow in contaminated area.

Autochthonous bacteria proliferation and the increased of microbial degradation activity in soil were enhanced by the use of integrated technology, in fact, land farming and biostimulation were used to inject oxygen in order to improve environmental conditions for microbial growth and bioaugmentation to increase the total biomass (Fig. 3.13).

In Table 3.4 and Fig. 3.14, we have showed microorganisms content at different steps of integrated strategy, with an increase at the end of trial, after bioaugmentation of microbial consortium that are able to remediate TPHs.

The total compounds present in soil before, during and after the application of Multi-Process System were analyzed using GC-MS and matched with the limits established by Italian environmental legislation (D. Lgs. 152/06). The preliminary characterization has showed the amounts of PAHs; BTEX and heavy metals (HMs) (Table 3.5) were below the established limits by law.

Fig. 3.13 Sampling of soil with little core drill in order to monitor the effects of multi-process steps

Table 3.4 Autochthonous bacteria proliferation (values of CFU)

Sample (depth)	T_0	T_1	T_2	T_f	Δ (CFU/g dry)
A1 (0–1)	1.27E + 05	1.63E + 07	1.57E + 07	6.79E + 06	6.66E + 06
A1 (1–2)	1.27E + 07	2.94E + 07	1.1E + 07	–	−1.61E + 06
A2 (0–1)	2.63E + 06	4.46E + 07	2.13E + 07	–	1.86E + 07
A2 (1–2)	2.65E + 07	5.91E + 07	2.35E + 07	–	−2.98E + 06
A3 (0–1)	5.63E + 06	5.95E + 07	1.25E + 07	–	6.88E + 06
A3 (1–2)	5.17E + 07	6.11E + 07	1.81E + 07	–	−3.36E + 07
B1 (0–1)	1.119E + 07	5.49E + 06	5.12E + 07	–	3.94E + 07
B1 (1–2)	9.3E + 06	1.06E + 08	1.95E + 07	–	1.02E + 07
B2 (0–1)	2.28E + 07	2.11E + 07	5.77E + 06	3.19E + 07	9.09E + 06
B2 (1–2)	4.61E + 07	7.17E + 07	3.07E + 07	5.63E + 06	−4.04E + 07
B3 (0–1)	1.04E + 07	6.33E + 06	3.19E + 07	3.33E + 07	2.3E + 07
B3 (1–2)	2.70E + 07	3.1E + 07	1.59E + 07	–	−1.11E + 07

TPHs represented the only contaminant class present into area and at significant concentration level; a specific analytical method was allowed to identify four different groups: low molecular weight hydrocarbons ($C \leq 12$), aliphatics (C_{13}–C_{18} and C_{19}–C_{36}), aromatics (C_{11}–C_{22}), and high molecular weight hydrocarbons ($C > 12$) (Table 3.6). Long chain hydrocarbons are less biodegradable and more recalcitrant to biological actions as well as they are less volatile and soluble in the water (decreased of migration into environment). Changes in the residual of THPs of soil samples treated are showed in Fig. 3.15, quantified in top and deep layer. We have reported the contamination values as percentage ratio compared to starting contamination (T_0). In panel (a) we have showed a significant TPHs concentration decrease (about 50%) after land farming and biostimulation step (T_1). The pollutants removal

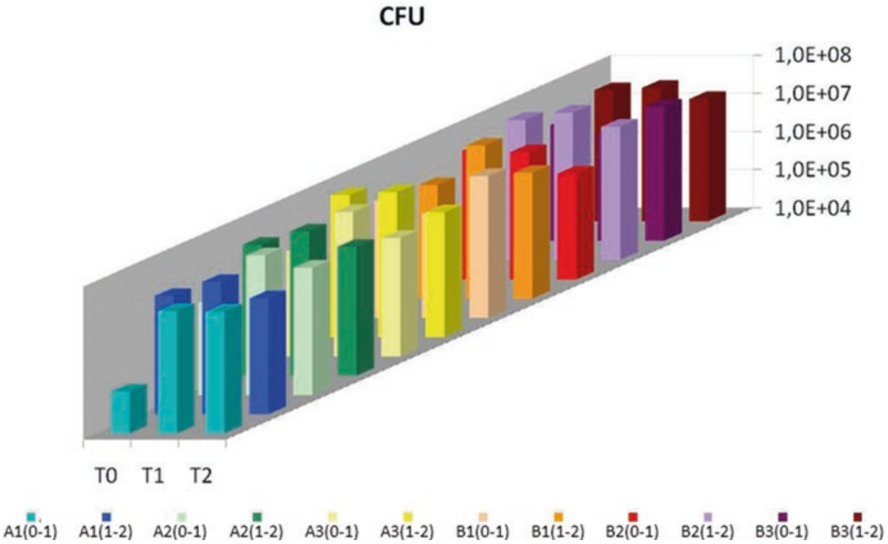


Fig. 3.14 Autochthonous bacteria proliferation

speed after 50 days is decreased due to the lower amounts in soil. Each stage of the applied technological process led to a reduction value of TPHs content always higher than 40%, highlighting a significant removal speed at each phase. At the end of the trial test (T_f), the remediation speed was found higher than 85% with respect to the initial contamination. Instead, the removal rate showed in panel (b) is too slow compared to the first step of remediation into top layer (T_1); this is due to a lower soil aeration which leads to a slower activation of biological processes. The following phases of biosparging and bioaugmentation showed a faster removal rate, comparable to that observed for top layer, confirming the high efficiency of the two combined processes. Finally, despite the first slower phase, we observed a remediation of about 80% (T_f) with respect to the initial contamination (T_0).

TPHs concentrations were always below the Italian legal limits at the end of the trial (T_f), both top or deep layer. All the results did not show any substantial difference in removal rate between C_{13}–C_{18} and $C_{19–36}$ fractions, confirming the effectiveness of the selection of autochthonous microorganisms in the laboratory. The further step of consortium cultivation, carried out in laboratory, likely allowed a high specific adaptation to the particular substrate made available at that stage: the high selective pressure generated a strong adaptation of microorganisms, with a potential considerable removal rate at optimal conditions of growth in view of full-scale remediation.

All results of bioremediation method are monitoring after 150 days and this time is too short to analyze the activity of plants; however, we have shown the rooting and the germination of plant species (Fig. 3.16). So, we can estimate that the root system is in an early growth stage and therefore, it is not able to influence the remediation process.

Table 3.5 Total amount of inorganic compound into soil at T_0 and limit values (D. Lgs 152/06)

Sample (depth)	As	Cd	Cr (III)	Cr (IV)	Fe	Mn	Hg	Ni	Pb	Cu	Sn	Zn
T_0 (mg/kg)												
A1 (0–1)	11.2	<0.20	15.0	<0.20	11,309	765	0.74	25.4	18	13	0.86	98
A1 (1–2)	5.1	<0.20	13.4	<0.20	5988	272	0.61	21.7	3	4	<0.50	61
A2 (0–1)	9	<0.20	18	<0.20	11,585	981	0.88	30.8	23	16	0.92	115
A2 (1–2)	6.6	<0.20	20.6	<0.20	8415	503	0.79	34.4	4	5	<0.50	80
A3 (0–1)	8.9	<0.20	17.9	<0.20	11,407	768	0.89	28.4	86	20	2.31	283
A3 (1–2)	4.5	<0.20	16.2	<0.20	5942	306	0.93	27.2	5	5	<0.50	92
B1 (0–1)	10.4	<0.20	14.5	<0.20	10,556	996	0.80	25.1	10	10	0.71	89
B1 (1–2)	5.4	<0.20	18.4	<0.20	7334	531	0.88	29.8	4	6	<0.50	85
B2 (0–1)	7.6	<0.20	15.8	<0.20	10,579	741	0.85	27.8	53	27	1.33	137
B2 (1–2)	5.2	<0.20	17.3	<0.20	7544	329	1.70	32	6	9	1.02	160
B3 (0–1)	8.2	<0.20	23.6	<0.20	13,748	729	0.87	30.8	74	26	1.71	137
B3 (1–2)	5	<0.20	31	<0.20	10,026	399	3.46	44.9	6	12	2.21	359
Limit D. Lgs. 152/06	50	15	800	15	–	–	5	500	1000	600	350	1500

Table 3.6 Total amount of TPHs T_0 and T_f; we do not show low molecular weight hydrocarbons ($C \leq 12$) and aromatics C_{11}–C_{22} because we have parameters too low (data for $C \leq 12$: sample A2 (0–1) = 89.9 mg/kg and B1 (1–2) = 32.7 mg/kg; for other samples, we have values lower than legal limit)

	Aliphatics C_{13}–C_{18} (mg/kg)				Aliphatics C_{19}–C_{36} (mg/kg)				TPHs ($C > 12$) (mg/kg)			
Sample (depth)	T_0	T_1	T_2	T_f	T_0	T_1	T_2	T_f	T_0	T_1	T_2	T_f
A1 (0–1)	204	171	103	80	363	326	242	116	567	497	345	196
A1 (1–2)	157	88	13	1	314	233	142	8	471	321	155	9
A2 (0–1)	2357	1415	172	72	4173	732	287	187	6530	2147	459	259
A2 (1–2)	353	287	94	44	376	297	142	96	729	584	236	140
A3 (0–1)	1291	524	506	160	2239	1232	723	328	3530	1756	1229	488
A3 (1–2)	306	214	120	20	332	262	153	52	638	476	273	72
B1 (0–1)	1405	651	223	133	1852	943	536	206	3257	1595	759	339
B1 (1–2)	439	391	113	1	477	427	122	10	916	818	235	11
B2 (0–1)	902	463	353	203	1520	781	616	216	2422	1244	969	419
B2 (1–2)	281	222	75	75	403	354	274	74	684	576	349	149
B3 (0–1)	1429	876	723	624	1958	1269	1078	678	3387	2145	1801	1314
B3 (1–2)	224	226	418	238	307	317	359	179	531	543	777	417

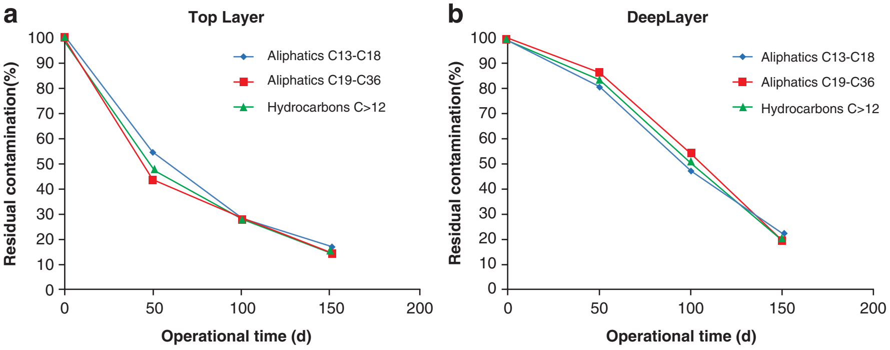


Fig. 3.15 Removal trend expressed as percentage over trial time (**a**) top layer; (**b**) deep layer

Fig. 3.16 Images of plants at T_f; (**a**) *P. australis*, (**b**) *F. arundinacea,* and (**c**) *P. nigra*

F. arundinacea has covered homogenously all pilot field; in parcel A, young shoots of *P. australis* are visible and in parcel B, the little tree of *P. nigra* have responded well to the transplant operation.

3.4 Conclusion

In our study, we applied a total biologically integrated bioremediation system in situ based on aerobic degradation by microorganisms (autochthonous and inoculated) and plants, on petroleum hydrocarbons-contaminated soil at a former refinery. Our results have highlighted that the integration of different bioremediation methods increased the effects of aerobic hydrocarbon-degrading bacteria activities, with the developed of optimal environment conditions for the microorganisms growth and low energy consumption, before laboratory trials and after in situ.

Our integrated technology was designed and developed in order to produce a diversified enzymatic degradation activity to reduce the amount of TPHs molecules without risk of pollutant residues and inverse toxic transformation. The primary laboratory steps are important to establish the best environment condition for plants and bacteria activities as well as the optimum condition for technology transfer in large scale.

So, not all remediation steps are involved into TPHs-degradation, in particular, the presence of the plants could play a potential role as support for biological activity, how reported in literature [15, 27], and contributing to a more development of suitable environmental conditions, reducing time of remediation. A well-developed root system and the oxygen and fertilizer injection could support microbial activity in less time just as the trial is monitored step by step in order to highlight the potential of bioremediation system with the contribution of phytoremediation. The microbial degradation is linked to enzymatic activities of microorganisms into soil, and the bioremediation of polluted area is based on catabolic metabolism that is capable of using organic contaminants as carbon source and energy. The organic compounds can be completely degraded to carbon dioxide and water, or mineralized or biotransformed into less toxic compounds. The integrated bioremediation system is the possible future in situ application in order to clean up contaminated areas (in large field scale) and decontaminated areas can return to society.

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Chapter 4
Phytoremediation of Petroleum-Contaminated Soil in Association with Soil Bacteria

Prayad Pokethitiyook

Abstract Unprecedented progress in industrial activities over the last century has directly contributed to the discharge of huge amounts of petroleum hydrocarbons into the environment. It has been estimated that about 1.7–8.8 million metric tons of oil is released into the environment every year. More than 90% of this oil pollution is caused by accidents due to human errors and also deliberate disposal of the waste containing hydrocarbons. Generally, petroleum and its products get into the environment through natural seepages, transportation, accidental spills, deliberate disposal, offshore production, and breakage of pipelines. Presence of petroleum hydrocarbon compounds in the environment can affect both on human health and the environment. Therefore, their presence in nature is of great concern today, and they need to be cleaned from the environment in the best possible way. Many research works have been carried out to determine the eco-toxicity of these pollutants but biological method has been reported to be more suitable to determine the possible hazards of pollutants in soil on the ecological and environmental bases.

Keywords Rhizosphere bacteria • Plant–microbe interactions • Bioremediation • Phytoremediation • Petroleum • PAH

4.1 Introduction

Unprecedented progress in industrial activities over the last century has directly contributed to the discharge of huge amounts of petroleum hydrocarbons into the environment. It has been estimated that about 1.7–8.8 million metric tons of oil is released into the environment every year [1]. More than 90% of this oil pollution is caused by accidents due to human errors and also deliberate disposal of the waste containing hydrocarbons. Generally, petroleum and its products get into the environment through natural seepages, transportation, accidental spills, deliberate disposal, offshore production, and breakage of pipelines [2–4]. Presence of petroleum

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hydrocarbon compounds in the environment can affect both on human health and the environment [5]. Therefore, their presence in nature is of great concern today, and they need to be cleaned from the environment in the best possible way. Many research works have been carried out to determine the eco-toxicity of these pollutants but biological method has been reported to be more suitable to determine the possible hazards of pollutants in soil on the ecological and environmental bases [6].

The effort to clean up these contaminants in the environment through various green technologies has become a prioritized search for both the scientific community and the industries. One of the promising technologies is phytoremediation [7]. Phytoremediation can be performed in the soil, air, groundwater, or surface water environment depending on the plants or the process settings. However, the toxicity of low molecular weight petroleum hydrocarbons is considered to inhibit plant growth and development. Furthermore, total carbon concentration of roots significantly decreased with increasing petroleum concentration [8]. Moreover, petroleum hydrocarbons had been found to be positively correlated to the abundance of bacterial genes responsible for biodegradation in the soil system [8–10]. Therefore, biological remediation of hydrocarbon-contaminated soils by environmental scientists and engineers need to be explored further.

Bioremediation and phytoremediation of petroleum-contaminated soils have been studied extensively in the past few decades and have been proved to be effective techniques [11, 12]. Rhizoremediation, a subset of phytoremediation, is the use of synergy between plant and its associated rhizosphere microbes to degrade pollutants in soil, has been recently found to be an effective technology. Several studies on remediation of petroleum contamination conducted both in situ and ex situ using plant–microbe interactions have proven to be effective [13–15].

Several studies have concentrated on the plant–microbe interaction using indigenous microorganisms [16–18]. The use of plants in cooperation with hydrocarbon (HC)-degrading bacteria or plant growth-promoting bacteria (PGPB) offers an enhanced potential for the bioremediation of TPH-contaminated soil [19–22].

Hence, plant-associated bacteria, such as rhizosphere bacteria (RB) and endophytic bacteria (EB), have been shown to contribute to biodegradation of toxic organic compounds in polluted soil and could have potential for improving the tolerance of plants in phytoremediation due to their possession of alkane or benzene biodegradation pathways and their metabolites [19, 23]. Additions of these oil degrading bacteria to the root zone might be able to enhance the remediation efficiency of plants as well [24, 25]. Since this is a new innovation in environmental biotechnology, a lot remains to be explored to make the technology even more effective. Examples of the role of rhizosphere bacteria in the improvement of plant fitness in petroleum-contaminated soils as well as the site-specific selection of plants for soil remediation by promoting rhizosphere bacteria for field use will be described.

4.2 Petroleum Spills into the Environment

Petroleum hydrocarbons are generally divided into two groups: aliphatic and aromatics. Aliphatic hydrocarbons are the compounds whose carbon atoms are joined together in straight or branched open chains but not in rings. Aliphatic hydrocarbons (alkanes, alkenes, and alkynes) in gasoline, crude, diesel, and lubricating oils constitute a substantial part of organic contamination in the environment [26]. This set of contaminant comprises saturated and unsaturated hydrocarbons having linear or branched open-chain structures. When total petroleum hydrocarbon (TPH) is directly released to water through spills or leaks, certain TPHs fractions will float in water and form thin surface films. Other heavier fractions will accumulate in the sediment at the bottom of the water, which may affect bottom-feeding fish and organisms. If the TPH spills occur in soil, physico-chemical processes will influence the fate and behavior of it [27]. The properties of hydrocarbons especially in crude, diesel, and lubricating oils will influence the degradation by microorganisms.

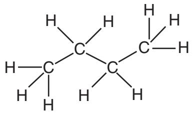
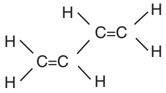
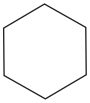
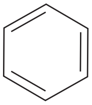
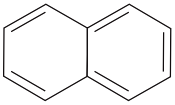
Diesel oil is composed of middle end distillates of crude oil with boiling points between 200 and 300 °C. Hydrocarbons in diesel oil are generally found to be in the C_8 to C_{26} range, which comprises an estimated 60–90% alkanes and cycloalkanes, less than 5% alkenes and 10–30% aromatics [9]. Lubricating oil is an important product obtained from the residue of crude oil, which accounts for 60% of crude oil derivatives. It is a petroleum product typically characterized by a very high boiling point of more than 350 °C. Its typical carbon ranges are C_{20} to C_{45+} comprising around 90% alkanes and 10–30% aromatics [28]. Chemical structures of various categories of hydrocarbons are shown in Table 4.1 [28].

Apart from the much visible and attention gaining large-scale accidental discharge of petroleum into both terrestrial and marine environment, the seemingly insignificant regular discharge from effluents, urban runoff, cleaning operations, and other oil treatments make up an estimated 90% of the total petroleum pollution brought about by anthropogenic activities. On the other hand, localized large-scale discharge like tanker accident and pipeline breaks make up 5–10% of the total anthropogenic petroleum spill. Of the petroleum spills, taking into account the amount of petroleum handled being more on land, discharge on land can be more or even greater than into the marine environment [11].

4.3 Fate of Hydrocarbons in Soil

Petroleum spill on land is followed by rapid vertical infiltration downward until it meets the water table. Once it reaches the water it spreads out laterally over it. Two important features of that influences the percolation of total petroleum hydrocarbons (TPHs) are its viscosity and porosity of the soil. Light petroleum like gasoline infiltrate rapidly into porous soil while the case is not the same with heavy

Table 4.1 Chemical structure of various categories of hydrocarbons

Hydrocarbon classification	Description	Chemical structure and example
Aliphatic		
Alkanes	Carbon chain with single bond	 n-Butane
Alkene	Carbon chains with at least one carbon–carbon double bond	 Butadiene
Alkynes	Carbon chains with at least one carbon–carbon triple bond	$HC \equiv CCH_2CH_3$ 1-Butyne
Cycloalkanes	Single-bonded carbon ring structure	 Cyclohexane
Aromatics		
Monoaromatics	The benzene ring made up of six carbon atoms with alternating single and double bonds	 Benzene
Polycyclic aromatic hydrocarbon (PAH)	Aromatic compounds having two or more benzene rings fused together	 Naphthalene

lubricating oil spills [11]. Most soils are described to be a multiphasic system characterized by the presence of ionic solid mineral matrix and associated organic matter that is enveloped by a film of water (Fig. 4.1). In unsaturated soils, technically termed as vadose zone, a gas phase occupies the pore spaces while in the spaces in saturated soils are occupied by aqueous phase. Fresh spills of TPHs on land are initially subject to volatilization, especially from the less porous surfaces (Fig. 4.1) while the heavier hydrocarbons may be partially oxidized by auto-, thermal-, and photo-oxidation in addition to biodegradation [12].

Petroleum hydrocarbons released into the environment are subject to degradation process with time. The processes that degrade TPHs include evaporation, leaching (transfer to the aqueous phase), chemical oxidation, and microbial degradation [13]. Petroleum hydrocarbons by virtue of their nature are generally biodegradable. Even the ones in natural reservoirs, the site of petroleum formation, are subject to biodegradation [14]. The alteration in the composition of petroleum hydrocarbons in the soil brought about by various physical, chemical, and biological factors are collectively called weathering [15].

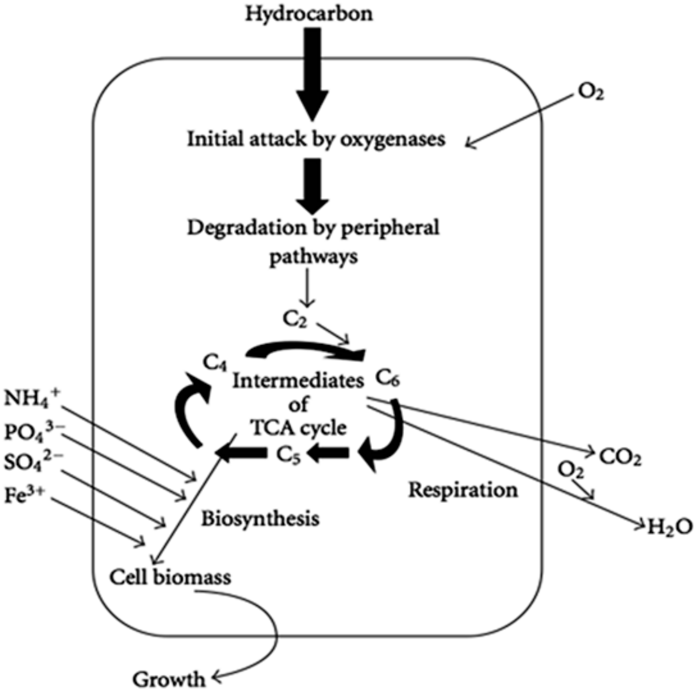


Fig. 4.1 Principles of aerobic degradation of hydrocarbons by microorganisms (Fritsche and Hofrichter [18])

4.4 Treatment of Petroleum-Contaminated Soil

Over the years, many treatment methods have been developed and practiced to treat petroleum-contaminated soil, which can be broadly classified into physical, chemical, and biological processes [17].

4.4.1 Phytoremediation

Phytoremediation is a term used to describe a set of technology employed to clean up contaminants utilizing plants. The term “phytoremediation” was first used in 1991, which was the product of research efforts in constructed wetlands, oil spills, and agricultural plant accumulation of heavy metals [25]. It is also defined to be a technology that uses plants with its associated rhizosphere microorganisms to remove, transform, or stabilize contaminants found in soils, sediments, and water bodies. At present, the technology is used for decontaminating many categories of contaminants including petroleum hydrocarbons [16].

Table 4.2 Predominant bacteria in soil samples polluted hydrocarbons

Gram-negative bacteria	Gram-positive bacteria
Pseudomonas spp.	*Nacardia* spp.
Acinetobacter spp.	*Mycobacterium* spp.
Alcaligenes spp.	*Corynebacterium* spp.
Flovobacterium/Cytophage group	*Arthrobacter* spp.
Xanthomonas spp.	*Bacillus* spp.

There are various mechanisms proposed by researchers over the years that have been theorized and experimented in phytoremediation (see [25]).

4.4.2 Microbial Degradation of Petroleum Hydrocarbons

Biological degradation of pollutants is founded on the principles that sustain all ecosystems. The processes involve circulation, transformation, and accumulation of energy and matter in nature. The soil microbes that predominantly degrade use TPH as a carbon source and electron donor for generating energy. Through laboratory tests and microbial characterization of bioremediation works, many bacteria have been identified to be active in biodegradation among which a dominating population is found in the genus *Pseudomonas* (Table 4.2) [18]. Although many bacteria are capable of degrading contaminants, a single bacterium may not possess the enzymatic capability to degrade all pollutants in soil. For the complete and successful cleanup of contaminated sites characterized by complex environment and contaminant composition, mixed microbial consortiums are needed [18, 24].

4.4.3 Bioremediation

Bioremediation can be defined as the use of microorganisms such as bacteria to remove environmental pollutants from soil, water, or gases [26]. It can also be defined as the utilization of the natural ability of microbes to use waste materials in their metabolism and change them into harmless end products. Bioremediation requires special kind of bacteria and also special operation conditions to accelerate the natural biodegradation rates by overcoming the limiting factors. It is the controlled manipulation of environment to produce proper enzymes for catalyzing the desired reactions to break down contaminants. Basically, it is the application of chemistry in a more intricate manner as it involves the crucial role of specific enzymes to run the reactions, which are introduced into the system by specific microorganisms [24]. The primary aim of bioremediation is to degrade the TPHs fully by microbes to carbon dioxide and water. This technology comes with added advantages over other methods of treating pollutants. Some notable advantages are low-cost operation, reduced health, and ecological ramifications [17]. The terms and technologies involved are summarized in Table 4.3.

Table 4.3 The terms and technologies involved in bioremediation technology (Juwarkar et al. [87])

Terms	Technology involved
Bioaugmentation	Addition of bacterial cultures to a contaminated medium; frequently used in bioreactors and ex situ systems
Biofilters	Use of microbial stripping columns to treat air emission
Biostimulation	Stimulation of indigenous microbial populations in soils and/or groundwater; may be done in situ or ex situ
Bioreactors	Biodegradation in a container or reactor; may be used to treat liquids or slurries
Bioventing	Method of treating contaminated soils by drawing oxygen through the soil to stimulate microbial growth and activity
Composting	Aerobic, thermophilic treatment process in which contaminated material is mixed with a bulking agent; can use static piles, aerated piles, or continuously fed reactors
Land farming	Solid-phase treatment system for contaminated soils may be done in situ or in a constructed soil treatment cell

Bioremediation technology is labeled to be efficient and cost-effective method to clean up TPH-contaminated soil in both in situ and ex situ after excavation of the soil. If in situ treatment is not feasible accounting to any reason, especially of environmental concern, the TPH-contaminated soils are removed from the site of contamination and are treated biologically using land treatment units, composting, biopiles, or slurry bioreactors [15].

Advantages of bioremediation

- In situ treatment is possible
- Permanent removal of contaminants
- Economically cheap and feasible
- Positive public acceptance
- Long-term liability risk eliminated
- Minimum disturbance to the site of contamination
- Can be clubbed with other treatment methods

Disadvantages of bioremediation

- Some pollutants cannot be broken down by biological processes
- Extensive monitoring should be put in place
- Site-specific requirements
- Toxicity of contaminants hamper the method
- Potential production of unknown by-products in the process [24].

4.4.3.1 Mechanism of Microbial Degradation of TPHs

In bioremediation, hydrocarbon substrate serves as the food (carbon) source for energy and growth of microorganisms, which is made available following two major ways: oxidation and/or reduction. However, hydrocarbons being already reduced chemically and stable, further reduction is not the main mode for bioremediation, even under anaerobic conditions [14].

Basically, aerobic respiration of hydrocarbons entails the need for enzyme oxygenase. The role of oxygenase is to incorporate molecular oxygen into the reduced hydrocarbon substrate. The initial products are alcohols that are subject to sequential oxidation producing carboxylic acids, which undergo β-oxidation [14, 15, 18]. Microorganisms engaged in biodegradation of TPHs require oxygen at two points in the metabolic pathway; first at the initial oxidation of the substrate and then at the end of the respiratory chain [18].

To have the fast and better degradation of TPHs under aerobic conditions, some characteristics of aerobic microbes must be met.

1. They must have metabolic process to increase the contact between the microbes and the TPHs. For biodegradation, TPHs must be bioavailable to the microbes. For example, TPHs must be accessible and also in the form that microbes can start working on. For example, TPHs must be water soluble by the oxygenase enzymes or by biosurfactants produced by microbes themselves.
2. The first step of degradation requires microbes to work on TPHs by enzyme activation and incorporating oxygen into the chains. It is an oxidative process by oxygenases and peroxidases enzymes.
3. TPHs are converted into intermediates compounds via the metabolic pathways of tricarboxylic acid (TCA) cycle and β-oxidation.
4. Production of cell biomass from central intermediary metabolites such as acetyl-CoA, succinate, and pyruvate. Sugar is produced from gluconeogenesis, i.e., used for biosyntheses and growth.

The aerobic degradation of hydrocarbons is illustrated in Fig. 4.1 [18].

4.4.4 Rhizoremediation

The treatment of hydrocarbon-contaminated soils by the combined ability of plants and their associated microorganisms is referred to as rhizoremediation and has been demonstrated to be the primary mechanism responsible for plant-mediated hydrocarbon degradation. Basically, it is the breakdown of soil contaminants by microbial activity, which is enhanced in the plant root zone [27]. With the plant exerting changes in the physical, chemical, and biological properties of the soil effecting degradation of contaminants, rhizoremediation is a parallel and inseparable term from phytoremediation [29]. It is also known by other terminologies like plant-assisted degradation, plant-assisted bioremediation, plant-aided in situ biodegradation, and enhanced rhizosphere biodegradation [25].

It is a treatment technology that combines phytoremediation with bioremediation. Besides the plant itself undertaking phytodegradation of the TPHs, it increases microbial numbers in the rhizosphere that undertakes biodegradation. This phenomenon is termed rhizosphere effect. A previous study done to see the effect of ryegrass and alfalfa microbial population and diversity in petroleum-contaminated soil found a significant increase in heterotrophic bacteria in planted soils as compared to bulk soil over a 7-week period [9].

A symbiotic relationship, based on evolutionary significance, between plants and the microorganisms play a key role in the degradation process. Root exudates from plants, besides directly degrading contaminants, act as substrate for soil microorganisms thereby enhancing microbial activity that results in increased rate of biodegradation. On the other hand biotransformation of contaminants by microorganisms helps detoxify chemicals that may be deleterious to the plant itself [25, 30].

4.5 Phytoremediation of Petroleum-Contaminated Soil

It has long been recognized that plants can remove metal contaminants from the soil and water during the last three decades. However, the abilities of plants to tolerate and degrade petroleum hydrocarbons are rather new. It has opened up possibilities for researchers to explore deeply into more details on how to use plants more effectively for remediation of TPH-contaminated soil [31, 32]. Plants vegetated in contaminated soil can uptake small quantity of TPHs and accumulate them in the root and shoot parts [33, 34]. Once inside the plant, these TPHs may have multiple fates; some TPH compounds can be sequestered in root tissue, some can be transported into shoots and leaves, where they can be stored in the vacuole or volatilized into the surroundings [33, 35]. The metabolic processing to clean up any xenobiotic compounds by plants was compared to mammalian liver and was termed the "Green Liver" by [36]. The green liver treats xenobiotics into three phases: transformation by enzyme activities (Phase I), conjugation to form moieties of conjugates (Phase II), and storage of the final products in the vacuoles (Phase III). However, Phase III in mammals the conjugates are excreted in urine or feces instead of being stored [36]. Considering this ability of plants to store toxic chemicals, one can therefore use plants as the sink for chemical hazard materials.

Soil is normally heterogeneous and form from weathered bedrock. The way in which TPH compounds partition differently among the different soil horizons depends on their individual constituents and the disposal sites. Some TPHs cannot move considerably into plants from soils due to partitioning coefficient of that substance. How much TPHs can dissolve into water before being transported into plants depends on the n-octanol/water partition coefficient, K_{ow}. The higher the K_{ow}, the more nonpolar the compound is. Log K_{ow} is generally used as a relative indicator of the tendency of an organic compound to adsorb to soil. Log K_{ow} values are generally inversely related to aqueous solubility and directly proportional to molecular weight. Many TPHs cannot move considerably into plants from the soil when log $K_{ow} > 4$ [37].

Vegetation growing on a soil can significantly affect many of these characteristics and responses. Depending on the nutrient sources, for example, plant roots can make the soil near them either more acidic or more alkaline than the soil at a distance from the root. This is because the root exchanges anions or cations with the soil as part of the root's uptake of essential plant nutrients [38]. Smiley [38] measured the rhizosphere pH (pH_r) of field and container-grown wheat plants and compared it with the non-rhizosphere pH (pH_b). The pH_r, was generally lower than pH_b

when ammonium was supplied as a fertilizer, higher when nitrate was supplied, and remained relatively unchanged when both forms were added together. In the rhizosphere, plants support TPH-degrading microbes involving in the biodegradation of TPHs [39–42]. A number of plant and grass species were studied for the abilities to tolerate and remove TPHs from the soil. Among them, alfalfa (*Medicago sativa* var. Harpe), ryegrass (*Lolium multiflorum* L.), birdsfoot trefoil (*Lotus corniculatus* var. Leo), sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), Bermuda grass (*Cynodon dactylon* L.), legumes, and beggar ticks (*Bidens cernua* L.) have been shown to be suitable for the TPH removal [3, 43–45]. All of these plant species have one thing in common the fibrous root system. Plants used for the cleanup of soil contaminated with TPHs should enclose the ability to tolerate their high concentrations and possess the extensive root system.

The previous above studies have been focused on the use of grasses for the remediation of TPHs due to their ability to tolerate the high concentration of the TPHs, extensive fibrous root system, large root surface area, and deep penetration of the root system into the soil [21, 46]. Processes and mechanisms taking place in the areas surrounding the roots provide an ideal environment for TPHs degradation. These processes include the exchange of gases, provision of water, and the increase in the bioavailability of TPHs by decreasing the surface and volume of soil micropores [47, 48]. There was also the enhancement of bacterial population, diversity, and some activities. Overall activities consequently in favor to TPH biodegradation [7].

Apparently, plants enhance soil microbial population and activity through the release of organic compounds, e.g., amino acids, sugars, enzymes, organic acids, and carbohydrates, or the so-called Root Exudates, from the root system [49, 50]. Several compounds released by roots act as inducer for microbial genes or co-metabolite involving in TPH biodegradation [51, 52]. It was reported that root exudates supported the development of high diversity of bacteria containing known hydrocarbon-degrading genes [53]. Since considerably higher numbers and diversity of HC-degrading bacteria were observed in rhizosphere soil as compared to bulk soil (the fungal abundance is 10–20 times higher and the bacterial abundance 2–20 times higher) [54–56]. Therefore, enhanced phytoremediation of TPH-contaminated area might be due to an increase in the population and activities of TPH-degrading bacteria in the rhizosphere [34, 57, 58].

4.6 Phytoremediation of Petroleum-Contaminated Soil in Association with Soil Bacteria

The remediation of soils containing diverse organic pollutants, including organic solvents, pesticides, and petroleum, is possible with the use of plants and their rhizosphere processes or the so-called phytodegradation. Phytodegradation of petroleum hydrocarbons may be enhanced by bacterial activities. In this process, plants interact with soil microorganisms by providing nutrients in the rhizosphere which leads to an increased microbial activity and degradation of organic pollutants.

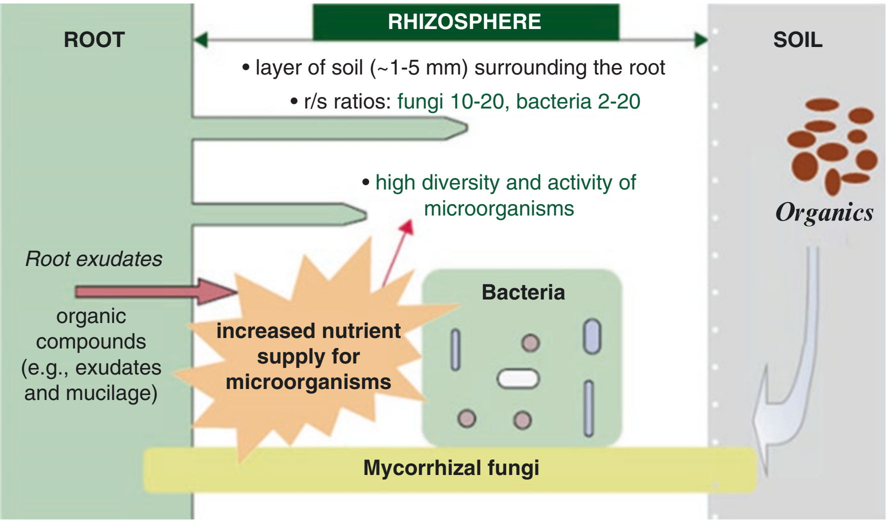


Fig. 4.2 Rhizosphere microorganisms as a critical link between plants and soil (adapted from Hrynkiewicz and Baum [50])

A narrow zone of soil affected by the presence of plant roots is defined as rhizosphere [50]. The rhizosphere is known to be a center of microbial activities. This is due to an increase in nutrient supply for microbes by the release of some extracellular organic compounds from the root system, namely, exudates and mucilage [59]. Therefore, rhizosphere is a soil matrix with a high microbial diversity resulting in a high microbial diversity. This microbial activity in turn affects the root development and plant growth in general. In general, the microbes serve as mediator between the plant and the soil. Since, in general, plant requires soluble mineral nutrients but often soil contains the necessary nutrients in low concentrations and in complex and inaccessible forms. Thus rhizosphere microorganisms, as a mediator, can provide a critical link between plants and soil plus organic compounds attached to soil (Fig. 4.2, [50, 60]).

4.6.1 *Plant Growth-Promoting Rhizobacteria (PGPR)*

Rhizosphere bacteria continuously metabolize various organic compounds from root exudates. As a result, there are quantitative and qualitative alterations of the released root exudates. Bacteria in the rhizosphere can significantly influence the nutrient supply of plants by competing for mineral nutrients and by mediating the turnover and mineralization of organic compounds. Therefore, bacteria in the rhizosphere can be a leading control of the turnover of nutrients in the soil [61]. Rhizosphere bacteria can influence plant growth also directly by releasing a variety of compounds, e.g., phytohormones or antimicrobial compounds [62] or biofertilizers [63].

Plant growth-promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria and can be plant specific but varies over time [64, 65]. Diversity of bacteria is affected by the plant age, the season, and the soil conditions [66]. For a long period, PGPR were largely applied in agriculture for facilitating plants to uptake nutrients from the environment or preventing plant diseases [67]. The combined use of plants and pollutant degrading and/or PGPR is relatively a new concept in the field of bioremediation of contaminated soil and water [46, 57, 67, 68].

PGPR can increase the availability of nutrients to plants by enzymatically nutrient mobilization from organic matters or soil and the production of siderophores [69, 70]. Some rhizosphere bacteria also produce siderophores which can be absorbed as the bacterial Fe^{3+}-siderophore complex by a number of plant species in the deficiency of iron [63]. Microbial siderophores in the rhizosphere can significantly contribute to the biocontrol of soil-borne pathogens due to their competitive effects [71] and the mobilization of metals to plants [70].

Some PGPR are acting as biofertilizers. Biofertilizer is defined as a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Whether the existence of a microorganism increases the growth of plants by replacing soil nutrients or making nutrients more available (by solubilization of phosphates) or increasing plant access to nutrients (by increasing root surface area), as long as the nutrients available to plants have been enhanced by the microorganism, the substance that was applied to the plant or soil containing the microorganisms, is referred to here as a biofertilizer [63].

PGPR producing extracellular degrading enzymes are major decomposers of organic matter. They contribute essentially to the soil aggregation and nutrient availability [72]. In soils with low phosphate, bacteria facilitate the release of phosphate ions from low-soluble mineral P crystals and from organic phosphate sources. These bacteria slowly release organic acids that dissolve the P crystals and exude enzymes that split organophosphate [63, 73].

PGPR are usually in contact with the root surface and improve growth of plants by several mechanisms, e.g., enhanced mineral nutrition and disease suppression [73]. PGPR can also promote the root growth. Rhizobacteria produces phytohormones such as indole-3-acetic acid (IAA), cytokinins, gibberellins, ethylene which promote cell division and cell enlargement, extension of other morphological changes of roots [50].

Petroleum hydrocarbon pollutants can be biodegraded by plants through biochemical reactions taking place within the plants and in the rhizosphere. The remediation of soils containing diverse TPHs, including crude oil, fuel oil, and lube oil, is possible with the use of plants and their rhizosphere processes (phytodegradation) [50]. Phytodegradation of organic pollutants may be enhanced by bacterial activities. In this process, plants interact with PGPR by providing nutrients in the rhizosphere which leads to an increased microbial activity and degradation of TPHs as describe earlier.

Endophytic bacteria, bacteria colonizing healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease, can produce extracellular enzymes, including pectinase, cellulose, lipoidase, proteinase, phenoloxidase, and lignin catabolic enzymes. All these enzymes are necessary to penetrate and colonize the host plants. Degradation of organic pollutants also occurred by rhizosphere and/or endophytic bacteria [74].

The investigation of site-adapted cultivable microorganisms in unfavorable or contaminated soils will contribute to identify the site-specific microbial populations and to provide fundamental knowledge and strain collections for subsequent selections and applications of plant growth and site remediation promoting microbial strains.

4.6.2 *Microorganisms and Its Selection for the Promotion of Plant Growth and Soil Bioremediation*

Applications of bacterial inoculation provide a great challenge in the future to increase plant growth and remediate contaminated soils. PGPR bacteria are the most important group capable of improving phytoremediation of petroleum hydrocarbons contaminated soil [21, 47, 75]. They are ubiquitous in the environment and play an important role in biodegradation of TPH contaminants from the soil, water, and air [5, 44, 76]. However, several obstacles must be overcome to achieve the successful applications of such treatments. Theoretically, microbial inoculum should be relatively universal for various plants and soils and its effectiveness should be relatively easy to evaluate on a standard scale. Practically, many experiments were plant-specificity and soil-specificity instead of being universal [63].

Information on the diversity of microorganisms at polluted sites is supposed to be valuable for a future selection of microbial inoculum for those sites. Information on microbial diversity and activity may not only provide evidence of ecosystem degradation but it might also be a valuable source of information for future application as inoculums for PGPR bacteria. Some molecular techniques, e.g., denaturating gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) can provide detailed information on the taxonomic and phylogenetic relationships of bacteria found on the contaminated sites. This information can describe the co-evolution between plants and bacteria in the field [77].

For PGPR to have a beneficial effect on plant growth through an enhancement of the nutrient status of their host, there obviously needs to be an intimate relationship between the PGPR and the host plant. However, the degree of intimacy between the PGPR and the host plant can vary depending on where and how the PGPR colonizes the host plant. As rhizobacteria themselves can be categorized into two groups: (1) rhizospheric and (2) endophytic bacteria [63].

Soil microorganisms (95–99%) is known to be at least so far nonculturable [78]. However, the basic criterion for the selection and application of this microbial inoculum useful for plant-growth promotion is cultivable and easily multiplication of bacteria. Information of critical factors influencing plant–microbe interactions with TPHs in soils could lead to an improved selection of bacterial inoculum for a bacterial-assisted phytoremediation of TPHs. A fundamental basis for the subsequent on-site applications of selected microorganisms is their safety for the environment and humans. Therefore, before field applications, all selected microorganisms have to be precisely identified and toxicologically assessed. Few microbial taxa have been reported so far for their capability to promote plant growth at contaminated soils. Also little is known on the microbial diversity which might be relevant to promote plant growth in those soils. In general, numerous species of soil bacteria which inhabit the rhizosphere can promote plant growth [63], e.g., by enzymatic nutrient mobilization from soil or organic matter (mostly P and N) and production of siderophores [70]. The study on barley (*Hordeum vulgare* L.) have uncovered that PGPR can contribute essentially to soil aggregation and nutrient availability which is often important for contaminated soils [72]. Therefore, enzyme activities can be suitable selection criteria for microbial inoculum for plant growth promotion in disturbed soils.

Microbial enzyme activities in the soil were predominantly measured as total potential activities rather than at the level of isolates within a community. As a matter of facts, investigations of single strains are also necessary for the selection of potential inoculum [66]. Acid phosphatases contribute to the P mobilization from organic matter. These enzymes cause the release of phosphate from a variety of substrates as inositol phosphate, polyphosphates, and phosphorylated sugars into the soil solution [79]. The production of these enzymes is species- and strain-dependent and often stimulated by deficiency of mineral phosphate. Beside the phosphatase activity, cellulolytic and pectolytic activities have been used for selection of microorganisms for promotion of plant growth and mycorrhiza formation. High cellulolytic and pectolytic activities of mycorrhizal fungi and rhizosphere bacteria allow the disintegration of living and dead plant tissue and, consequently, can enable microorganisms to enter roots. High cellulolytic and pectolytic activities can be used as a distinguish factor in selecting the rhizobacteria.

In many rhizospheric relationships, the PGPR actually attached to the surface of the plant. Scanning electron micrograph of bacteria on the surface of plants roots is a good scientific tool for proof checking of the microbial existence [63, 80]. In endophytic relationships, microorganisms actually reside within apoplastic spaces inside the host plant. Although there is rare evidence of endophytes occupying intracellular spaces [81].

Quantitative PCR (qPCR) has been used to monitor the presence of specific HC-degrading bacteria in any environment (e.g., by looking at the abundance of alkB gene in the rhizosphere and endosphere of plants growing in TPH-contaminated soil) and to monitor defined functional activity (e.g., alkane-degrading alkB gene

Table 4.4 Plant growth-promoting rhizobacteria (PGPR) for which evidence exists that their stimulation of plant growth is related to their ability to fix N2 (Vessey [63])

PGPR	Relationship to host	Host crops
Azospirillum sp.	Rhizospheric	Maize, rice, wheat
Azoarcus sp.	Endophytic	Kallar grass, sorghum, rice
Azotobacter sp.	Rhizospheric	Maize, wheat
Bacillus polymyxa	Rhizospheric	Wheat
Burkholderia sp.	Endophytic	Rice
Cyanobacteria[a]	Rhizospheric	Rice, wheat
Gluconacetobacter diazotrophicus	Endophytic	Sorghum, sugarcane
Herbaspirillum sp.	Endophytic	Rice, sorghum, sugarcane

[a]Numerous species; predominantly of the genera *Anabaena* and *Nostoc*

expression during phytoremediation of HC-contaminated soil). Studies regarding the abundance and expression of alkB and CYP153 genes in rhizosphere, and shoot and root interior of plants vegetated in HC-contaminated soil indicated that bacteria carrying these genes are not only able to colonize the rhizosphere and plant interior but are also metabolically active in HC degradation [21, 82–84]. Moreover, these studies concluded that survival and metabolic activities of HC-degrading bacteria varied distinctly between different strains, plants species, plant development stages, and plant compartments. Greater numbers of HC-degrading bacteria possessing alkB and tol genes were also found at the vegetative growth stages of ryegrass (*Lolium multiflorum* L.) [8].

It has been concluded that the use of bacteria with both pollutant degrading as well as plant growth-promoting properties worked better than using the bacteria having either pollutant degrading or plant growth-promoting properties only. PGPR showing 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity can decrease ethylene amounts produced by plants under stress and consequently reduce stress symptoms leading to improved plant growth and development [84, 85].

Inoculation of plants with bacteria possessing both HC-degrading and plant growth-promoting activities, with both seed inoculation and soil method, has been successfully applied in the laboratory, greenhouse, and field for their mediation of HC-contaminated soil and water [46, 75, 82, 83]. Most PGPR promote plant growth through their ability to fix N_2 in situ [63]. A list of these PGPR is shown in Table 4.4. Some recent successful examples of rhizobacteria application for the phytoremediation of petroleum hydrocarbon-contaminated soil are shown in Table 4.5. The potential benefits of using genetic engineered bacteria to improve recalcitrant organic pollutants biodegradation are summarized by Newman and Reynolds [86]. Field trials will need to be done to determine if this advantage remains stable in the field-grown plants. Moreover, the concept of releasing engineered bacteria into the environment must be addressed and monitor with a rigorous surveillance program. Although these organisms have been transformed using naturally occurring bacterial genes, their function in the host system might be different or distorted.

Table 4.5 Examples of successful remediation of hydrocarbons from soil by combined use of plants and rhizobacteria

Plant used	Rhizobacteria	Bacterial characteristics	Reference
Maize (Z. mays L.)	*Pseudomonas* sp. UG14Lr	Hydrocarbon degradation	Chouychai et al. [88]
Italian ryegrass (*L. multiflorum* var. Taurus)	*Pantoea* sp. strain BTRH79	Hydrocarbon degradation and ACC deaminase activity	Afzal et al. [46]
Alfalfa (*M. sativa* L.)	*R. meliloti* (strain ACCC17519)	Hydrocarbon degradation	Teng et al. [20]
Maize (*Z. mays* L.)	*Rhizobacterium, Gordonia* sp. S2RP-17	Hydrocarbon degradation, ACC deaminase, and siderophore synthesizing activities	Hong et al. [89]
Sorghum (*S. bicolor*)	*Sinorhizobium meliloti*	Hydrocarbon degradation, auxin production	Golubev et al. [90]
Ryegrass (*L. multiflorum*)	*Acinetobacter* sp. strain	Hydrocarbon degradation	Yu et al. [91]
Italian rye grass (*L. multiflorum* var. Taurus) and birdsfoot trefoil (*L. corniculatus* var. Leo)	*Pantoea* sp. strain BTRH79, *Pseudomonas* sp. strain ITRH76	Hydrocarbon degradation	Yousaf et al. [44]
Winter rye (*Secale cereale L.*), alfalfa (*M. sativa L.*)	*Azospirillum brasilense* SR80	Hydrocarbon degradation, indole-3-acetic acid (IAA) production	Muratova et al. [92]
Italian ryegrass (*L. multiflorum* var. Taurus)	*Rhodococcus* sp.strainITRH43	Hydrocarbon degradation	Andria et al. [93]
Sorghum (*S. bicolor* L. Moench)	*S. meliloti* P221	Phenanthrene degradation, indole-3	Muratova et al. [94]
		Acetic acid (IAA) production	
Maize (*Z. mays* L.)	*P. putida* MUB1	Hydrocarbon degradation	Chouychai et al. [19]

Annual ryegrass (*L. perenne*), tall fescue (*F. arundinacea* var. Inferno), barley (*Hordeumvulgare*)	*Pseudomonas* strains, UW3 and UW4	ACC deaminase production	Gurska et al. [75]
Rice (*Oryza sativa* L.)	*Acinetobacteria* sp.	Hydrocarbon degradation	Li et al. [95]
Barley (*H. sativum* L.)	*P. putida* KT2440	Hydrocarbon degradation	Child et al. [96]
Barley (*H. sativum* L.)	*Mycobacterium* sp. strain KMS	Hydrocarbon degradation	Child et al. [97]
Wheat (*Triticum* spp.)	*Pseudomonas* sp.GF3	Phenanthrene degration	Sheng and Gong [98]
Wheat (*Triticum* spp.)	*A. lipoferum sp.*	Hydrocarbon degradation and indole-3	Muratova et al. [99]
		Acetic acid (IAA) production	
Common reed (*P. australis*)	*P. asplenii* AC	ACC deaminase production	Reed and Glick [100]
White Clover (*T. repens* L.)	*R.. leguminosarum*	Hydrocarbon degradation	Johnson et al. [101]
Tall fescue grass (*F. arundinacea*)	*A. brasilense* Cd, *Enterobacter cloacae* CAL2, *P. putida* UW3, *P. putida*, *Flavobacterium* sp., *P. aeruginosa*	Hydrocarbon degradation and ACC deaminase activities	Huang et al. [82]
Barley (*H. sativum* L.)	*P. fluorescens*, *P. aureofaciens*	Hydrocarbon degradation	Anokhina et al. [102]
Barmultra grass (*L. multiflorum*)	*P. putida* PCL1444	Naphthalene-degrading bacteria	Kuiper et al. [103]

4.7 Conclusion

The use of rhizosphere bacteria contributes significantly to the improvement and sustainability of agriculture and agroforestry as well as the phytoremediation of organic contaminated soils. Selection and promotion of desirable rhizospheric processes requires a fundamental understanding of the complex microbial interactions in the rhizosphere. Rhizobacteria belong to the microorganisms in the rhizosphere, which contribute essentially to increase the soil fertility and remediate chemically contaminated soils.

Inoculation of soils with selected plant growth and soil remediation PGPR has the capacity to improve the plant fitness in polluted soils with unfavorable conditions and increase the biodegradation of organic pollutants. The successful use of such inoculum in the contaminated field with natural environmental conditions and competition will be a great challenge. In this regard, for efficient petroleum hydrocarbon remediation, it is of primary importance that the inoculated hydrocarbon-degrading bacteria colonize the rhizosphere and/or plant cells so as to initiate their effects on plant growth and hydrocarbon biodegradation. Rhizobacteria and endophytic bacteria showing hydrocarbon degradation capacity and/or plant growth-promoting ACC deaminase activity are more effective in petroleum hydrocarbon phytoremediation since they are enhancing plant growth and simultaneously encouraging hydrocarbon degradation. The bacterial ACC deaminase activity accelerates root growth, as a result a better access to nutrients and water and consequently faster initial growth, which enable plants to better counteract stress responses caused by hydrocarbon contamination.

At present, it seems necessary to use always site-specific selections of inoculum since a general suitability of inoculum for diverse site conditions seems rather unlikely. Combined use of plant and effective and specific rhizobacteria seems to be a more promising technique for the remediation of petroleum hydrocarbon-contaminated soil as compared to only bioaugmentation (only use of microorganisms) and phytoremediation (only use of plants).

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Part II
Higher Plants in Biomonitoring and Environmental Bioremediation

Chapter 5
The Use of Higher Plants in Biomonitoring and Environmental Bioremediation

Svetlana Vladimirovna Gorelova and Marina Vladimirovna Frontasyeva

Abstract This chapter provides basic information on the use of higher plants for biomonitoring and bioremediation in the world. It contains a large amount of material of the authors' own research on the possibility of using woody plants for biomonitoring and phytoremediation of environment anthropogenic pollution with heavy metals. The species of woody plants are revealed, which are recommended for use in biomonitoring of anthropogenic pollution of the environment in temperate latitudes (the study of biogeochemical parameters of leaves): *Acer platanoides*, *Aesculus hippocastanum*, *Betula pendula*, *Cotoneaster lucidus*, *Populus nigra*, and *Salix fragilis.* The following species are recommended for phytoremediation of soils from heavy metals: *Betula pendula*, *Cotoneaster lucidus*, *Syringa vulgaris*, *Sorbus aucuparia*, *Philadelphus coronarius*, and *Larix sibirica.* The species of woody plants—bioindicators of air and soil pollution by heavy metals—are revealed. The chapter also shows the significance of the statistical analysis for the detection of the main element pollutants of the environment.

Keywords Biomonitoring • Bioremediation • Heavy metals • Woody plants • Bioaccumulation • Soil and air pollution • Statistical analysis

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5.1 The Use of Higher Plants for Biomonitoring (Basic Information)

Biomonitoring is a system of control and obtaining quantitative characteristics of biological objects (biomonitors) in time for the assessment of the environmental changes. The main objective of biomonitoring is to prevent the adverse effects of sequences of the environmental changes and forecasting of developments of events at the level of individual populations as well as of biogeocenosis and the biosphere as a whole.

Monitoring can be carried out at different levels: molecular, tissue, organ, organism, population, species, ecosystem, and biosphere. Depending on the venue, it can be local, regional, national, and international. It may also vary according to the objects of research and may define the parameters, being passive (carried out directly in the wild nature) or active (requires setting of the experiment by a researcher) [1–5].

To conduct biomonitoring, the major role belongs by the choice of the environmental monitors (markers) of the environmental state, the development of uniform methods of sampling, sample preparation, and the proper selection of analytical methods for different types of contaminants. Due to the fact that the spectrum of the environmental pollutants comprises more than 400,000 items, use of chemical methods of analysis only is too costly, and it does not allow to get the whole picture of their cumulative impacts on biota; so it is more cheaper to apply methods of bioindication, biological testing, and biomonitoring. However, none of biological object may be a universal indicator or monitor sensitive to various substances to the same degree.

Basic requirements for plant biomonitors are summarized as follows [2, 3]:

1. Widespread and long vegetation period and high degree of bioaccumulation of elements of the environment (passive biomonitoring), the ability to good growth in standardized conditions (active biomonitoring)
2. A clearly marked and reproducible response to certain changes in the environment and bioaccumulation of toxic elements in an amount reflecting the situation in the environment
3. High sensitivity to pollutants (diagnosis effect at low levels of contamination)

There are no universal biomonitors that meet the requirements with respect to all possible contaminants; therefore, an important task for biomonitoring is the selection of species that can be used for biomonitoring of various parameters of the environment.

Markers for biomonitoring may be molecular mechanisms: the study of the structure of DNA changes, the genetic response, and synthesis of substances [6]. Biomonitoring can be carried out at a biochemical and physiological level: determination of the content of low molecular antioxidants, involved in detoxification mechanisms when the radicals produced under stress. Such antioxidants are ascorbic acid, glutathione and proline [7–13]. The stress level can be determined by the change of activity of antioxidant enzymes superoxide dismutase, peroxidases, catalase, and glutathione reductase [14–18]. However, these plant reactions are not always specific to certain toxicants and depend on the species of the plant [19, 20].

The most commonly used is an identification sign of quantification of chlorophylls and carotenoids in plants [21–23], as well as their ratio and the response of the light phase of photosynthesis [24–26]. At the level of organelles, membrane structure, chloroplasts, and mitochondria (transmission electron microscopy, TEM) are known [27]. When studying the plants at the tissue level, histochemical methods are applied using dyes that are specific to a particular metal, which helps to determine the localization in the tissue and way of their movement and accumulation in the plant [28, 29]. As biomarkers in model experiments on determination of the effects of various concentrations of toxic substances in the environment on the plant, individual organs of plants can be used, where the biomass growth (shoots), parameters such as germination and vigor (seeds), the root test, and definition of tolerance index are studied [30–33]. In passive monitoring at the organ level, the development and percentage of leaf necrosis and chlorosis, development of deformation of shoots and leaves, and modifications of the leaf blade (the appearance of the blades in simple leaves, the absence of leaf share, threadlike leaves, etc.) are determined. Besides, one can determine the leaf square, the degree of xeromorphism, and determine the percentage of dead shoots and dry crown of trees [34, 35]. At the organism level, vitality of species in the altered environmental conditions is determined [21, 36, 37]. However, when it comes to polymetallic pollution of the environment with heavy metals and metalloids, most significant is determination of elemental (biogeochemical) composition of plants and plant organs, which may reflect the situation in the environment [36], if the plant is an *indicator* [37–40]: it adsorbs and bioaccumulates metals in the process of growth, develops mechanisms of resistance to toxic elements, and does not belong to *excluders* (which exclude) or bioaccumulators in accordance with the classification proposed by AJM Baker [41, 42].

From the plant physiology point of view, biomarker of the pollution stress effect at the level of phytocoenosis, to some extent could be chlorophyll fluorescence [24, 25]. For biomonitoring of ecosystems, geobotanical methods are also applied: analysis of the number and types of species and their vitality, crown density, and density of herbaceous (or moss-lichen) cover and the analysis of the presence of anthropogenic weeds in phytocenosis, which makes it possible to conclude about the degree of digression of the community.

According to their response to the content of toxic components in the environment, bioindicators and biomonitors may be sensitive (respond to the impact of a significant deviation from the norm) or bioaccumulative (feedback manifests itself gradually, and pollutant accumulates in the body or individual organs and tissues) [1–3].

Most often to biomonitor atmospheric deposition the higher spore plants – mosses – are used. The idea of using terrestrial mosses for the analysis of atmospheric deposition of heavy metals has been proposed in the late 1960s of the twentieth century by Rühling and Tyler [43–45]. It is based on features of moss anatomic structure and physiology. The leaves of moss are composed of 1–3 layers of cells, they lack cuticles on the leaves preventing the penetration of pollutants, they have no roots, and they readily absorb water and nutrients from wet and dry deposition by rhizoids.

Mosses effectively accumulate heavy metals and other compounds due to the large specific surface area and slow growth. As a passive biomonitor in most cases, they help to identify the impact of pollutants at the ecosystem level. Ideas of moss monitoring in Europe have been developed by Rühling et al. [46, 47], Steinnes [48, 49], Steinnes and Andersson [50], Steinnes et al. [51], Steinnes and Frontasyeva [52], Rühling and Steinnes [53], Berg and Steines [54], Schröder et al. [55], and Harmens et al. [56–60].

Since the 1970s, in the Scandinavian countries, and in the last 20 years in the Eastern, Central, and Western Europe, passive briomonitoring receives support of targeted state grants and programs, and it is held regularly every 5 years in the framework of the UN Convention on Long-Range Transboundary Air Pollution (LRTAP) [53, 58, 60–64]. Coordination of moss biomonitoring in Europe, Russia, and Asia is carried out through the United Nations program (UNECE ICP Vegetation).

Based on the monitoring results, the atlases of atmospheric deposition of pollutants are edited and published, which allow estimating the cross-border transfer of elements, reveal sources of pollution and their impact on the environment, as well as trace the retrospective distribution of elements in the atmosphere [58, 60, 61, 65].

In Russia, conducting biomonitoring first started in the northwestern regions: Leningrad region [66, 67], Kola Peninsula, and Karelia [54, 68]. Since the late 1990s of the twentieth century, biomonitoring was carried out on the basis of the analytical complex of the Joint Institute for Nuclear Research for a number of central regions of Russia: one-time study conducted in Tula region [69, 70], Tver, Kostroma, part of Moscow and Ivanovo regions [71–74], Ural [75, 76], Udmurtia [77], as well as Kaliningrad region [78–82].

In 2014 the coordination of the moss surveys in the UNECE ICP Vegetation has been transferred from the UK to Russia, Joint Institute for Nuclear Research (Dubna, Moscow Region) to M. V. Frontasyeva, so far JINR has direct access to the member-states in which the UNECE ICP Vegetation is interested in the Caucasus region and Asia: Azerbaijan, Georgia, Kazakhstan, Mongolia, Vietnam, and Moldova in the southern east. Currently, the study area of atmospheric deposition by passive biomonitoring greatly increased, and GIS mapping and transport models build on the data submitted by teams from different countries; it will be possible to make more global conclusions on the transboundary transport of substances (2015–2016 moss survey) and to create a database on the content of elements in mosses on a global scale, which can be replenished in the future [83]. In addition to atmospheric deposition of heavy metals, this method also allows evaluating the contamination of nitrogen, persistent organic pollutants (POPs), and radionuclides [58, 59, 83–87].

Besides higher spore plants for biomonitoring, woody and herbaceous plants of genera *Gimnospermae* and *Angiospermae* can be used. They reflect the state of soil, air and water may due to change of their biochemical, physiological, and morphological parameters and their ability to bioaccumulate the toxic elements from the environment [21–23, 34, 35, 88–105].

Using the higher seed plants for biomonitoring purposes has advantages over the use of spore plants: they are easily identified (e.g., than mosses) and grow in urban ecosystems, and some of them have extensive habitat areals and can be used for the diagnostics of transboundary transport of elements between countries and continents.

Table 5.1 Higher plants for bioindication and biomonitoring of the environment (Applied Ecobiotechnology [106]; Gorelova [107]; Gorelova et al. [97, 104, 105])

Species	Used in bioindication, symptoms	Used in biomonitoring	Substance to which the given type reacts
Taráxacum officinále	+, necrosis and chlorosis of leaves, the change of physiological parameters (photosynthetic pigments content of low molecular weight antioxidants)	+, accumulation of heavy metals in the body depending on the degree of pollution	Heavy metals, ozone
Gladiolus gandavensis, *Tulipa gesneriana*, *Iris germanica*, *Petroselinum crispum*	+, regional and apical necrosis, accumulation of fluorine in the dry matter	–	HF
Urtica urens	+, necrosis strips on the underside of leaves	–	Peroxyacetyl
Nicotiana tabacum, *Spinacia oleracea*, *Glycine max*, *Trifolium pratense*, *Trifolium angustifolium* and subsp.	+, necrotic changes in leaves (spots), pink spots on the leaves (reaction on pollution by ozone), interveinal necrosis (reaction on nitrogen oxides)	+	O_3, NO_2
Poa annua	+, necrosis strips on the leaves	+	NO_3^-
Medicago sativa, *Fagopyrum esculentum*, *Plantago major*, *Pisum sativum*, *Trifolium incarnatum*, *Pinus sylvestris*, *Quercus* subsp., *Platanus* subsp., *Populus* subsp., *Acer* subsp., *Fagus sylvatica*, *Fraxinus excelsior*	+, interveinal point and extensive necrosis and chlorosis. Marginal necrosis and chlorosis, early death of needles and dry crown dieback (*Pinus*)	+	SO_2
Spinacia oleracea, *Phaseolus vulgaris*, *Latuca sativa*	+, chlorosis, change the structure of chloroplasts, photosynthesis violation		Cl_2
Lepidum sativum	+, reduction of biomass, germination and growth energy, dying roots		NaCl, heavy metals
Pleurozium schreberi, *Hylocomium splendens*, *Polytrichum* sp., *Brachythecium* sp., *Hypnum cupressiforme*, *Sphagnum* subsp.		+, accumulation of atmospheric deposition	Radionuclides Sr^{90}, Cs^{137}, K^{40}

Species	Used in bioindication, symptoms	Used in biomonitoring	Substance to which the given type reacts
Pleurozium schreberi, *Hylocomium splendens*, *Polytrichum* subsp., *Brachythecium* subsp., *Hypnum cupressiforme*, *Sphagnum* subsp.	+, necrosis at high concentrations of heavy metals in the environment	+, accumulation in organs from atmospheric deposition	Heavy metals and metalloids
Fagus sylvatica, *Ailanthus altissima*, *Robinia pseudoacacia*, *Picea abies*	needle chlorosis, the appearance of dead branches in the crown	+, accumulation of toxic elements in leaves	Heavy metal
Aesculus hippocastanum	+, regional and interveinal necrosis	+, accumulation of Cu, Ni, Pb. Reduction of amount of photosynthetic pigments, decrease of ascorbic acid content, increased synthesis of phenolic compounds	Heavy metals
Betula pendula	+, asymmetry of leaves, necrosis	+, Mn, Ni, Zn, Cd, Pb, reduction of the amount of photosynthetic pigments, decreased ascorbic acid content, synthesis of phenolic compounds	Heavy metals
Populus nigra	+, decrease in vitality, necrosis of leaves, early defoliation, dry crowns	+, Fe, Ni, Zn, Cd, Pb, reduction of the amount of photosynthetic pigments, decrease of ascorbic acid content, synthesis of phenolic compounds	Heavy metal
Tilia cordata	+, regional and focal necrosis	+, reduction of photosynthetic pigments, reduction of ascorbic acid, increased synthesis of phenolic compounds	SO_2, NO_2, Na Cl, heavy metals
Acer platanoides	+, regional and focal necrosis, damage by fungi	+, accumulation of Fe, Mn reduction of photosynthetic pigments, reduction of ascorbic acid, increased synthesis of phenolic compounds	Heavy metals
Quercus sp.	+, necrosis of leaves, reduced vitality, insect damage	+, accumulation of heavy metals	Complex (SO_2, NO_2, heavy metals)
Thuja occidentalis	+, death of needles, dying of shoots, reduced vitality, loss of landings	+, accumulation of heavy metals V, Cr, Fe, Ni, As, and Mo	SO_2, NO_2, Na Cl, heavy metals
Juniperus scopulorum Skyrocket	+, death of needles and shoots, reduced vitality	+, accumulation V, Cr, Fe, Ni, and Sb	SO_2, NO_2, Na Cl, heavy metals
Taxus baccata	+, dying of shoots, reduced vitality, loss of landings	+, accumulation of Cr, Fe, Ni, Zn, As, and Cd in shoots	SO_2, NO_2, Na Cl, heavy metals

The list of species used for biomonitoring and bioindication purposes on a global scale is relatively large. Some of these species are shown in Table 5.1.

5.2 The Use of Higher Plants for Bioremediation (Basic Information)

5.2.1 Phytoremediation

Phytoremediation is the restoration of ecosystems, or their individual components, contaminated with heavy metals, radionuclides, NaCl, petroleum ore, and other toxic organic products by the use of herbaceous and woody plants. The advantage of phytoremediation over other methods of purifying ambient environment is its relatively low cost (50–100 times less), the ability of the implementation in situ, environmental safety, and the ability of further use of obtained biomass to extract valuable elements [108]. One of the drawbacks is the time required for full soil recovery.

Selection of plants for phytoremediation is determined by their ability to absorb toxic compounds from the soil or water systems or transfer them from the surface to volatile forms, the growth rate, and the volume of produced biomass during vegetation and depth of root system.

For phytoremediation, plant hyperaccumulators could be used which accumulate high concentrations of toxic compounds (heavy metals, nonmetals, radionuclides) in biomass [109, 110]. They have developed mechanisms to adapt to high concentrations of metals in organs: representatives of galmain flora (*Viola lutea* var. calaminaria, *Thlaspi* Zn), “tin flora” *Trietaris europea*, *Gnaphalium suaveolens*, accumulating Ni, *Alyssum bertolonii*, *Sebetaria*, copper acuminators *Cyanotis cupricola*, *Sopubia metallorum*, *Gypsophila patrinii*, and others.

Such plants, as a rule, are usually characterized by low biomass. Lately more and more attention of scientists is directed to the use of plants with the medium potential for bioaccumulation of toxic elements, but creating more biomass in the process of vegetation (e.g., C4 plants and woody plants) [111–116]. So far the feasibility of using plants-accumulators of heavy metal is determined by the metal accumulation rate (mg/kg of biomass), multiplied by their biological productivity (kg/ha per year). Economically justifiable plants for phytoremediation are those in which the yield of biomass reaches at least 250 kg/ha per year and metal content in biomass of at least 1% (dry weight) [108].

There are several ways to plant uptake of toxic elements from the environment:

5.2.2 Phytoextraction

Phytoextraction is a process of conversion of heavy metals or metalloids by plants into the form of complex compounds (chelates) and their accumulation in tissues and organs (overground or root system) [117].

To increase the ability of plants to absorb heavy metals from soils, chelators (e.g., ethylenediaminetetraacetic acid, citric acid and oxalic acid, malic acid, salicylate, succinate, tartrate, and other compounds) or inoculation of plants by symbiotrophic microflora (fungi and bacteria), are used [118–120]. Furthermore, the studies are known where the genes of bacterial cells are introduced to the organism of higher plants to increase their ability to absorb heavy metals from the substrate.

The effectiveness of phytoextraction is influenced by several factors: by the content of humus in the soil (the binding of toxic components into complexes increases; their availability for plants decreases), activity of soil microorganisms, introduction of sorbents (iron oxides, manganese, organics, clay, fly ash, coal, vermiculite sawdust, and others) into soil, the pH value of the soil solution (pH reduction leads to an increase in mobility of many heavy metals, Cd, Zn, Ni, etc., as well as binding them with organic soil components), liming (this leads to reduction of the solubility of Fe, Cu, Ni, Co, Zn, and Cd and their availability for plants), introduction of organic acids and complexing agents (enhances uptake of heavy metals by plants), application of plant growth stimulators (heteroauxsin, succinic acid, etc.), and interaction between the ions in the soil solution (formation of insoluble compounds) [106].

5.2.3 Phytotransformation and Phytodegradation

Phytotransformation is an ability of plants to convert toxic compounds (organic pollutants—xenobiotics) in the process of the plant fermentative enzymatic reactions to nontoxic form and their subsequent transfer to the vacuole or binding to lignin and other components of the cell.

5.2.4 Rhizodegradation (Rhizosphere Biodegradation Ore Phytostimulation)

Rhizodegradation (rhizosphere biodegradation ore phytostimulation) is decomposition of toxic organic compounds in the soil in the process of enzymatic degradation in the interaction of the rhizosphere of plants and microorganisms. Thus, the roots of plants affect xenobiotic root exudates, stimulate the increase of the number of microorganisms in the rhizosphere, and accelerate the transfer of toxic compounds in the root zone due to the difference in osmotic pressure between root cells and the soil solution [121, 122]. It is used for soil purification from oil products (not more than 2%): the PAH, PCBs, other hydrophobic aromatic compounds, and pesticides [106].

5.2.5 Phytovolatilization

Phytovolatilization is conversion of toxic components into nontoxic volatile compounds using enzymatic reactions in biochemical cycles of plants and their subsequent release (selenium, mercury) (*Liriodendron tulipifera*) [106].

5.2.6 *Phytostabilization*

Phytostabilization is transfer of metals into the insoluble stable compounds due to synthesis and release by plant compounds that reduce the spread of pollutants (binding to lignin or organic soil components) (conversion into insoluble forms) [123] and precipitation of heavy metals and metalloids (Cd, Cr, Cu, Hg, Pb, Zn, andAs) in the root zone in the form of carbonates, phosphates, and hydroxides. It is used as a step in soil remediation together with the introduction of lime, organic fertilizers, and structurants (phosphates, synthetic resin, clay, bentonite, fly ash, zeolites, aluminosilicates, hydroxides of Fe, Al, and Mn) [106].

5.2.7 *Rhizofiltration*

Rhizofiltration is absorption, concentration, and precipitation of heavy metals and hazardous chemicals by plant roots. This is a most often used technology for water purification from toxic substances and radionuclides. For rhizofiltration rafts in ponds with terrestrial and aquatic plants (in situ) or special tanks for water treatment with platforms (gratings) for plants (ex situ) are used [124–126].

An example of the integrated use of living organisms for bioremediation of water is so called “living machines”: a system of tanks for anaerobic treatment, aerobic treatment using microorganisms and planktonic animals, containers with higher plant hydrophytes and hygrophytes (*Lemna minor, Eichornia crassipes, Phragmites australis, Typha latifolia, Gliceria fluitans, Calla palustris, Alisma plantago-aquatica, Sagittaria* spp., and others), through which the contaminated water flows. As a result, the water is purified from organic and inorganic pollutants.

After rhizoextraction of rhizofiltration, the biomass of plants containing metals can be used for extraction of metals by chemical means (Ni, Cu, Au) or for energy generation [127–129].

At present, scientists intend to create greenbelts of the plant in the industrial zones, which serve as a barrier to heavy metal and serve as phytoremediation of the environment by absorbing heavy metals and radionuclides from the air and soil. The most promising for this are woody plants which possess a combination of features: a deep (or surface) root system of a large volume, a large volume tree crown (height 1.5–30 m), ability to accumulate a large biomass of leaves during the growing season, the possibility of absorption and bioaccumulation of heavy metals (mainly Pb and V) by leaves from atmospheric deposition, durability, and possibility to use wood.

See Table 5.2 for higher plants v.

Many woody plants meet all the requirements of biomonitors listed by Markert:

- High abundance
- Widespread

Table 5.2 Higher plants used for phytoremediation (Baker and Brooks [109]; Wenzel et al. [130]; Glass [131]; Palmer et al. [132]; Prasad [108, 133, 134]; Trace elements [135]; Applied Ecobiotechnology [106]; Favas and Pratas [136])

Species (genera)	Accumulated elements	Substrate
Acacia dealbata	Cu, Pb	Soil
Agrostis tenuis	Cu, Pb, Zn	Soil phytostabilization
Agrostis capillaris	Cu, Pb, Zn	Soil phytostabilization
Agrostis lanatus	Fe, Cu, Zn, As, Pb	Soil
Alyssum sp.	Ni	Soil
Alnus glutinosa	Cu, Pb	Soil
Amaranthus retroflexus, *Amaranthus tricolor*	^{137}Cs, Zn	Soil
Armeria maritima	Pb	Soil
Atemisia absinthium	Zn, Cu, Cr	Soil
Artemisia vulgaris	Zn, Ni, Cu	Soil
Atriplex prostrata	NaCl	Soil
Alisma plantago-aquatica, *Calla palustris*, *Gliceria fluitans*, *Sagittaria* spp	HM, organic compounds	Storm water ditch, sewage wetlands, water
Berberis	Xenobiotics	Soil rhizodegradation
Beta vulgaris	Ni, Cu, Zn, Cr	Soil
Brassica canola	^{137}Cs	Soil
Brassica juncea	Pb, Zn, Cr, Cd, Ni, Cu,^{90}Sr, Se, U	Soil phytoextraction (U—only with organic acids), phytotransformation (Cr^{+6}-Cr^{+3}); phytostabilization
Brassica nigra	Zn, Pb	Soil
Buxaceae	Ni	Soil
Calamagrostis epigejos	Pb, Zn, Cu	Soil
Cardamonopsis hallerii	Heavy metals (HM) (Zn, Cd)	Soil, hydroponics
Cynodon dactylon	Xenobiotics	Soil rhizodegradation
Eucalyptus sp., *Eucalyptus globulus*	Na, As, Cu, Pb	Soil
Eichhornia crassipes	Pb, Cu, Cd, Fe	Storm water ditch, sewage wetlands, water
Festuca arundinacea	Xenobiotics	Soil rhizodegradation
Festuca rubra	Pb, Zn	Soil phytostabilisation (with $CaCO_3$)
Fagopyrum esculentum	Ni	Soil
Juncus compressus	Zn, Cd, Pb	In the roots
Haumaniastrum katangense	Co	Soil
Chenopodium album	Zn, Cu	Soil
Helianthus annuus	Cr, Mn, Cd, Ni, Zn, Cu	Soil, storm water ditch, sewage wetlands, water (rhizofiltration)
Helianthus annuus	Pb, U, ^{137}Cs, ^{90}Sr, Cu (mutant forms)	Soil
Hydrocotyle umbellata	Pb, Cu, Cd, Fe	Soil
Kochia scoparia	Radionuclides (RN)	Soil

(continued)

Table 5.2 (continued)

Species (genera)	Accumulated elements	Substrate
Lemna minor	Pb, Cu, Cd, Zn	Storm water ditch, water
Linum usitatissimum	Cu, Ni, Cd, Cr, Pb	Soil (phytoremediation + raw material for plant fiber)
Lycopersicon lycopersicum	Pb, Zn, Cu	Soil
Lolium perenne	Radionuclides, xenobiotics	Soil rhizodegradation
Medicago sativa	Ni (HM), Pu, xenobiotics	Soil (symbiosis with bacteria)
Melilotus officinalis	Zn, Ni, Cu	Soil
Miscanthus giganteus	Cu, Ni, Cd, Cr, Pb	Soil (phytoremediation + raw material for plant fiber)
Morus sp.	Xenobiotics, HM	Soil rhizodegradation
Phalaris arundinacea	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Polygonum sp., *P. sachalinense*	Cd, Pb, Zn, ^{137}Cs, ^{90}Sr, Cu	Soil
Populus sp.	Hg, Fe, Ni, Zn, Cd, Pb, herbicides	Soil phytoextraction, soil rhizodegradation
Pinus pinaster	Fe, Zn, As, Pb, W	Soil
Phalaris arundinacea	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Quercus ilex, *Quercus rotundifolia*, *Quercus suber*	Ni, As, W, Zn, Pb	Soil
Rhus typhina	Polycyclic aromatic hydrocarbons	Soil rhizodegradation
Salix sp.	Ni, Zn, Cd, Pb, perchlorate	Waste water, filtrates
Scirpus sylvaticus	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Secale cereale	Zn, Pb	Soil (only with the introduction of bacteria *Rhodococcus equi*)
Silene latifolia	Zn, Cu	Soil
Sorgo bicolor	Zn, Cu, Pb	Soil
Trifolium sp.	Xenobiotics	Soil rhizodegradation
Typha latifolia	Cu, Zn, Cd, Pb	Storm water ditch, sewage wetlands, water
Thlaspi caerulescens	Zn, Cd	Soil
Thuja occidentalis	V, Cr, Fe, Ni, As, Mo	Soil
Urtica dioica	Cu, Ni, Cd, Cr, Pb	Soil
Vetiveria zizanioides	Cr, Cu, Ni, Zn, As, Cd, Pb	Water, soil

- Easy to identify
- Easily available
- Analytically accessible and low detection and determination thresholds with current analytic technology
- Accumulation of pollutants

And features mentioned by Bargagli [3]:

- Long vegetation period
- Clearly marked and reproducible response to certain changes in the environment

In addition, they have a number of advantages for phytoremediation [136, 137]:

- A high yield of biomass (at a density of 10,000–20,000 per hectare) to 15 tonnes of dry matter/ha per year, which enables efficient phytoextraction with moderate amounts of accumulation of toxic elements [138]
- Famous cultivating agricultural technology (*Salix* and *Populus* trees grown on a short rotation system: the harvest in 3–5 years with a total duration of 30 years of cultivation), which can be adapted for use at contaminated lands
- Ability after bioremediation to be used as biofuels (direct combustion, anaerobic digestion processing, fermentation in liquid fuels), for the production of wood, ethanol, biogas, biofortified, biochar, chipboard, paper, constructions, and technical production [139–141]
- Ability to use in urban landscapes
- Creation of greenbelts and phytocenoses for remediation [142–144]
- The stabilization of the substrate under cultivation: soil protection from water and air erosion; prevent metal leaching to protect surface and groundwater

5.3 Possibilities of Woody Plant Use for Biomonitoring of Antropogenic Pollution of Environment

Selection of species for environmental assessment is dictated by a number of necessary conditions: they must be sufficiently widespread in the study area and well reflect the state of the environment by changes of qualitative or quantitative characteristics (e.g., the development of necrosis and chlorosis, the change of physiological parameters, morphological or anatomical changes, changes at the molecular level, etc.). Based on these characteristics the assessment of the environment can be carried out which includes physiological and biogeochemical characteristics of species [19–23, 34, 35, 88–105].

An important issue is the expansion of the list of species of woody plants, which can be used for phytoremediation of environment in conditions of complex pollution of air and soil by heavy metals in industrial centers.

We carried out integrated monitoring of ecosystems with varying degree of anthropogenic load at the territory of a model region of the central zone of Russia—Tula region. The parameters of the woody plants growing in natural habitats (forests and forest-steppe ecosystems) and in polluted urban environment of the regional center of industry (protection zones of motorways, the territory of the metallurgical enterprises) were determined [103].

The parameters of bioaccumulation of toxic elements of trees growing in contaminated and clean areas of Tula region (Russia) were studied.

5.3.1 *Study Area: Objects of Investigation*

The model region of the study was Tula region and the city of Tula. In the study area, three natural territories are located: coniferous-deciduous forests in the north, deciduous forests in the center of the region, and forest steppe and steppe in the south. The region is characterized by a well-developed industry: mechanical engineering and metal working, chemical industry, defense industry, ferrous metallurgy, construction materials, light industry, and food industry. Districts of Tula region are characterized by a varying degree of anthropogenic impact. Tula industry (ferrous metallurgy and mechanical engineering) and Novomoskovsk (chemistry) account for more than 2/3 of the regional production. A great contribution to the pollution of the region is from the chemical industry centers Schekino, Efremov, and Aleksin and the center of the electricity Suvorov. The remaining 20 districts of the region account for 10% of industrial production. According to the concentration of industrial enterprises, the Tula region is the second after Moscow, and it is among the five most ecologically unfavorable regions of Russia, ten times exceeding the amount of emissions to the atmosphere of the surrounding Kaluga and Oryol regions. 94% of all emissions are due to the city of Tula and Aleksinskiy, Suvorovskiy, Efremovskiy, Novomoskovskiy, Uzlovsky, and Schekinsky districts where the largest number of industrial enterprises is clustered. 52% of pollutants in the atmosphere fall to the share of industrial enterprises.

The regional center—the city of Tula—is located 180 km south of Moscow. This ecosystem includes the city area of 154 km^2 and a population more than 500,000; it represents an area with developed metallurgical, chemical, engineering, and defense industries with the city's infrastructure and network of roads with heavy traffic. The first stage of investigations was focused on revealing the geochemical anomalies of soil and the analysis of atmospheric air. The results of these investigations showed that more than 40% of the territory of the selected ecosystem was characterized by excess of maximum permissible levels (MPL) of the set of heavy metals in the environment (Fig. 5.1).

The main element pollutants of urban (Tula city) soils were:

Mn (in sampling point 1 up to 50% of soil exceeded MPL)
Fe (high gross concentration all over)
Cu (24% soil exceeded MPL up to 3–6 times)
Zn (28% of soil showed excess of MPL by 15–62%)
As (38% of soil excess of MPL by 36–62%)
Pb (12% soil excess of MPL by 10–50%)

The total index for grading soil contamination identified 20 areas of moderately hazardous category (28% of soils) and 4 of extremely dangerous category (6% of soil) [145]. The map (Fig. 5.1) presents geochemical anomalies in soils of the city on the total pollution index. In the most polluted areas, the analysis of atmospheric air was carried out. The high content of Fe in the form of oxides and sulfates at all sampling points was revealed, which exceeded the MPL average concentrations of Fe by several hundreds of times. The copper concentration was higher than the

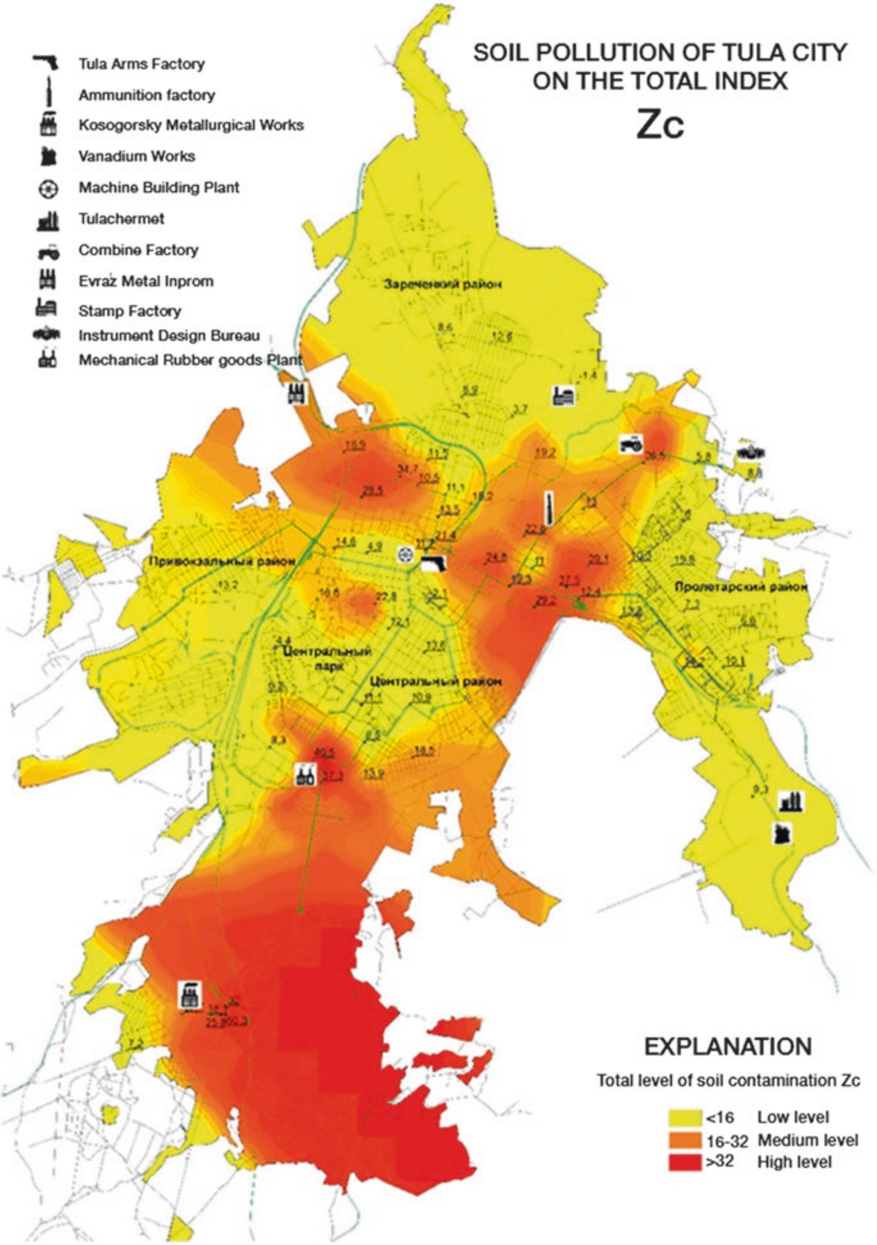

Fig. 5.1 Characterization of Tula city soil pollution

maximum single MPL by 56% of the surveyed zones and exceeded the daily average by 1.5–3.3 times and maximum single—in 3–9 times. Pb content exceeds the average daily rate of MPL sampling points close to Kosogorsky Metallurgical

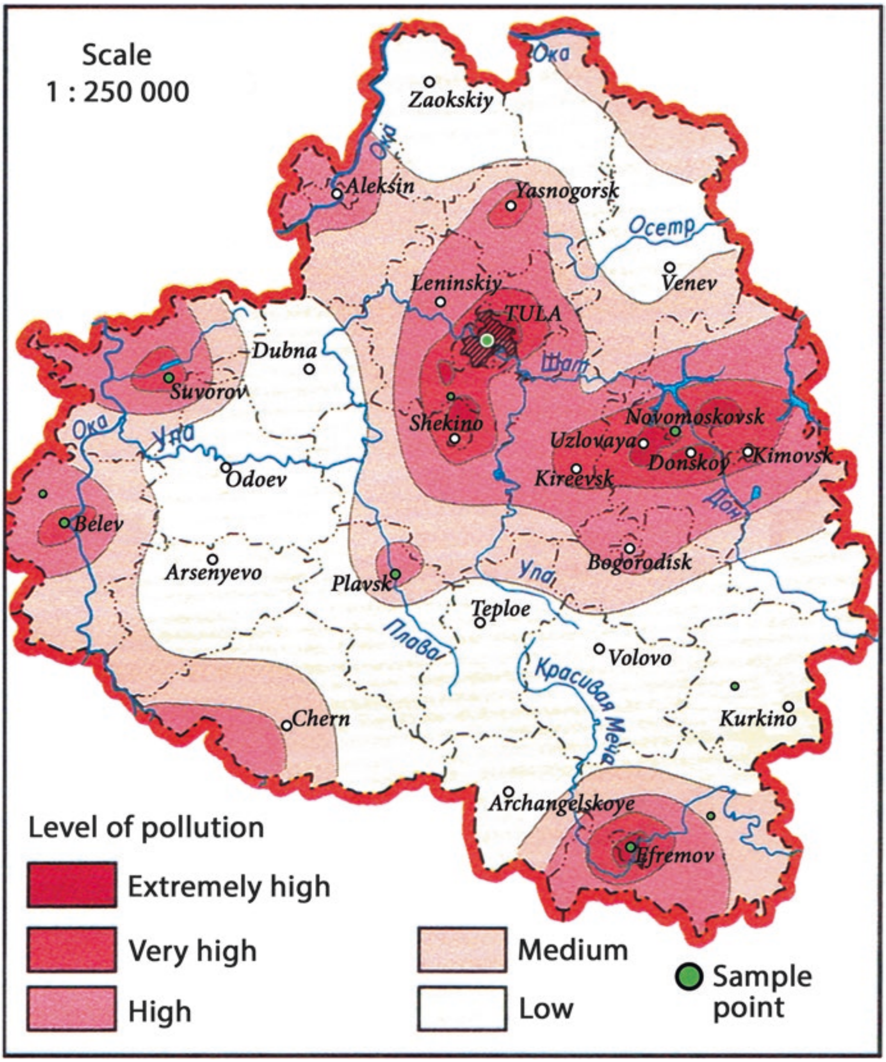

Fig. 5.2 Characterization of Tula region soil pollution and sampling points

Works (ferromanganese production), the Mogilev square (Pedagogical University), and at the intersection of two main roads of the city (Krasnoarmeyskiy Avenue) [104, 105, 145].

To investigate the possibility of using woody plants for biomonitoring the environmental situation, seven areas with different levels of anthropogenic pollution of the region and the regional center—the city of Tula—were chosen (Fig. 5.2, Table 5.3). The objects of biogeochemical parameters in the study (content of elements in the leaves) were native tree species: *Tilia cordata*, *Acer platanoides*, *Salix fragilis*, and *Picea abies*.

Table 5.3 Characterization of different areas of Tula region

Sampling point	Description of environmental conditions
City of Tula, sanitary-protective plantings along roads	Industrially developed urban ecosystem with a high level of technogenic pollution, exceedance of MPL of HM metals in soils by 40% of the territory, a high level of dust and exceedance of the MPL of HM in the air sampling points
Tula, sanitary-protective zone of metallurgical enterprises, KME and Tulachermet	In soil samples of the sanitary-protective plantings observed, an excess of MPL of HM on a number of elements, Mn (twofold), Pb (1.5 times), and Zn (2 times), was observed
Kulikovo Pole	The area is located in forest-steppe vegetation zone of Tula region and characterized by low level of human impact (a historical place reserve museum area). Agricultural using with fertilizers is the main form of anthropogenic activity for the soils
Yasnaya Polyana	The area of museum reserve “Yasnaya Polyana” is located in deciduous forests and influenced of metallurgical and chemical enterprises (Kosogorsky metallurgical plant, Shchekinoazot)
Plavsk town	The city is located in the forest-steppe part of the region. The state of ecology is affected by a distillery “Plavsky” emissions of which contaminate the river
Belev town	The city is located in coniferous-deciduous forest area and experiences recreational and vehicle load, and among the industrial enterprises is the plant Transmash
Belevskiy area (forest)	The region is located in a strip of coniferous-deciduous forests, and there are no large industrial enterprises
Novomoskovsk town	The city with high level of industrial pollution (Nitrogen, Procter & Gamble—Novomoskovsk, Knauf Gypsum Novomoskovsk, Orgsintez, Polyplast, Novomoskovskaya GRES)
Suvorov town	The industrial city is located in coniferous-deciduous forest area and influenced of Cherepetskaya hydropower station, Cherepetskaya precast concrete plant, Mitinskaya Iron Works and recreation
Suvorovskiy area (Varushizi)	The city is located in coniferous-deciduous forest area and influenced of Cherepetskaya hydropower station
Efremovskiy area (Shilovo)	The region is located in the forest-steppe part of the region. The enterprises have a negative impact on the environment and are the production of synthetic rubber and household chemicals (Novomoskovskbytkhim and Procter & Gamble) and Efremov thermal power station

The sampling sites to determine the suitability of species for phytoremediation of soils in sanitary-protective planting of metallurgical enterprises of Tula are: point I, JSC “Kosogorsky Metallurgical Works” (KME) (ferromanganese production), and point II, complex of enterprises of JSC JV “Tulachermet” and “Vanadium” (Tulachermet) (production of pig iron, vanadium, and chromium). For relatively pristine (background or control) zone, the area of the Central Park of Culture and Leisure was chosen. The distance between point I and control zone is 2–3 km and between point II and control zone is 5–6 km. Distance of sanitary-protective planting from aerosol emission sources is 30–400 m.

The objects of investigation of woody plant feasibility for bioremediation are seven tree species and eight shrubs dominating in the sanitary-protective zone of the metallurgical enterprises: *Sorbus aucuparia*, *Acer platanoides*, *Populus nigra*, *Aesculus hippocastanum*, *Tilia cordata*, *Larix sibirica*, *Betula pendula*, *Crataegus sanguinea*, *Crataegus monogyna*, *Cornus alba*, *Cotoneaster lucidus*, *Symphoricarpos albus*, *Syringa vulgaris*, *Philadelphus coronarius*, and *Physocarpus opulifolius.*

5.3.2 Sampling, Sample Preparation, and Methods of Research

Sampling to determine the ability of wood to bioaccumulation of toxic elements was carried out in the third decade of July during the vegetation peak over the perimeter of the tree crowns at a height of 1.5–2 m in the plant communities of different districts of the region and urban ecosystems of the city of Tula. The minimum number of trees (shrubs) in each type of sampling points was ten. The minimum number of leaves from each tree (shrubs) was ten.

Leaves of woody plants were washed in running water, and then they were washed twice in distilled water. This way of sample preparation, as opposed to the use of unwashed samples, in our opinion, allows to avoid large errors in sample preparation that may occur due to loss of the dust particles in the course of operations, packaging, grinding, weighing, and pressing samples and eliminates dependence on climatic factors (washings by rains, the wind emission) during the sampling and before it. It allows to perform a comparative description of the research results.

Washed samples were dried at room temperature and brought to constant weight in an oven at a temperature of 60 °C. The samples were averaged and were packed in paper bags with a label. Sample preparation for instrumental neutron activation analysis (INAA) (grinding, weighing, pressing, and packing containers of samples) took place in a chemical laboratory sector neutron activation analysis LNP JINR.

Part of the elements in plant samples (Mn, Fe, Ni, Cu, Zn, Cd, Pb) was determined in the laboratory of chemical analysis of the Geological Institute (GIN RAS) by atomic absorption spectrometry using “QUANT-2A” (KORTEK, Moscow) equipped with deuterium corrector of nonselective absorption and relevant hollow-cathode lamps; determination of heavy metals in the samples was carried out in accordance with standardized methods [146]. Determination of Zn, Pb, Cu, and Cd was performed in “propane-air” flame and Fe, Mn, and Ni in “acetylene-air” flame. Quality control was provided by using certified reference materials IAEA-SOIL-7, IAEA-336 (lichen), SRM 1572 (*citrus* leaves), and SRM 1575 (*pine* needles).

INAA of plant samples was carried at the IBR-2 reactor at JINR LNP using activation with epithermal neutrons along with the full energy spectrum. To determine the long-lived isotopes, samples of leaves of about 0.3 g were packed in aluminum foil. The containers with samples were irradiated for 4–5 days in a cadmium-screened channel (epithermal neutron activation analysis). After exposure, the samples were repacked in clean plastic containers for measurement of induced activity.

Induced gamma activity of the samples was measured twice: after 4–5 days after irradiation (for determination of As, Br, K, La, Na, Mo, Sm, U, and W) and after 20 days (to determine Ba, Ce, Co, Cr, Cs, Fe, Hf, Ni, Rb, Sb, Sc, Sr, Ta, Tb, Th, Yb, and Zn). Measuring time was 40–50 min and 2.5–3 h, respectively.

To determine the short-lived isotopes of elements (Al, Ca, Cl, I, Mg, Mn, and V), samples of 0.3 g weight were packed in polyethylene packs and irradiated for 3–5 min. Induced gamma activity of the samples was measured after 5–7 min of cooling twice: for 3–5 and 10–5 min, successively.

Measurement of the induced gamma activity was carried out by gamma spectrometers with Ge (Li)—detectors with a resolution of 2.5–3 keV of the gamma line 1332 keV of ^{60}Co and HPGe detector with a resolution of 1.9 keV of the gamma line 1332 keV of ^{60}Co.

A software package developed in the Frank Laboratory of Neutron Physics of JINR was used for processing gamma spectra of induced activity and calculating the elemental concentrations. The concentrations of elements were determined by relative methods (by comparison with the standards) [147]. Certified reference materials (pine needles, NIST) were irradiated and measured together with samples.

The uncertainties in elemental determinations of Na, K, Cl, As, Sr, Fe, and Pb are in the range of 5–10% and for V, Ni, Cu, Se, Mo, Cd, and Sb are 30%.

So far for vegetation there are no identified MPLs and the data on the elemental content in the different studies are very different in dependence on the used methods and sample preparation [21, 34, 90–95] (some studies are made using unwashed plant material), to assess the biochemical characteristics of the investigated samples, they were compared with the average data of the Reference plant (RP) [148].

5.3.3 Results and Discussion

The results carried out in seven districts of the Tula region showed that the leaves of woody native species can be used as bioindicators and biomonitors of biogeochemical parameters in determination of the degree of anthropogenic load on ecosystems.

The two studied species in the cities accumulate more chlorine in the leaves than in the steppe and forest communities. Thus, the chlorine content in the leaves of *Tilia cordata* and *Acer platanoides* in the towns of Plavsk, Novomoskovsk, and Tula varied in interval of 3270–6400 mg/kg, that is, 1.5–3 times higher than the critical concentrations and mean values for vegetation [148–150] and 2–17 times higher than the values for forest and steppe of the region (370–1520 mg/kg) (Fig. 5.3). The accumulation of high concentrations of chlorine in leaves of trees in urban areas may be due to the use of NaCl on the sidewalks followed by washing the salt melt water into the soil in winter and early spring as well as by deposition of the aerosol particles due to the impact of the chemical industry.

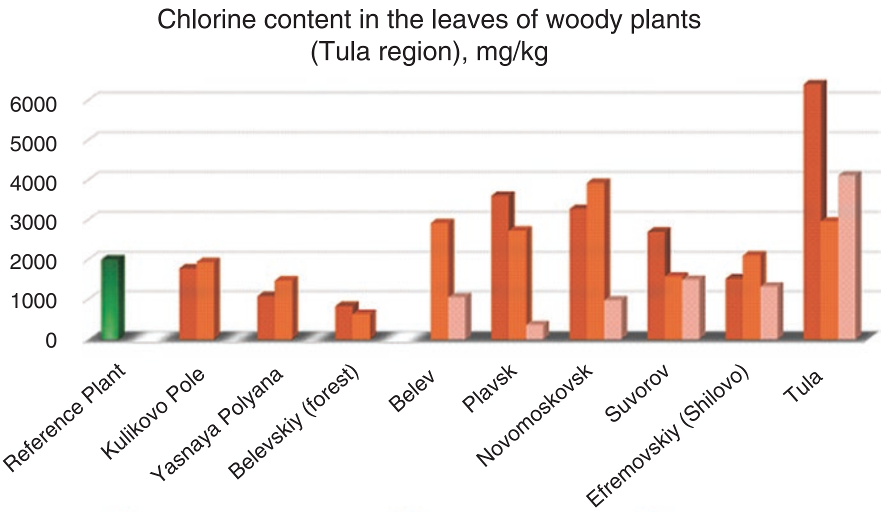

Fig. 5.3 The chlorine concentration in the leaves of woody plants from Tula region ecosystems with varying degrees of anthropogenic load

Acer platanoides, *Salix fragilis*, and *Picea abies* well reflect an increase in the concentration of vanadium in the environment. So, in Belev and in deciduous forest near the city, as well as in the city of Tula (sanitarian-protective zone of motorways), V content in the leaves and needles of the enlisted species ranged from 0.7 to 1.5 mg/kg of dry matter, that is, 1.3–3 times higher than in the reference plant (RP) (0.5 mg/kg). It could be connected with aerosol emissions from enterprises of “Vanadium,” Instrument Design Bureau, NGO “Fusion” (Tula), and JSC “Transmash” (Belev) (Figs. 5.4, 5.5, and 5.6).

The concentration of chromium in leaves of all investigated deciduous woody plants was 1.5–2.7 times higher than in RP (2.3–5.0 mg/kg dry weight) at all sampling points examined except Belevskiy area (Figs. 5.4, 5.5, and 5.7). This fact may be an evidence of air emission of this element, and it also confirms our previous assumption that woody plants concentrate more chromium in organs rather than herbaceous plants [38–40, 148, 149].

The given fact evidences that the woody plants are sensitive indicators to the content of the given element in the environment in time and they can reflect the spatial distribution of an air emission and absorb elements from deeper soil horizons.

Two deciduous species *Acer platanoides* and *Salix fragilis* react to high iron content in the soil and air (Figs. 5.4 and 5.5). The greatest sensitivity characterizes *Acer platanoides* (element content of the leaves increases up to 1250 mg/kg (Tula), that is, twofold higher than the concentrations of toxic element for vegetation [149, 150] and eight times higher than the RP). Such intense absorption of iron along with other heavy metals can lead to the development of necrotic changes in the leaf and to reduction of vitality of the species in terms of polymetallic soil pollution of industrial cities.

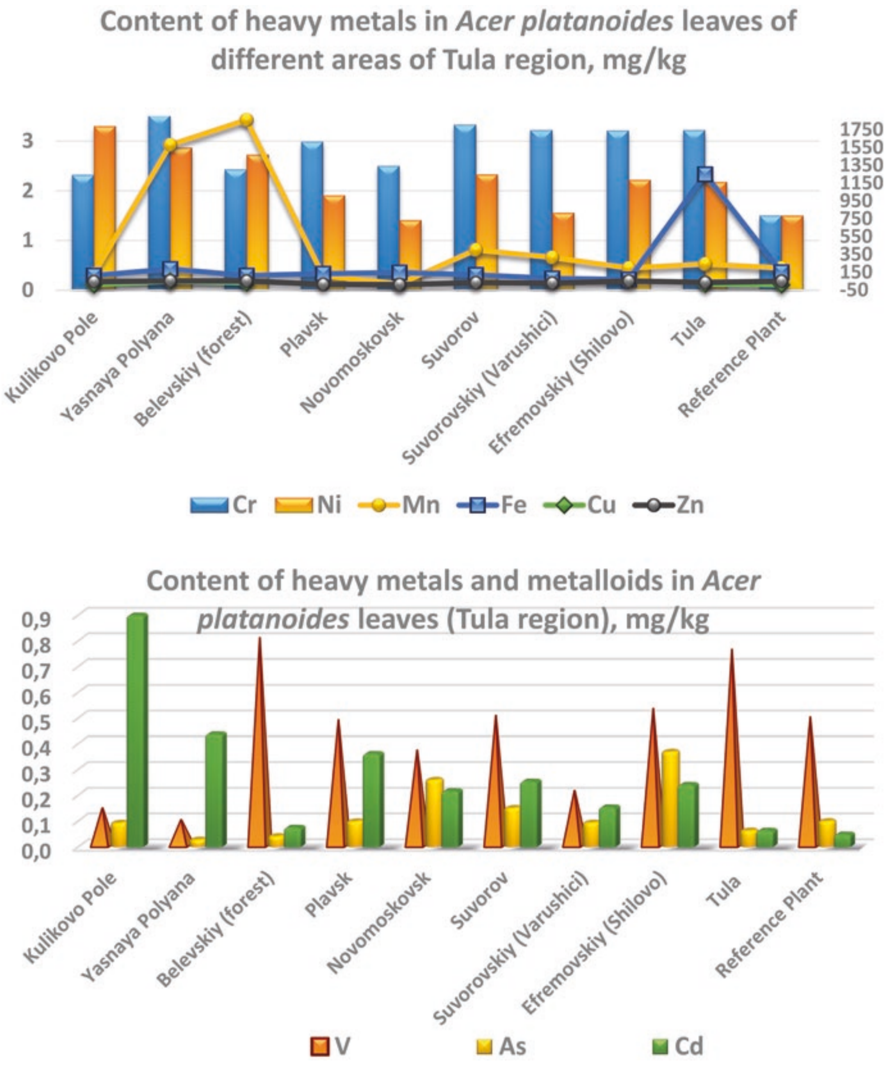

Fig. 5.4 The content of heavy metals and metalloids in *Acer platanoides* leaves grown in different areas of Tula region

Bioaccumulation of copper in leaves of woody plants in Belevskiy area (city and forest), Yasnaya Polyana, and the regional center—the city of Tula—reached 24–53 mg/kg of dry weight, that is, 2.5–5 times higher than the values of RP. The high concentration of the element in the wood plants is conditioned by its high content in the air and soil due to the impact of metallurgical industry and metalworking [104, 105, 145].

Accumulation of arsenic was noticed in the leaves of deciduous trees at the sampling points in Novomoskovsk and Efremovskiy area (Shilovo village) (Figs. 5.4 and 5.7). Its concentrations of 0.21–0.37 mg/kg of dry weight are 2–3.5 times higher than in the reference plant. *Picea abies* needles accumulate in two times less arsenic than RP regardless of the point of sampling (Fig. 5.6).

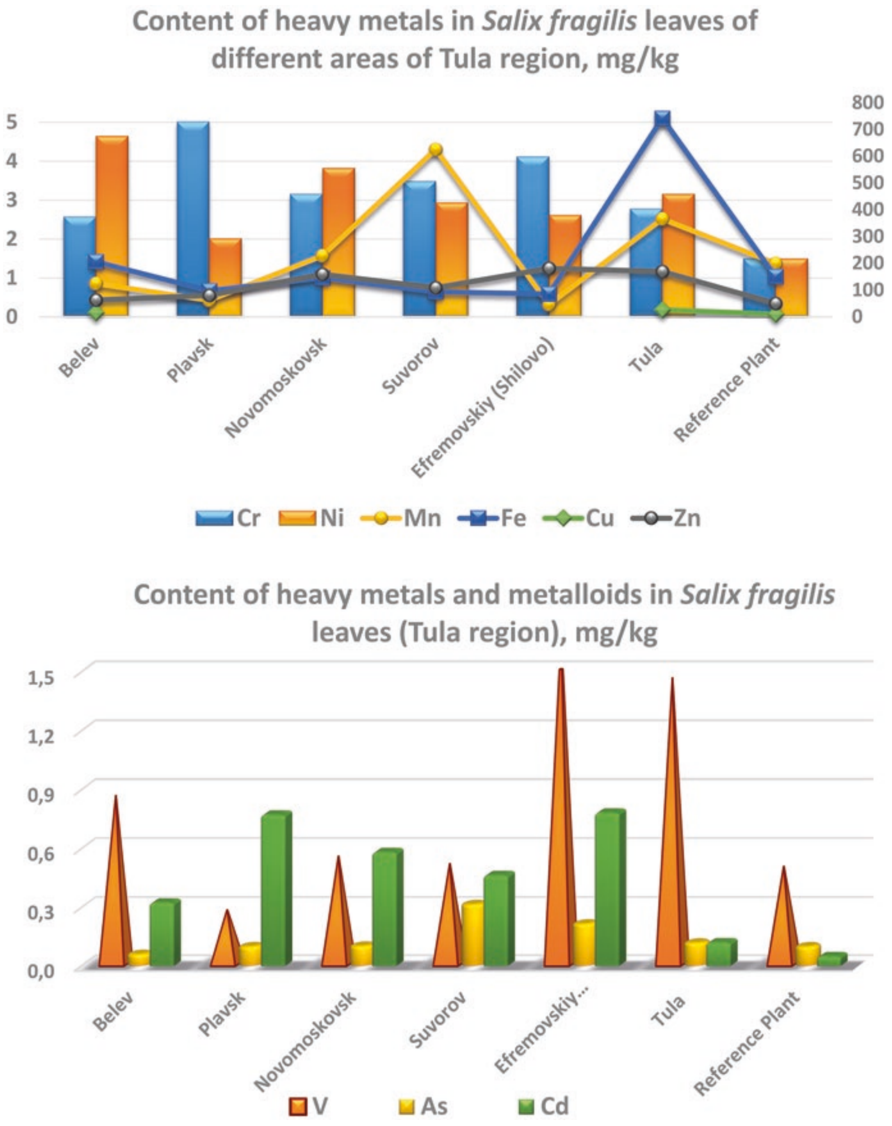

Fig. 5.5 The content of heavy metals and metalloids in the leaves of *Salix fragilis* (Tula region)

The trend in accumulation of cadmium is similar to the arsenic one: *Picea abies* does not accumulate element in the needles, while all deciduous woods are investigated at all points, except with sampling growing in sanitary-protective zone along Tula roads and in the forest area of Belevskiy, accumulated from 0.16 to 0.92 mg/kg of the element in the needles that exceeded by 3–18 times the average data for plants (Fig. 5.6). The highest concentration of the element was observed in *Acer platanoides* leaves growing in the Kulikovo Pole, as well as in the leaves of *Salix fragiles* (Figs. 5.4 and 5.5). The best biomarker for this element is *Salix fragilis*. The fact of high

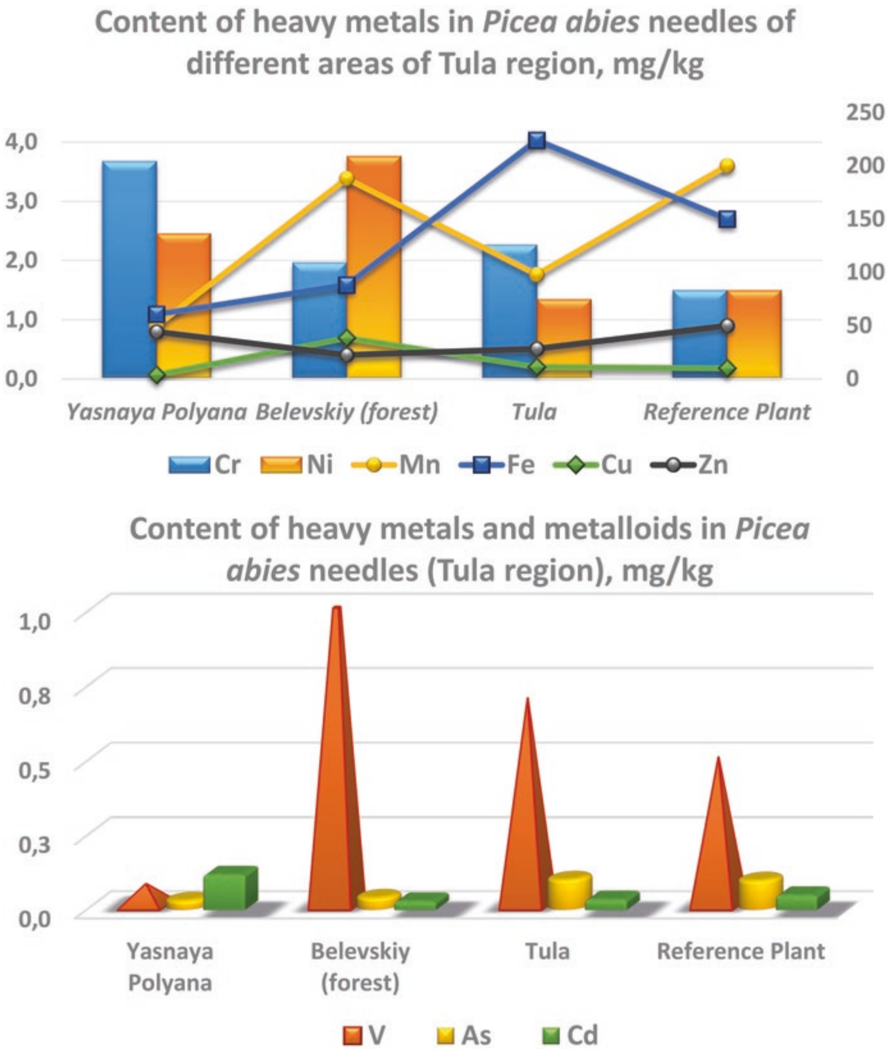

Fig. 5.6 The content of heavy metals and metalloids in the needles of *Picea abies* in different ecosystems of Tula region

accumulation of cadmium by leaves of woody plants can be explained by air emission of the element by motorway, as well as, apparently, by better degree of absorption of the elements at alkaline soils (Efremovskiy area (Shilovo), Kulikovo Pole). The trend of low bioaccumulation of cadmium in leaves of trees growing in Tula, in the soils of multi-element anomaly, can be explained by the antagonism of the ions when accumulated from the environment, as well as the low level of cadmium in the soil.

Taking into consideration the difference in bioaccumulation of toxic elements by leaves of plants growing in different districts of the region, one may conclude that the chosen plant for bioindication and biomonitoring reflects the environmental

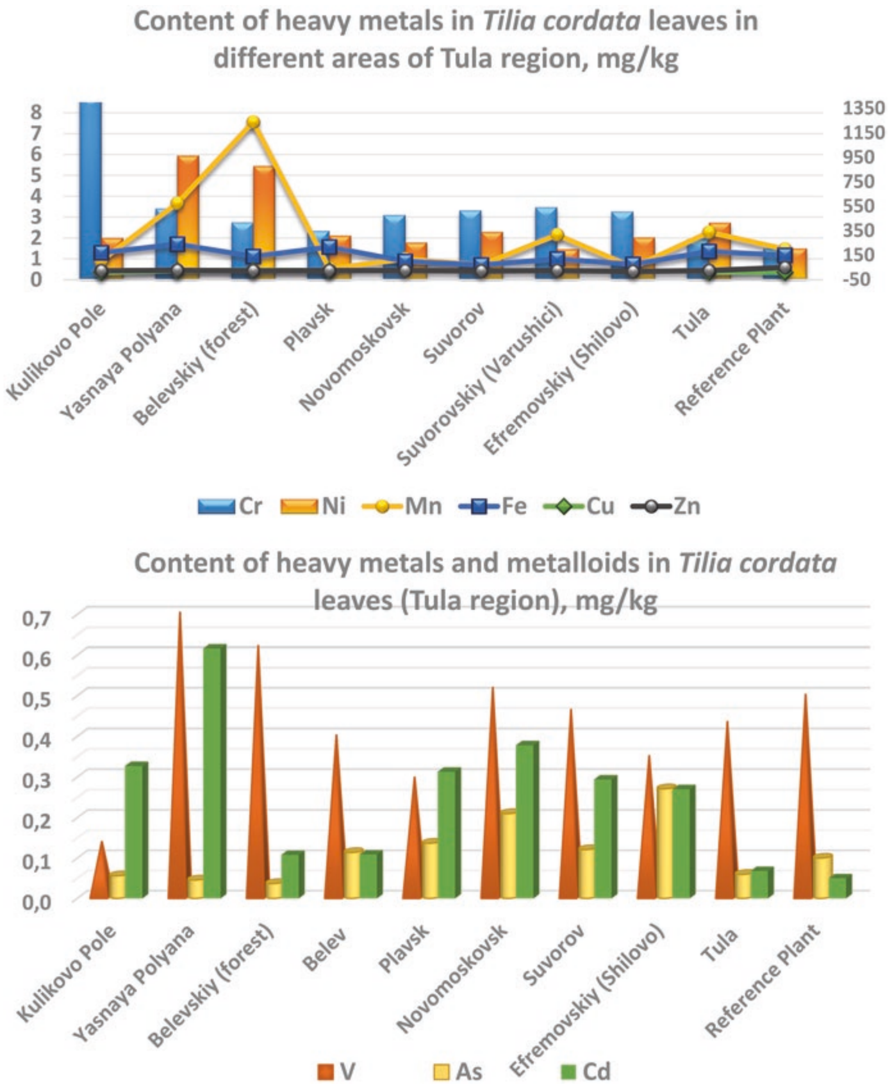

Fig. 5.7 Heavy metal bioaccumulation in leaves of *Tilia cordata* (Tula region)

situation in ecosystems with different levels of anthropogenic load and can be used as biomonitors.

At the same time the largest range of changes in biogeochemical activity is typical for species *Acer platanoides* and *Salix fragilis*, which are preferably used for biomonitoring based on biogeochemical parameters of variability options when changing of the main sources of environmental pollution occurs. Aboriginal species of Gymnospermae—*Picea abies*—is less favorable for the chosen purposes. Below are details of the biogeochemical activity of the studied species (Table 5.4).

Table 5.4 Biogeochemical activity of leaves of woody plants reflecting the anthropogenic load on ecosystems

Species	Elements which accumulated at elevated technogenic loads
Acer platanoides	Cl, V, Mn, Fe, Ni, Cu, As, Cd
Tilia cordata	Cl, Mn, Ni, Cd
Salix fragilis	Cl, V, Mn, Fe, Ni, Zn, As, Cd
Picea abies	V, Mn, Fe

Analysis of correlation bindings at bioaccumulation of elements by leaves of plants revealed strong correlation between accumulated elements. Al correlates with V, Zn, Sm, Hf, Th, and U (accumulation of elements of technogenic soil contamination); Mn with Cu; Cd with W; V with Th and U; and Zn with Th and U (impact factor of metallurgical defense and metal processing industries).

5.4 Accumulation of Heavy Metal in Conditions of Polymetallic Contamination of Industrial Areas (Metallurgical Plants) by Woody Plants of Moderate Climate: Possibilities of Their Application for Soil Phytoremediation

At present a lot of investigations on the use of woody plants for phytoremediation of soil from heavy metals were undertaken. The genera *Salicaceae: Populus* and *Salix* [113, 137, 151–158], *Pinus* [136, 159–161], *Acer* [162, 163], *Betula* [164–168], *Quercus* [136, 169, 170], *Morus alba* [115], *Acacia retinoides* and *Eucaliptus torquata* [171, 172] for soil of subtropical climate were investigated.

For phytoremediation of the environment from radionuclides the resistant to them *Juglans mandshurica* and characterized by high ecological plasticity *Phellodendron amurense* were used.

An important issue is the expansion of the list of species of woody plants, which can be used for phytoremediation of environment in conditions of complex air and soil heavy metal pollution in industrial centers (in a temperate continental climate).

Prior to the beginning of our biogeochemical studies, we have evaluated the vitality of species of sanitary-protective plantations of metallurgical enterprises and highways, the presence of necrotic and chlorotic leaf damage, as well as their ability to accumulate dust emissions on the leaf surface.

In assessing the vitality, the scale was proposed by T. V. Chernenkova [94]: 1 point, “healthy”; 2 points, “weakened”; 3, “severely weakened”; 4 points, “moribund”; and 5 points, “deadwood.” Assessment of the vitality showed that the most morphologically adapted species to the conditions of polymetallic contamination are *Larix sibirica*, *Syringa vulgaris*, *Caragana aerorescens*, *Ligustrum vulgare* (vitality 1), *Philadelphus coronarius* (vitality of 1–2), *Sorbus aucuparia*, and *Acer platanoides* (vitality 2). All studied species in the sanitary-protective plantations showed necrotic and chlorotic leaf change (from 7 to 98%—the most in the genus *Populus*), expressed in the point and edge necrosis and interveinal chlorosis.

Fig. 5.8 Necrosis and chlorosis of tree leaves growing in the area of influence of metallurgical enterprises (*Sorbus aucuparia*, *Aesculus hippocastanum*, *Tilia cordata*)

Manifestation of damage in the leaves of various species was different. For example, *Sorbus aucuparia*, *Betula alba*, *Acer platanoides*, *Tilia cordata*, and *Cotoneaster lucidus* showed the appearance of the point of necrosis on the leaf blade (Figs. 5.8, 5.9, and 5.10). In *Aesculus hippocastanum, Cotoneaster lucidus, Crataegus, Cornus alba*—edge and interveinal necrosis of leaves appear (Figs. 5.8 and 5.10).

All these symptoms may be indicative of the direct damage of leaf tissue by toxic concentrations of iron, manganese, nickel, and chromium [34]. The development of necrosis of leaves can be used for bioindication of the environment at affected areas by the polymetallic pollution with the help of the leaves of wood. Leaf chlorosis may be caused by an imbalance in the leaves with an excess of magnesium ions of other substituents. At the level of the leaf anatomy noted manifestation of was observed kseromorphism symptoms (reduction of the leaf square, Fig. 5.9), An increase in the number of stomata, sheet thickness and diameter of the stomata to compensate for exchange in dusty conditions and high concentrations of heavy metals in the environment of the sampling sites took place [35].

The ability to accumulate dust by leaves of woody plants is different for trees and shrubs and is ranging from 8 to 206 mg/dm^2 for trees and from 17 to 423 mg/dm^2 for shrubs.

The maximum ability to accumulate dust in sanitary-protective plantations belongs to:

- *Tilia cordata* (till 115 mg/dm^2)
- *Populus nigra* (till 115 mg/dm^2)
- *Salix caprea* (till 141 mg/dm^2)

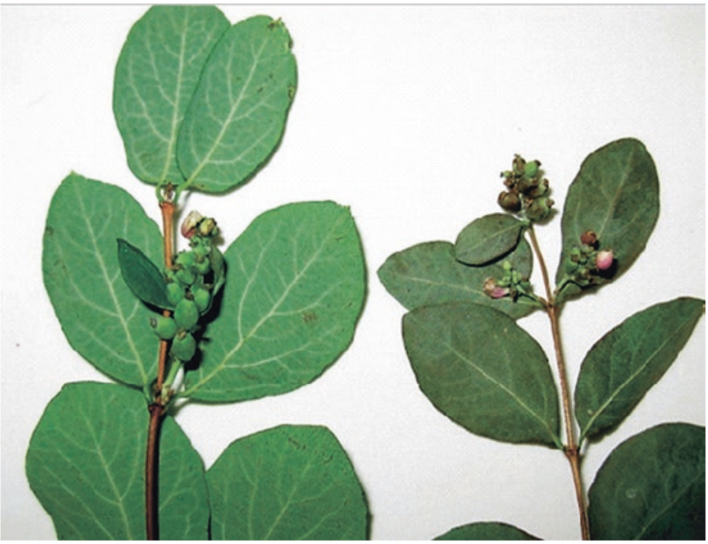

Fig. 5.9 Decrease of leaf area of the *Symphoricarpos albus*, which grows in the area of influence of metallurgical enterprises (*right*) in comparison with the control zone (*left*)

Fig. 5.10 Necrosis and chlorosis of shrub leaves growing in the area of influence of metallurgical enterprises (*Cornus alba*, *Cotoneaster lucidus*, *Crataegus monogyna*)

- *Sorbus aucuparia* (till 206 mg/dm^2)
- *Crataegus sanguninea* (till 107 mg/dm^2)
- *Philadelphus coronarius* (till 149 mg/dm^2*)*
- *Symphoricarpos albus* (till 153 mg/dm^2)
- *Syringa vulgaris* (till 189 mg/dm^2*)*
- *Cornus alba* (till 296 mg/dm^2)
- *Ligustrum vulgare* (till 423 mg/dm^2*)*

We have determined the dust particle content on the surface of leaves using the method of electron scanning microscopy in the laboratory of geochemistry and mineralogy of soil of Federal State Institute of physical, chemical, and biological problems of pedology of RAS (operator E. I. Elfimov).

The distribution of elements on the leaves surface is random, but the greatest number of dust particles concentrates at the bottom, along the edge of the leaf surface and along the main veins of leaves (Fig. 5.11). Analysis of the spectra showed

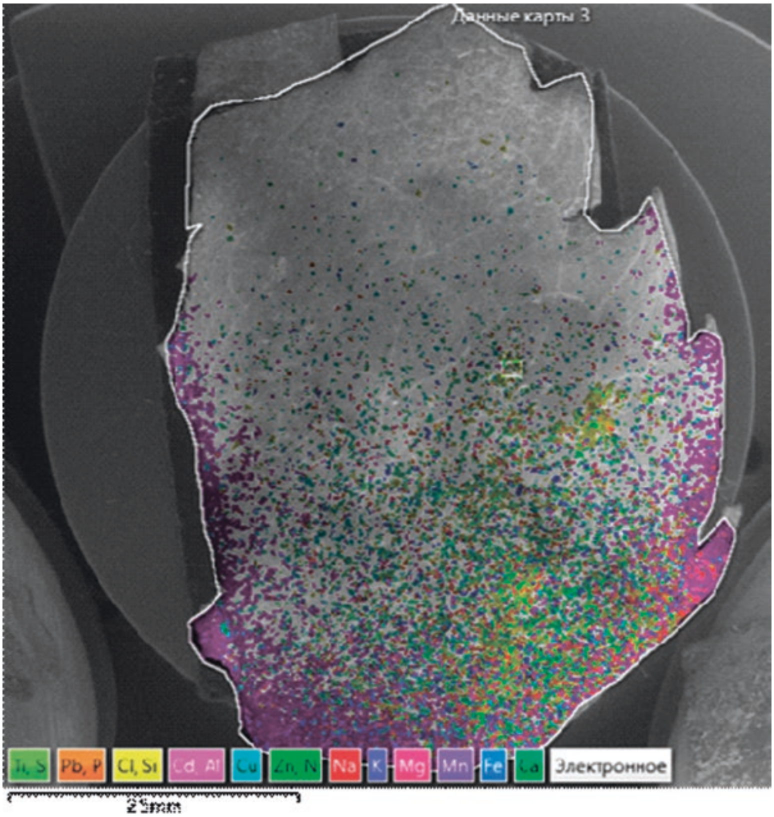

Fig. 5.11 Distribution of dust emissions on a fragment of leaf surface of *Crataegus sanguinea* which grows in the area of metallurgical production (scanning electron microscope)

that most of the adsorbed particles are those compounds of organic nature, and about 1–3% accounted for by soil components, the proportion of heavy metals Fe, Cu, Mn, Cd, Zn, and Pb on the leaf surface may vary from 0.02 to 5% for each element. The method might be the qualitative for analysis of components of aerosol emissions and allows one to establish the presence and ratio of the components on the surface of the leaf that reflects the air pollution of the studied zone.

5.4.1 Bioaccumulation of Heavy Metals by the Trees and Shrub Leaves

5.4.1.1 Manganese

Analysis of the Mn content in leaves of woody plants showed that its concentration ranges from 23 to 385 mg/kg (Table 5.5, Fig. 5.12). That is a rather strong variation of the element probably depending on the species features, growing conditions, and the element concentrations in the environment . Low concentrations of the element (23–150 mg/kg) are characteristic for species such as *Aesculus hippocastanum*, *Tilia cordata*, *Betula pendula*, *Cornus alba*, *Physocarpus opulifolius*, and *Philadelphus coronarius* growing in urban conditions in relatively pristine areas. However, the impact of emissions from the steel industry resulted in increase of the content of elements by two times in *Philadelphus coronarius* and *Tilia cordata* (sample point KME), four times in *Aesculus hippocastanum*, and eight times in *Betula pendula* and reached a value of 340 mg/kg, that is, on average, 1.5 times higher than in the reference plant [148]. However, for *Larix sibirica* in excess of the concentration of the element in the environment overrelatively permissible concentrations, increase of its content in the leaves was not noticed. This may serve as evidence for inclusion of protective mechanisms in the absorption and transport of this element by plant root system, and it also can be caused by antagonism with Fe ions in the process of element uptake by root system.

The role of *Betula pendula*, *Pinus sylvestris*, and *Larix sukaczewii* in the absorption of Mn from heaps of mining enterprises has been described [34]. The maximum element concentration in the leaves of the studied species of woody plants was characteristic for *Acer platanoides*, *Betula pendula*, *Tilia cordata* (180–340 mg/kg), and *Cotoneaster lucidus* (336 mg/kg dry weight). When the concentration of elements in soil exceeds maximum permissible level (MPL) by a factor of 4.7 (site affected by metallurgical enterprises), the contents of the element in the leaves of *Acer platanoides*, *Tilia cordata*, and *Betula pendula* increased by 2–7 times, but not as significantly as in *Aesculus hippocastanum*.

Thus, among the studied species, the maximum accumulation of Mn in the leaves is characteristic of *Acer platanoides*, *Betula pendula*, and *Cotoneaster lucidus*, which is a peculiarity of species.

Table 5.5 Content of heavy metals in the leaves of woody plants, mg/kg of dry weight

Element		**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
RP (Markert [148])/terrestrial vegetation (Kabata-Pendias [149])		**200**	**150**	**1.5**	**10**	**50**	**0.05**	**1**
		15–500	18–1700	0.1–3.7	5–30	1.2–73	0.08–028	0.1–10
Woody plants (zone of metallurgical enterprises and ore dumps) *Acer rubrum*, *Betula pendula*, *Pinus sylvestris*, *Picea*, Lukina, Nikonov [90] Chernenkova [94], Kulagin, Shagieva [34]		197–1055; 157–1050; 195–1130	88–698; 2140–4790; 1630–3680	14–98; 4.5–16; 0.9–11	16–37; 5.1–57; 9.3–26	19–54; 5.6–56; 93	0.14–0.64; 0.42–0.97	1.2–16.3
Acer platanoides	I (KME)	180±22	810±7	0.7±0.3	5.7±0.4	31±6	0.09±0.01	2.6±0.2
	II (Tulachermet)	260±28	1250±41	3.2±0.1	10.1±0.9	44±8	0.16±0.02	0.6±0.1
	Control zone	120±17	310±16	2.2±0.2	8.2±0.7	38±7	0.11±0.02	0.6±0.1
Aesculus hippocastanum	I	53±16	1065±54	4.5±0.7	15.4±1.3	41±9	<0.04	5.6±0.2
	II	91±11	1388±78	1.5±0.2	12.8±0.8	33±8	<0.04	0.6±0.1
	Control zone	83±14	230±12	1.1±0.2	10.7±0.8	32±5	<0.04	0.6±0.1
Betula pendula	I	240±19	800±28	4.3±0.2	6.3±0.5	156±24	0.15±0.02	5.6±0.2
	II	340±7	940±85	6.1±0.4	6.8±0.4	99±12	0.14±0.03	0.5±0.1
	Control zone	45±8	180±24	3.1±0.2	4.3±0.2	53±8	<0.04	0.5±0.1
Populus	I	190±21	1580±62	5.0±0.3	6.8±0.5	171±19	0.58±0.03	8.1±0.2
	II	92±17	500±27	7.2±0.4	10.9±0.9	127±16	0.66±0.04	<0.5
	Control zone	27±5	195±14	1.5±0.1	4.6±0.2	124±18	0.27±0.02	<0.5
Sorbus aucuparia	I	110±16	1780±54	1.2±0.1	6.2±0.4	29±4	0.08±0.01	6.9±0.2
	II	120±14	2570±78	1.8±0.2	6.0±0.3	29±4	0.04±0.01	<0.5
	Control zone	51±7	550±24	1.5±0.1	8.4±0.7	29±3	0.10±0.01	0.9±0.1
Tilia cordata	I	306±54	5082±72	3.5±0.2	7.6±0.6	35±3	0.09±0.01	1.1±0.1
	II	130±14	970±35	1.8±0.1	8.0±0.5	41±3	0.08±0.01	<0.5
	Control zone	130±15	220±25	2.3±0.3	9.0±0.6	41±4	0.10±0.01	0.7±0.1
Larix sibirica	I	110±17	1210±64	0.9±0.1	6.0±0.4	33±2	0.09±0.01	15.2±0.3
	II	120±32	3640±126	4.2±0.4	5.1±0.4	42±4	<0.04	1.6±0.1
	Control zone	410±38	840±32	3.1±0.4	6.5±0.5	41±3	0.06±0.01	1.9±0.2
Excess concentration (toxic concentration) (Cabata-Pendias, 1989)		**≥500**	**≥500; >1000**	**10–100**	**20–40**	**≥300**		**≥60**
Normal concentration (range of normal regulation) (Cabata-Pendias, 1989)		**20–60**	**19–250**	**0.1–2.1**	**3–12**	**20–60**		

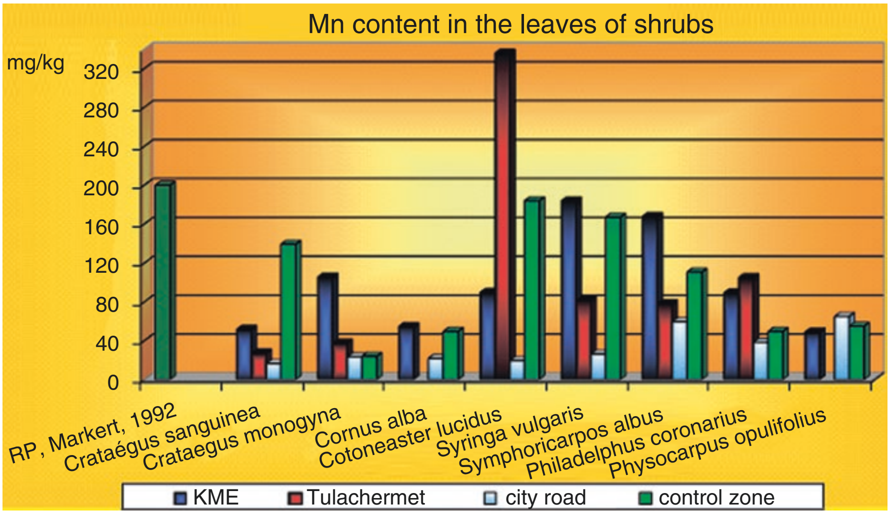

Fig. 5.12 Comparison of Mn content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

5.4.1.2 Iron

The results of investigations showed that the Fe content in leaves of woody plants in urban conditions, in the absence of additional sources of pollution (emissions of enterprises), varies from 48 to 2062 mg/kg of dry weight. In the absence of additional pollution from industries, the minimum iron content is characteristic for leaves of *Populus nigra* (48–195 mg/kg of dry weight) (Table 5.5). The maximum from trees was observed in *Larix sibirica* needles (840 mg/kg) and *Cotoneaster lucidus* leaves (687 mg/kg of dry weight). The content of Fe in the leaves of *Aesculus hippocastanum* and *Tilia cordata* is characterized as close to the average of 142–290 mg/kg, which exceeds the average values for the reference plant [148]. The leaves of *Betula pendula* are characterized by a minimum range of Fe concentrations (154–180 mg/kg).

Moreover, the maximum content of Fe was observed in leaves of *Larix sibirica*, *Sorbus aucuparia*, *Tilia cordata*, *Cornus alba*, and *Cotoneaster lucidus* (Table 5.5, Fig. 5.13).

In general, *Larix sibirica*, *Sorbus aucuparia*, *Tilia cordata*, *Aesculus hippocastanum*, *Acer platanoides*, *Populus nigra*, *Cotoneaster lucidus*, *Cornus alba*, and *Crataegus sanguina* can be considered the best bioaccumulators of Fe when its content in soil is high. The content of Fe in the leaves of these woody species growing in industrial areas is 1250–8930 mg/kg, that is, 10–60 times higher than the values for the reference plant (Table 5.5).

According to some authors, organ concentrators of iron are the roots and bark of trees growing in dumps of polymetallic deposits [94]. The content of Fe in the organs

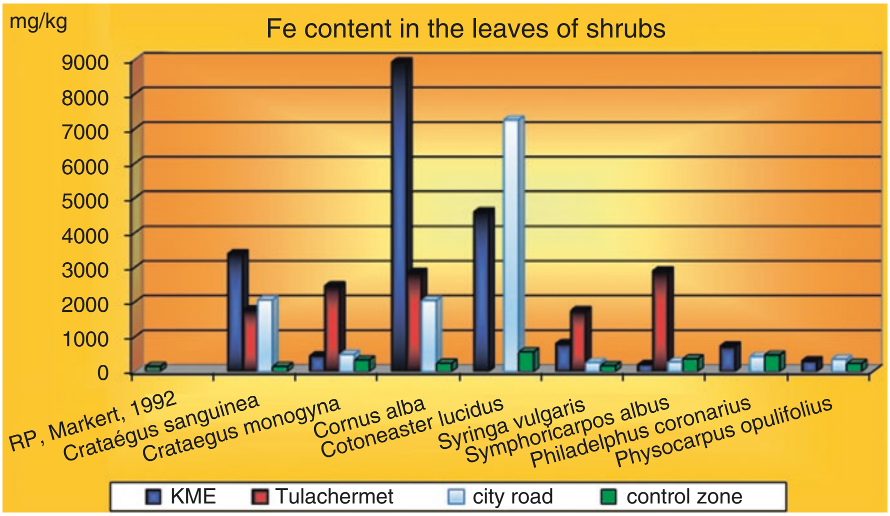

Fig. 5.13 Comparison of Fe content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

(*Betula, Pinus*) amounts to some thousand ppm [34, 90, 94]. Thus, one should expect that the total accumulative ability of trees in the industrial site in relation to iron would be even higher due to accumulation of iron in the perennial organs.

The ratio of Fe/Mn, apparently, is a decisive factor of plant resistance to toxic elements [149]. In this connection, the establishment of relationships in the intake of a given pair of elements in different plant species is of interest. The results obtained show that the ratio of pairs of elements in the accumulation of leaves of woody plants has species peculiarities. Thus, the lowest ratio of Fe/Mn is characteristic for leaves of *A. platanoides*, 0.5–2.5; *Populus nigra* in the Balkan countries, 1–1.4 [98, 99]; *Betula pendula*, 1–4; and *Syringa vulgaris* and *Crataegus sanguina*, 1–1.1 (in buffer zone conditions). A high content of iron relative to manganese is specific for *Aesculus hippocastanum*, 2.5–7; *Tilia cordata*, 2–12; and all other species of investigated shrubs, 3–14. Under the impact of polymetallic contamination, the ratio increases by up to 5 in *Acer platanoides*, 7–16 in *Tilia cordata*, 8 in *Populus nigra*, 16–21 in *Sorbus aucuparia*, 11–30 in *Larix sibirica*, 65 in *Crataegus sanguine*, 9–165 in *Cornus alba*, and 52 in *Cotoneaster lucidus* leaves. There species should be preferably used for phytoremediation of soils from an excess of Fe.

5.4.1.3 Zinc

The concentration of Zn in leaves of woody plants in urban ecosystems is ranging from 16 to 175 mg/kg (Table 5.5, Fig. 5.14). The minimum content of the element in the leaves is characteristic for *Aesculus hippocastanum*, 16–32 mg/kg; Tilia

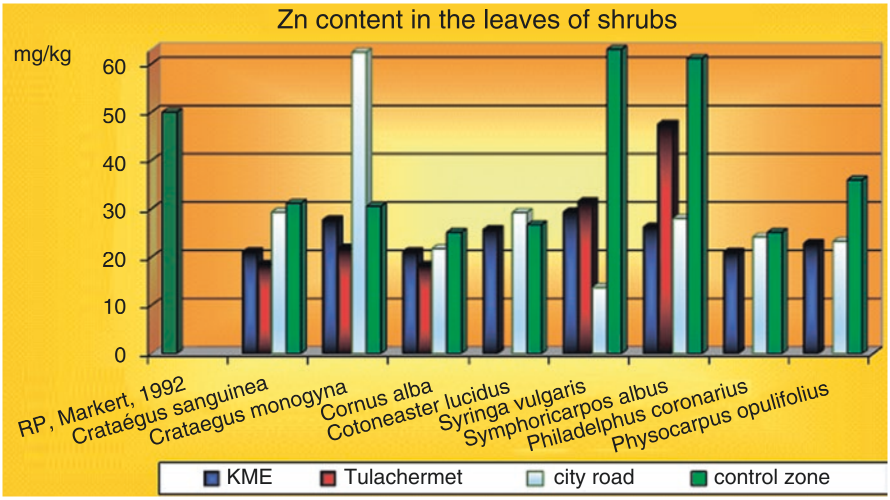

Fig. 5.14 Comparison of Zn content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

cordata, 19–41 mg/kg; and *Crataegus sanguina*, *Cornus alba*, and *Cotoneaster lucidus*, 18–31 mg/kg. Close values of element contents in the leaves are also characteristic for *A. platanoides*. All seven species exhibit a relative resistance with increasing content of Zn in the environment (site of emissions of metallurgical enterprises) that may be due to the known antagonism between the Fe and Zn ions. Data on the antagonism intake of Fe and Zn in the plant organisms [149] are not confirmed for all types of woody plants.

For example, in *A. platanoides*, *Aesculus hippocastanum*, and *Tilia cordata*, increase in accumulation of iron in the leaves takes place along with even a slight accumulation of zinc, i.e., iron absorption dominates over the absorption of zinc.

However, for the species accumulators of Zn, *Betula pendula* and *Populus nigra*, an increased concentration of all three element antagonists Fe, Mn, and Zn—in the site of polymetallic pollution—was revealed. The concentration of Zn increased up to 153–176 mg/kg that exceeds the average for terrestrial plants by a factor of 3–3.5. Such undiscriminating absorption of three elements at their high concentrations in the soil by the given species may be due to the absence of barrier function of the root system to absorption of Fe, Mn, and Zn that is a characteristic peculiarity of hyperaccumulators [29].

5.4.1.4 Nickel

Accumulation of Ni by leaves of woody plants was low and ranged from 0.1 to 4.5 mg/kg of dry weight within urban ecosystems (Table 5.5, Fig. 5.15). The values of Ni content in the leaves of trees exeeds average values for the reference plants by a factor of 1.5–2 of the species *Acer platanoides, Betula pendula, Tilia cordata,* and *Larix sibirica.* However, in the site affected by the metallurgical

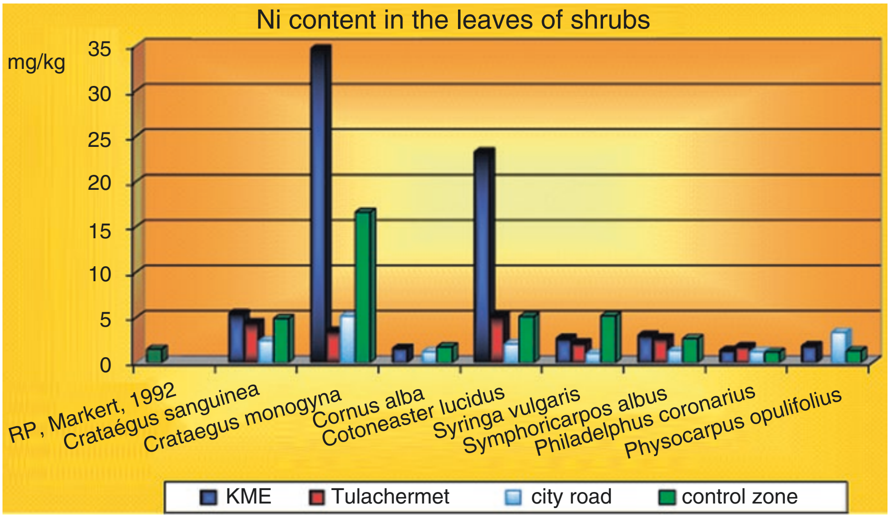

Fig. 5.15 Comparison of Ni content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

industry, the content of the element in the leaves of trees increased by a factor of 1.5–2 for the species *Acer platanoides*, *Tilia cordata*, and *Betula pendula* and more than a factor of 4–4.5 for *Aesculus hippocastanum* and *Populus nigra* (up to 4.3–7.2 mg/kg), exceeding the values for the reference plants by 3–5 times. High concentrations of nickel were found for shrubs *Crataegus*, *Cotoneaster lucidus*, and *Syringa vulgaris* in control zone with 4.9–16.6 mg/kg of dry weight. In the sampling sites affected by the metallurgical industry, the content of the element in the leaves of shrubs increased by a factor of 2–5 for such shrubs as *Crataegus monogina* (KME) and *Cotoneaster lucidus.* However, the total concentration of nickel in the tree leaves did not exceed the threshold of phytotoxicity of the element and was located in the middle of toxic concentrations for species of shrubs such as *Crataegus monogyna* and *Cotoneaster lucidus*. High concentration of heavy metals (nickel in particular) can cause necrosis of *Crataegus* leaves in case of low activity of antioxidant system [149].

5.4.1.5 Lead

Accumulation of Pb by leaves of studied trees and shrubs was low and ranged within 0.5–2.7 mg/kg (Table 5.5, Fig. 5.16). In the areas affected by emissions of metallurgical enterprises, when the concentration of the element in soil exceeds the maximum permissible level, *Tilia cordata* showed the greatest resistance, the concentration of elements in the leaves of which increased slightly compared with the relatively clean area. Possible low bioaccumulation of lead by *Tilia* leaves is due to the peculiarities of leaf surface. However, in the leaves of *Aesculus hippocastanum*, *Betula pendula*, *Populus nigra*, *Sorbus aucuparia*, *Larix sibirica* (sample point KME), *Philadelphus coronarius*, and *Cotoneaster lucidus* (sample point Tulachermet), the

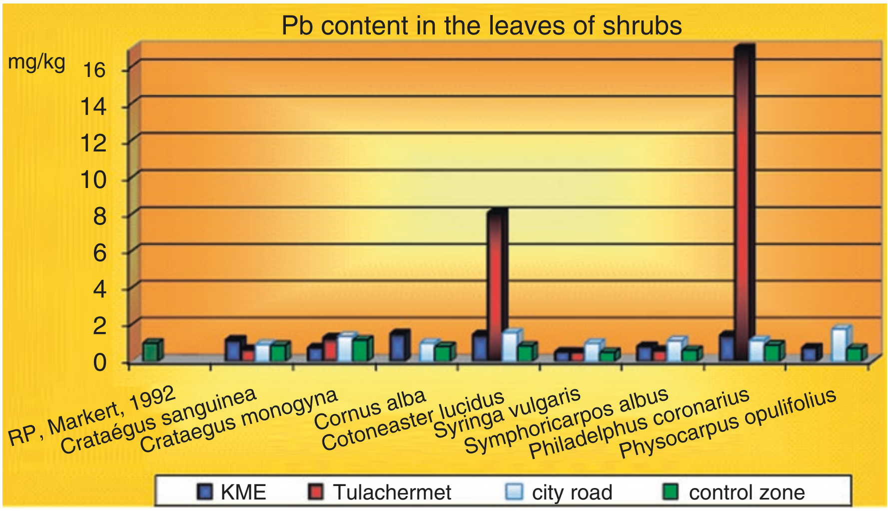

Fig. 5.16 Comparison of Pb content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

concentration of element in the leaves in the impact site of metallurgical enterprises increased by a factor of 9–16 and reached the values of 5.6–17 mg/kg, which is higher than in the “reference plant” by a factor of 5–17. However, in our case, the general element concentrations in leaves of most woody plants do not exceed the values for herbaceous terrestrial plants growing in nonmetallic areas (1–10 mg/kg) [149]. Only for *Larix sibirica* and *Philadelphus coronarius* grown under conditions of metallurgical enterprises, the value of lead concentrations in the leaves is above the average values (15–17 mg/kg). It is known that the transport of lead in the roots is passive, and the major part of the element is kept at a certain level in roots and in the tissue accumulators (inner and outer cortex) [29], but in its sufficient quantity, it can be absorbed by leaves from the air [149]. This is probably the main way of absorption and accumulation of lead by leaves of woody plants [173]. This confirms the fact of the higher lead content in the tree leaves of the cities of the Balkan countries, where sampling is performed in urban heavy traffic areas in comparison with the parks in Russia. For example, high concentration of many metals and toxic elements in dust in Sofia was observed, e.g., up to 192 mg/kg of Pb, 8 mg/kg of As, 123 mg/kg of Cu, 710 mg/kg of Zn, etc. [98].

It was established that leaves of woody plants are suitable for biomonitoring of elements in urban environments, and in the Mediterranean for that purpose such species as *Quercus ilex* was used [2, 3, 88, 174]. However, as it was demonstrated in our study, most of the examined species of trees and shrubs react to the polymetallic pollution accumulating some of these elements.

Of all studied species, only in *Aesculus hippocastanum*, *Betula pendula*, *Tilia cordata*, *Populus nigra*, *Crataegus sanguine* and *monogina*, and *Cornus alba*, combined effect of polymetallic contamination with Mn, Fe, Ni, Pb, and Zn causes stable morphological changes, namely, decrease of vitality, appearance of necrosis, and chlorosis of leaves (Figs. 5.8 and 5.10; Table 5.6).

Table 5.6 The possibility of using the studied species of woody plants for bioremediation of soils contaminated with heavy metals (Gorelova et al. [98, 99, 101, 102, 145, 175])

Species	Vitality/ steadiness to polymetallic pollution	Morphological changes	Ability to bioaccumulate heavy metals under the influence of polymetallic contamination, mg/kg/concentration factor in relation to the reference plants
Aesculus hippocastanum	2–3/–, the death of up to 30% of trees at planting	Regional and interveinal necrosis of leaves	Fe—970–1388/6.5–9 Ni—4.5/3 Cu—12.8–15.4/1.3–1.5 Pb—5.6 /5
Betula pendula	1–2/+	Point necrosis of leaves	Mn—240–340/1.2–1.7 Fe—800–940/5–6 Ni—4.3–6.1/3–4 Zn—99–156/2–3 Pb—5.6/5
Populus nigra	3/–	Necrosis of leaves, dry branches in the crown, dieback	Fe—500–1580/3–11 Ni—5–7.2/3–5 Zn—127–171/2.5–3.4 Cd—0.58–0.66/11–13 Pb—8.1/8
Sorbus aucuparia	2/+	Necrosis of the the leaf margin, chloroses	Fe—1780–2570/11–17 Pb—6.9/7
Larix sibirica	1/+	–	Fe—1210–3640/8–24
Crataegus sanguinea	2/+ in the absence of pruning	25–28% necrotic spots on the leaves	Cl—1720–2560/2 V—2.9–5.5/6–11 Fe—1780–3400/12–23 Ni—4.5–5.5/3–3.7 Cu—26/2.5
Crataegus monogyna	2/+ in the absence of pruning	10–37% regional and interveinal necrosis and chloroses of leaves	V—4.7/9 Fe—457–2470/3–16 Ni—16.6–34.7/11–23 Cu—14/1.4
Cornus alba	2/+ depends on the emissions components	24–28% necrosis of the the leaf margin, chloroses, pest insect damage (aphid)	Cr—6.5/4 Fe—2050–8930/14–59
Cotoneaster lucidus	1–2/+	9% regional and interveinal necrosis of leaves, chloroses	Mn—336/1.5 Fe—2860–7260/19–48 Ni—5–23/3–15 Cd—0.123/2.5 Pb—1.4–8.1/1.4–8
Symphoricarpos albus	1–2/+	4–5% point necrosis of leaves	V—4.4/9 Cr—4.2–5.6/3–3.5
Syringa vulgaris	1/+	4–5% necrosis of the the leaf margin	Cl—4460/22 V—3/6 Cr—5.2/3.4 Fe—810–2310/5–15
Philadelphus coronarius	1/+	7%—necrosis of the the leaf margin, chloroses	Cl—4830/24 V—5.3/10 Fe—430–735/3–5 Pb—1.4–17/1.4–17

This fact demonstrates preference of using the given species for bioindication. It is known that the meaning of bioindicator and biomonitor is not identical [148]. Due to this fact, the choice of biomonitors should be based on the whole set of features. The recommended biomonitors in heavy metal pollution areas are *Populus nigra*, *Betula pendula*, and *Cotoneaster lucidus* as concentrations of all studied elements in polymetallic pollution increase sharply in these species (Table 5.6). The other species can be used for biomonitoring selectively, given the species specificity of the absorption of elements.

On the contrary, for phytoremediation it is more reasonable to use species which have directed adaptive changes and which preserve normal vitality in the conditions of polymetallic contamination.

The number of accumulating elements in this case diminishes, but duration of detoxication of the environment increases. According to the results obtained, *Betula pendula*, *Sorbus aucuparia*, *Tilia cordata* and *Larix sibirica*, and *Cotoneaster lucidus* belong to such species.

5.4.2 Transfer Factor of Elements in Trees and Shrub Leaves from the Soil

Heavy metals enter plants in two ways: by absorbing root system and by uptake through the aboveground organs. An objective criterion, which characterizes the efficiency of accumulation of chemical elements, is the factor of biological accumulation or transfer factor (TF) [176]. To identify possible sources of contamination, it is also important to know the level of air pollution [177]. Part of pollutants are rather hygroscopic and can penetrate the epidermis and stomata in the form of tiny particles as well as a concentrated solution, causing water shortages and promoting early defoliation [178].

The results of the calculation of transfer factor (TF) for woody plants in the buffer site of metallurgical enterprises are shown in Tables 5.7 and 5.8. For most heavy metals, the values of TF are within 0.01–0.2. However, for some species, specificity of accumulation of elements in leaves relatively their concentration in the soil was observed. For example, for *Populus* leaves known as accumulator of heavy metals, the TF for Cd > 1 and it is 5–15 times higher than for other species. These values of TF may be associated with foliar absorption. Poplar leaves secrete sticky substances that promote the transition of insoluble compounds of aerosol particles in the soluble forms of active transport in the leaves cells [94]. However, under the increase of Cd content in the soil, we observed decrease of TF in five times (sampling point I), which is clearly associated with increased barrier function of the root system. Because of bioaccumulation from the soil, the content of Zn in the *Populus* species was higher than its total content in the soil. However, when Zn concentration exceeded the maximal permissible level in the soil by two times, as well as in the case of cadmium, the TF decreased due to barrier function of the roots.

Table 5.7 TF of woody plant leaves in buffer site of metallurgical enterprises (Tula, Russia)

Species	Sampling point	Mn	Fe	Ni	Zn	Cd	Pb
Acer platanoides	I	0.03	0.06	0.01	0.13	0.08	0.03
	II	0.29	0.03	0.08	0.35	0.62	0.02
	Control point	0.16	0.03	0.08	0.58	0.65	0.02
Aesculus hippocastanum	I	0.01	0.06	0.10	0.17	0.03	0.06
	II	0.10	0.02	0.04	0.26	0.15	0.02
	Control point	0.11	0.02	0.04	0.49	0.24	0.02
Betula pendula	I	0.03	0.05	0.09	0.66	0.13	0.06
	II	0.37	0.03	0.16	0.79	0.54	0.02
	Control point	0.06	0.02	0.11	0.82	0.24	0.02
Populus nigra	I	0.03	0.11	0.11	0.73	0.50	0.09
	II	0.10	0.01	0.19	1.01	2.54	0.02
	Control point	0.03	0.02	0.05	1.91	1.59	0.02
Tilia cordata	I	0.01	0.07	0.07	0.15	0.08	0.01
	II	0.14	0.03	0.05	0.33	0.31	0.02
	Control point	0.17	0.02	0.08	0.63	0.59	0.02

Table 5.8 TF of shrub leaves in buffer site of metallurgical enterprises (Tula, Russia)

Species	Samling point	Mn	Fe	Ni	Zn	Cu	Cd	Pb
Symphoricarpos albus	Control point	0.14	0.04	0.09	0.94	0.22	0.0013	0.019
	I	0.02	0.01	0.09	0.11	0.10	0.0002	0.009
	II	0.09	0.08	0.07	0.38	0.11	0.0002	0.024
Syringa vulgaris	Control point	0.22	0.02	0.18	0.98	0.37	0.0018	0.015
	I	0.03	0.06	0.08	0.12	0.15	0.0007	0.006
	II	0.09	0.05	0.06	0.25	0.19	0.0004	0.020
Cotoneaster lucidus	Control point	0.24	0.06	0.18	0.41	0.31	0.0013	0.026
	I	0.01	0.32	0.65	0.06	0.10	0.0003	0.016
Philadelphus coronarius	Control point	0.06	0.05	0.06	0.39	0.27	0.0003	0.028
	I	0.01	0.05	0.04	0.09	0.10	0.0002	0.015
	II	0.12	0.07	0.05	0.11	0.28	0.0003	0.652
Crataegus monogina	Control point	0.03	0.04	0.59	0.47	0.21	0.0012	0.037
	I	0.01	0.23	0.97	0.09	0.30	0.0005	0.008
	II	0.04	0.07	0.09	0.17	0.12	0.0005	0.049
*Crataegus sanguine*a	Control point	0.18	0.02	0.17	0.48	0.25	0.0014	0.027
	I	0.01	0.01	0.15	0.21	0.17	0.0004	0.013
	II	0.03	0.05	0.12	0.15	0.68	0.0001	0.026
Cornus alba	Control point	0.06	0.02	0.06	0.39	0.20	0.0003	0.025
	I	0.01	0.61	0.04	0.09	0.11	0.0002	0.016
	II	0.01	0.08	0.04	0.15	0.09	0.0002	0.016
Physocarpus opulifolius	Control point	0.07	0.02	0.05	0.55	0.30	0.0014	0.022
	I	0.01	0.02	0.05	0.10	0.15	0.0015	0.008

A reduction of the TF, while excess of the maximal permissible levels (MPL) in the soil of the total content of Ni, Zn, and Cd for *A. platanoides*; of Mn, Zn, and Cd for *Aesculus hippocastanum*; and of Mn, Zn, Cd, and Pb for *Tilia cordata*, is also observed (Table 5.7). For phytoextraction of heavy metals from soils, most advisable species are those in which the barrier mechanisms are not working at high concentrations of elements in the environment; however, the mechanism of physiological adaptation and TF increases compared to the background site. When selecting plants for phytoremediation, it is important to consider also species specificity in the accumulation of individual elements. The results obtained showed that with increasing Fe concentration in the medium in all species studied increased the value of TF for the transport from soil to leaves (Tables 5.5 and 5.6). For the sampling site (I), an increase in the value of TF for Fe may be due to foliar absorption element from aerosol particles (the concentration of elements in the air increases). However, due to the fact that iron is an element, concentrated mainly in the roots [108], for all kinds of woody plants, TF values for Fe would be considered low compared with the values for Cd and Zn, due to the barrier function of endoderm and low transport capacity of elements in the acropetal direction.

The average TF for investigated woody plants is as follows:

Mn—0.01…0.37 and maximal for *Betula pendula*
Fe—0.01…0.61, maximal for *Cornus alba*
Ni—0.04…0.18, but for *Crataegus monogyna* 0.97
Zn—0.06…0.48, maximally for *Cornus alba*
Cu—0.09…0.68, maximally for *Crataegus sanguinea*
Cd—0.0002…0.0018
Pb—0.009…0.049 but for *Phyladelphus coronarius* 0.65

For the species which TF of elements is greater than 0.2 (Tab. 5.7, 5.8) the bioremediation of soils from heavy metals will last for 2–10 years (depending on leaves biomass formed during the growing season, water content in leaves, nutrition areas, and the species values of TF) in the absence of additional receipt of heavy metals from the environment.

5.5 Statistical Analysis of the Results

One of the methods to identify the main trends in the intake of elements from the environment is multivariate statistical analysis. We carried out correlation, cluster, and factor analyses of the results of the study, which allowed to identify the main elements—environmental pollutants and their groups. Figure 5.17 shows the dendrogram of groups of elements, which woody plants bioaccumulated from the environment.

Results of cluster analysis clearly distinguished group of elements, which can be divided into several categories:

Group 1: Ca-K are elements-antagonists which play an important role in creating an osmotic pressure in plant cells and the regulation of processes in the plant.

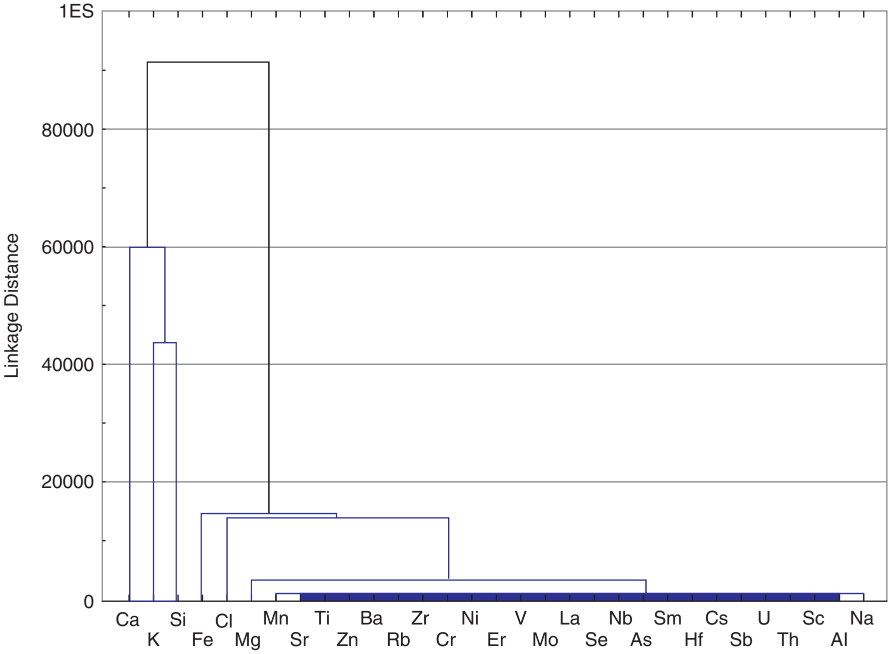

Fig. 5.17 Cluster analysis of bioaccumulation of the elements from the polluted environment by woody plants

Group 2: K-Si are biologically essential elements. Si can be included in the composition of cell walls of plants.

Group 3: Combined elements are organogenic (essential elements) Ca, K, Si, Fe, Cl, and Mg.

Group 4: Combined elements—environmental contaminants of the region Fe, Cl, Mn, Sr, and Ti, associated with soil and ferrous metallurgy.

Group 5: Soil elements and pollutants associated with the processing of ores.

Group 6: The components of the metallurgical and defense industry that can be combined into a conglomerate entering the plant, including root uptake from soil resuspention and atmospheric deposition.

Correlation analysis (Table 5.9) of bioaccumulation of elements by woody plants growing along highways of industrially contaminated city reflects the bioaccumulation characteristics of plants and components of the environmental pollution from the activity of enterprises, highlighting element group and links between them.

Ca-Mg are divalent essential elements with the same way of transport play an important role in the life of plants (Mg is a component of chlorophyll, the regulator of photosynthesis processes, Ca is a regulator of cellular immunity, enzymatic processes in the cell, component of microtubules and is a cell wall component).

Elements that can substitute each other in the biochemical processes in plants are K-Rb and Ca-Sr.

Elements associated with the soil particles are Na-Mg, Na-Al, and Ca-Al.

Table 5.9 Correlation analysis of the element bioaccumulation in woody plants growing near highways

	Na	Mg	Al	K	Ca	Sc	V	Cr	Mn	Fe	Ni	Co	Cu	Zn	As	Se	Rb	Sr	Zr	Mo	Ag	Cd	Nd	Sm	Eu	Tb	Yb	Hf	Ta	W	Hg	Th	U
Na	1.00																																
Mg	0.56	1.00																															
Al	0.74	0.63	1.00																														
K	0.47	0.46	0.37	1.00																													
Ca	0.48	0.74	0.57	0.35	1.00																												
Sc	0.16	0.05	0.30	0.31	0.13	1.00																											
V	0.42	0.34	0.72	0.05	0.34	0.14	1.00																										
Cr	−0.15	−0.16	0.08	−0.04	−0.24	0.26	0.17	1.00																									
Mn	0.09	0.34	0.32	0.02	0.32	0.12	0.52	−0.07	1.00																								
Fe	0.45	0.52	0.82	0.29	0.44	0.31	0.78	0.23	0.53	1.00																							
Ni	−0.21	−0.23	−0.02	−0.07	−0.28	0.04	0.17	0.86	−0.06	0.17	1.00																						
Co	0.17	0.11	0.31	0.05	0.06	0.49	0.27	0.62	0.08	0.30	0.28	1.00																					
Cu	0.19	0.13	0.12	0.21	0.24	−0.01	0.01	−0.23	0.16	0.00	−0.27	0.09	1.00																				
Zn	0.36	0.14	0.14	0.09	0.11	−0.14	0.00	−0.42	0.14	0.01	−0.40	−0.22	0.25	1.00																			
As	0.36	0.37	0.26	0.27	0.38	−0.08	0.07	−0.68	0.12	0.13	−0.58	−0.44	0.11	0.35	1.00																		
Se	0.09	0.12	0.27	0.19	0.07	0.62	0.17	0.54	0.03	0.31	0.28	0.59	−0.08	−0.14	−0.23	1.00																	
Rb	0.26	0.29	0.17	0.65	0.19	0.17	−0.08	−0.12	−0.12	0.12	−0.11	−0.08	0.19	−0.01	0.30	0.13	1.00																
Sr	0.49	0.45	0.51	0.29	0.68	0.14	0.31	−0.32	0.13	0.36	−0.33	−0.12	0.07	0.16	0.54	0.17	0.44	1.00															
Zr	0.01	0.01	0.19	0.17	−0.06	0.54	0.12	0.74	−0.05	0.25	0.35	0.84	0.06	−0.34	−0.50	0.68	0.04	−0.17	1.00														
Mo	0.06	0.01	0.03	−0.06	0.07	−0.11	−0.06	−0.16	0.15	−0.03	−0.14	−0.13	0.02	0.00	0.03	−0.11	−0.06	0.05	−0.15	1.00													
Ag	−0.06	−0.03	0.12	0.07	−0.14	0.47	0.09	0.76	−0.04	0.20	0.40	0.78	−0.01	−0.20	−0.56	0.72	−0.06	−0.24	0.92	−0.18	1.00												
Cd	0.11	0.24	0.28	0.34	0.18	0.37	0.22	0.54	0.10	0.29	0.28	0.61	0.07	−0.16	−0.43	0.55	0.09	−0.05	0.72	−0.11	0.76	1.00											
Nd	−0.04	0.19	0.16	0.13	0.06	0.39	0.06	0.36	0.14	0.21	0.08	0.46	0.03	0.20	−0.19	0.66	0.01	−0.03	0.57	−0.14	0.74	0.65	1.00										
Sm	0.36	0.44	0.68	0.24	0.47	0.37	0.64	−0.08	0.57	0.77	−0.13	0.13	0.07	−0.08	0.27	0.17	0.14	0.46	0.07	−0.02	−0.02	0.19	0.10	1.00									
Eu	−0.23	−0.10	−0.10	0.18	0.01	0.58	−0.06	0.17	−0.01	−0.02	0.10	0.20	−0.12	−0.32	−0.01	0.57	0.25	0.20	0.32	−0.17	0.34	0.33	0.40	0.22	1.00								
Tb	−0.06	0.10	0.15	0.02	0.11	0.30	0.11	0.04	0.29	0.23	−0.09	0.24	0.02	0.33	−0.04	0.42	−0.08	0.03	0.20	−0.12	0.43	0.39	0.76	0.19	0.30	1.00							
Yb	−0.23	−0.13	−0.03	0.02	−0.22	0.31	0.04	0.75	−0.07	0.09	0.64	0.46	−0.21	−0.14	−0.48	0.64	−0.08	−0.27	0.60	−0.22	0.78	0.62	0.66	−0.13	0.49	0.46	1.00						
Hf	−0.06	0.00	0.14	0.04	−0.11	0.44	0.09	0.76	−0.05	0.21	0.39	0.79	−0.02	−0.21	−0.57	0.67	−0.12	−0.28	0.94	−0.15	0.98	0.75	0.72	−0.04	0.25	0.38	0.73	1.00					
Ta	−0.18	0.00	−0.10	0.06	−0.07	0.19	−0.06	0.15	0.03	−0.04	0.03	0.23	−0.02	0.21	−0.17	0.43	−0.06	−0.15	0.33	−0.17	0.55	0.51	0.77	−0.09	0.45	0.71	0.68	0.50	1.00				
W	0.24	0.25	0.42	0.22	0.32	0.24	0.34	0.18	0.20	0.39	0.07	0.33	0.05	0.07	0.07	0.48	0.19	0.40	0.31	0.00	0.35	0.46	0.49	0.41	0.34	0.40	0.33	0.29	0.35	1.00			
Hg	−0.02	0.07	0.11	0.23	0.10	0.53	0.01	0.22	−0.08	0.14	0.03	0.32	−0.05	−0.29	0.11	0.54	0.27	0.29	0.51	−0.31	0.42	0.30	0.35	0.26	0.69	0.13	0.38	0.39	0.31	0.34	1.00		
Th	0.55	0.50	0.80	0.34	0.39	0.47	0.57	0.34	0.31	0.78	0.14	0.46	−0.09	0.08	0.06	0.54	0.14	0.35	0.44	−0.04	0.44	0.44	0.45	0.60	0.08	0.32	0.25	0.43	0.09	0.51	0.20	1.00	
U	0.65	0.50	0.81	0.30	0.38	0.36	0.65	0.11	0.24	0.63	0.00	0.36	0.08	0.05	0.24	0.33	0.15	0.41	0.23	−0.02	0.18	0.31	0.19	0.53	0.03	0.11	0.03	0.18	−0.04	0.39	0.18	0.72	1.00

Contaminants of city soil and particulate emissions into the atmosphere from components of metallurgical production and processing of ore are Fe-V, Fe-Mn, Cr-Ni, Cr-Co, and Sr-As.

Groups with a high correlation between heavy metals and rare earth element-pollutants of the atmosphere and soil of the city, originate from the sources of plant pollution—enterprises of the defense industry, instrumentation, and metallurgy.

Multivariate statistical analysis revealed three factors (Table 5.10):

Factor 1 is associated with ores used for production of steel and alloys.

Factor 2 can be attributed to technogenic pollution (metallurgical production) and soil particles.

Factor 3 is associated with physiological activity of plants.

Table 5.10 Factor analysis of elements bioaccumulation by woody plants

	Factor 1	Factor 2	Factor 3
Na	−0.14	0.73	0.11
Mg	−0.08	0.72	0.30
Al	0.11	0.92	0.05
S	−0.13	0.17	0.04
Cl	0.15	0.29	0.32
K	0.05	0.35	0.54
Ca	−0.16	0.67	0.34
Sc	0.53	0.22	0.34
Ti	−0.12	0.44	0.00
V	0.13	0.77	−0.21
Cr	0.83	0.00	−0.37
Mn	−0.03	0.56	−0.01
Fe	0.23	0.85	−0.05
Ni	0.53	−0.07	−0.44
Co	0.78	0.27	−0.11
Cu	−0.13	0.14	0.25
Zn	−0.31	0.20	0.13
As	−0.60	0.29	0.49
Se	0.77	0.17	0.27
Br	−0.03	0.30	0.62
Rb	−0.07	0.12	0.60
Sr	−0.24	0.51	0.47
Zr	0.90	0.10	0.03
Mo	−0.21	0.05	−0.14
Ag	0.96	0.03	0.02
Cd	0.76	0.23	0.21
In	−0.28	0.16	0.16
Sb	−0.14	0.56	0.21
I	−0.09	0.64	0.04

(continued)

Table 5.10 (continued)

	Factor 1	Factor 2	Factor 3
Ba	0.23	0.39	−0.04
Cs	0.54	0.38	0.16
Nd	0.72	0.11	0.34
Sm	0.04	0.72	0.23
Eu	0.46	−0.20	0.62
Tb	0.43	0.13	0.28
Dy	0.08	0.25	0.43
Yb	0.84	−0.15	0.06
Hf	0.94	0.04	−0.04
Ta	0.54	−0.17	0.38
W	0.38	0.38	0.38
Au	−0.04	0.20	0.14
Hg	0.46	−0.02	0.59
Th	0.43	0.77	0.06
U	0.18	0.77	0.07
Expl. var	9.35	8.25	4.12
Prp. totl	0.21	0.19	0.09

5.6 Conclusion

- Woody plants can be good bioindicators of the environmental pollution with heavy metals (air and soil) affecting the morphological parameters: the development of necrosis and leaf chlorosis between 25 and 98% for unresisting species to pollution. For the purposes of bioindication, species of woody plants such as *Populus nigra*, *Tilia cordata*, *Aesculus hippocastanum*, *Cornus alba*, *Crataegus monogina*, and *Crataegus sanguinea* can be used.
- Analysis of the dust particles on the surface of woody plant leaves using method of electron scanning microscopy can be used as the qualitative method for analysis of components of aerosol emissions and allows one to establish the presence and ratio of the components on the leaf surface that reflects the air pollution.
- Woody plant species can be used as biomonitor of technogenic emissions due to their ability to bioaccumulate Cl, V, Mn, Fe, Ni, Zn, Cu, As, Cd, and Pb which characterize the anthropogenic pollution of soil and air (compared to background values or control zone):
- *Acer platanoides*—Cl, V, Mn, Fe, Ni, Cu, As, and Cd
- *Aesculus hippocastanum*—Ni, Cu, As, and Pb
- *Betula pendula*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Cotoneaster lucidus*—Mn, Fe, Ni, Cu, Cd, and Pb
- *Crataegus monogyna*—Fe and Ni
- *Larix sibirica*—Fe and Pb
- *Philadelphus coronarius*—Pb and Sb
- *Populus nigra*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Salix fragilis*—Cl, V, Mn, Ni, Zn, As, and Cd
- *Tilia cordata*—Cl, Mn, Ni, and Cd

Most of the studied species are good biomonitors to study contamination of soil and air with iron.

- Woody plants which form a large biomass of leaves per season are able to absorb heavy metals to the extent exceeding several times the values characteristic for the reference plants. This allows to recommend them for phytoremediation of the environment from heavy metals:
- *Aesculus hippocastanum*—Ni, Cu, As, and Pb
- *Betula pendula*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Crataegus sanguinea* and *C. monogina*—Cl, V, Fe, Ni, and Cu
- *Cornus alba*—Cr and Fe
- *Cotoneaster lucidus*—Mn, Fe, Ni, Cd, and Pb
- *Syringa vulgaris*—Cl, V, Cr, Fe, and Cu
- *Sorbus aucuparia*—Fe and Pb
- *Philadelphus coronarius*—Pb and Sb
- Populus nigra—Fe, Ni, Zn, Cd, and Pb
- *Larix sibirica*—Fe and Pb
- Analysis of element transfer from the soil into the leaf biomass of woody plants (TF) has shown that for a number of elements (Ni, Cd, Zn), increase of their content in the soil leads to decreased transfer of these elements in the leaves of woody plants. That fact could be a sign of the barrier function of the root system.

Relatively high values of TF (0.2–1) for the elements Fe (species *Crataegus sanguinea* and *C. monogina*, *Cotoneaster lucidus*, *Cornus alba)*, Cu (species *Crataegus sanguinea* and *C. monogina*, *Philadelphus coronarius*), Zn (species *Acer platanoides*, *Aesculus hippocastanum*, *Betula pendula*, *Crataegus sanguinea*, *Betula pendula*, *Symphoricarpos albus*, *Syringa vulgaris*, *Populus nigra*), Cd (species *Acer platanoides*, *Betula pendula*, *Populus nigra*, *Tilia cordata*), and Pb (*Philadelphus coronarius*) in woody plants confirm the possibility of their use for phytoremediation from the enlisted elements.

- When selecting plants for phytoremediation, it is important to consider that species of *Aesculus hippocastanum*, *Crataegus* sp., *Cornus alba*, and *Populus nigra* are not resistant to the integrated pollution with heavy metals. Species *Betula pendula*, *Cotoneaster lucidus*, *Syringa vulgaris*, *Philadelphus coronarius*, *Sorbus aucuparia*, and *Larix sibirica* are resistant to high level of pollution (have normal vitality) and can be used in the creation of sanitary-protective zones of metallurgical enterprises and greenbelts.
- Methods of statistical analysis (correlation and factor analysis) in the processing of the biogeochemical composition of leaves of woody plants allow to clearly reveal group of elements polluting the study area.

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Chapter 6
Phytoremediation Applications for Metal-Contaminated Soils Using Terrestrial Plants in Vietnam

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Abstract In the past few decades, the association of economic growth and mining activities has led to an increase in areas of heavy metal-contaminated soils in Vietnam. As a developing country, Vietnam has the limited financial source for environmental restoration, so phytoremediation, a low cost and ecologically sustainable remedial technology, is considered to be a relevant option. To promote the application of phytoremediaton for heavy metal-contaminated soils in Vietnam, there have been several research programs conducted during the last decade. The studies identified two arsenic (As) hyperaccumulators, ***Pteris vittata*** and ***Pityrogramma calomelanos***, and four grasses suitable for treatment of lead (Pb)- and zinc (Zn)-contaminated soils, ***Eleusine indica***, ***Cyperus rotundus, Cynodon dactylon***, and ***Equisetum ramosissimum***, of which *E. indica* was found as Pb hyperaccumulator. All of these species are indigenous and naturally adapted to heavy metal-contaminated habitats. Three plant species, *P. vittata, P. calomelanos*, and *E indica* and one introduced plant species, ***Vetiveria zizanioides***, were subjected to further evaluation of their heavy metal removal potential under greenhouse and field conditions. The results of greenhouse experiments showed that two fern species, *P. vittata and P. calomelanos*, are effective in the accumulation of soil As in roots and fronds; ***E. indica*** can absorb high concentration of both Pb and Zn in roots. Under field conditions, the combination of ***P. vittata, P. calomelanos***, and ***V. zizanioides*** or ***P. vittata, E. indica*** and ***V. zizanioides*** is very effective in treatment of soils contaminated with low or moderate concentration of As and Pb in short time (3 years).

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Through these studies, phytoremediation has been demonstrated to be feasible for the remediation of heavy metal-contaminated soils in Vietnam.

Keywords Mining activity • Heavy metal-contaminated soil • Indigenous hyperaccumulator • Potential species for phytoremediation • Bac Kan and Thai Nguyen provinces

6.1 Introduction

An increasingly industrialized global economy and rapid rise in world population over the last century have led to dramatically elevated releases of anthropogenic chemicals, particularly heavy metals, into the environment [1]. The annual worldwide release of heavy metals reached 22,000 metric ton (t) for cadmium, 939,000 t for copper, 783,000 t for lead, and 1,350,000 t for zinc over recent decades [2]. Sources of heavy metal released into soil environments include mining, smelting of metalliferous, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, sewage sludge treatment, warfare and military training [3]. Hard-rock mining that is the largest producer of heavy metal waste takes place in all of the continents of the world with the exception of Antarctica [4].

Heavy metal-contaminated soils have caused serious problems threatening ecological systems and human health, recently attracted considerable public attention. Several metals, such as Cu and Zn, are essential for biological systems and must be present within a certain concentration range [5], at high concentrations they will become toxic. Other metals, such as Cd, As, Hg, and Pb, have not been found to have any function in plants and animals, and very toxic for biological life even occurred at low concentrations. Metals can act in a deleterious manner by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules [6]. Exposure to high levels of these metals can cause adverse effect on human and wildlife [7]. Toxic heavy metals can mutate DNA resulting in carcinogenic effects in animals and human [8, 9]. Lead causes neurological damage in children leading to reduced intelligence, loss of short-term memory, learning disabilities, and coordination problems [7]. The effects of arsenic include cardiovascular problems, skin cancer and other skin effects, peripheral neuropathy [10]. Cadmium accumulates in the kidneys and is responsible for a wide range of kidney diseases [10]. The principal health risks associated with mercury are damage to the nervous system, with such symptoms as uncontrollable shaking, muscle wasting, partial blindness, and deformities in children exposed in the womb [10].

Concentrations of heavy metals that have exceeded safety levels in soil should be treated [11]. There are several methods used for soil remediation, including chemical, physical, and biological techniques. Physical treatments involve removal from contaminated sites (soil excavation), deep burial (landfilling), and capping, while chemical methods use strong acids and chelators to wash polluted soils.

These approaches are expensive, impractical, and at times impossible to carry out, as the volume of contaminated materials is very large. Furthermore, they irreversibly affect soil properties, destroy biodiversity, and may render the soil useless as a medium for plant growth [7]. Recently, phytoremediation that refers to a diverse collection of plant-based technologies using either naturally occurring or genetically engineered plants to clean contaminated environments [12] represents a novel, environmentally friendly, and cost-effective technology and attracts the attention of publics and scientists worldwide.

The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia [13] and Chaney [14] and the first field trial on Zn and Cd phytoextraction was conducted by Baker et al. [15]. Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of indigenous metals in their tissues without symptoms of toxicity [15]. Depending on storage sites of heavy metals in plants, phytoremediation technology can be used for containment (phytostabilization) and removal (phytoextraction) purposes [16]. Phytostabilization involves plants to stabilize contaminants by heavy metal retention in roots. Phytoextraction uses plants to absorb metals from soils and translocate them to harvestable shoots where they are collected.

In Vietnam, the increase in mining activities associated with the economic growth has resulted in the increased areas contaminated with heavy metals in recent years. Mining, ore processing, and disposal of tailings provide obvious sources of heavy metal contamination in the mine area and surroundings. The contaminated soils require prompt remediation, and phytoremediation is considered to be one of the best demonstrated available technologies for such purpose [17]. Field applications of phytoremediation have only been reported in developed countries in spite of its cost-effectiveness and environment-friendliness. In most developing countries, it is yet to become commercially available technology possibly due to the inadequate awareness of government and public about its inherent advantages and principles of operation [18]. Since the last decade, therefore, there have been several groups of Vietnamese scientists studying on the use of plant for removal of heavy metals from soils in order to promote the application of phytoremediation. This chapter summarizes the recent research and application related to phytoremediation of heavy metal-contaminated soils in Vietnam, including investigation and selection of native hyperaccumulators of arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn) naturally grown on heavy metal-contaminated habitats; and evaluation of heavy metal removal potential of selected plants under greenhouse and field conditions.

6.2 Selection of Potential Plants for Heavy Metal Removal from Soils

The selection of plants species for phytoremediation is possibly the most important factor determining the heavy metal removal efficiency. Other aspects such as the ecological and environmental protection should be taken into account as selecting the phytoremediating plants.

The success of phytoextraction is dependent on two important characteristics of plants: the ability to produce large quantities of biomass rapidly and the capacity to accumulate large quantities of environmentally important metals in the shoot tissue [19–22]. Hyperaccumulators have been characterized by high heavy metal accumulating potential, small size, and slow growth, while the common non-hyperaccumulators have low potential for metal bioconcentration that is often traded off by the production of significant biomass [23]. In environmental aspect, most of hyperaccumulators are classified as weedy species that can be invasive and endanger the harmony of ecosystem in the new environments, while some crops are palatable and pose a risk to grazing animals. Therefore, the choice of metal hyperaccumulators or common non-accumulator species for phytoremediation is one of the most debated controversies in the field.

Many researchers have supported for the use of non-accumulator species, while others have promoted the application of natural hyperaccumulators. In the study of Ebbs et al. [23], *Brassica juncea* (also known as Indian mustard) was more effective in removing Zn from soil than *Thlaspi caerulescens* (a well-known Zn hyperaccumulator) although the Zn concentration in its biomass was about one-third the concentration of Zn in *Thlaspi caerulescens*. The advantage is due primarily to the fact that *B. juncea* produces ten times more biomass than *T. caerulescens*. Nevertheless, Chaney et al. [24] analyzed the rate of Zn and Cd removal by non-accumulators crops and came to the remark that these crops could not remove enough metal to support phytoextraction. In addition, the high concentrations of heavy metals at many contaminated sites may cause toxicity to crop species and significant biomass reduction. In support of this, several maize (one of the most productive crops) inbred lines have been identified which can accumulate high levels of Cd [25]. However, these lines were susceptible to Zn toxicity and, therefore, could not be used to cleanup soils at the normal Zn:Cd ratio of 100:1 [24]. In addition, when appropriate disposal is an important regulatory concern, the use of lower biomass producing hyperaccumulator species would be an advantage because less contaminated biomass will have to be handled. Moreover, the use of native plants for phytoremediation is more effective because such plants respond better to the stress conditions at the site than would plants introduced from other environments [26]. Consequently, the selection of native and hyperaccumulators for phytoremediation purposes is one of the most important steps to ensure the success of phytoremediation programs.

In order to select the indigenous hyperaccumulators of heavy metals, there were two investigations conducted at mining sites in Bac Kan and Thai Nguyen province, northern Vietnam, where the most mining activities are done in Vietnam. Soil analyses of mining sites in this region showed that soils have been heavily contaminated with a range of heavy metals, including Mn, Zn, As, Cd, and Pb (Table 6.1). Therefore, the objective of these surveys was to search for hyperaccumulators of Mn, Zn, As, Cd, and Pb.

Table 6.1 Family, species, and number of plant samples around and outside of the mine site

STT	Code name	Family	Species	*n*
1	Age	Asteraceae	*Ageratum houstonianum* Mill.	12
2	Bid	Asteraceae	*Bidens pilosa* L.	6
3	Dip	Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	9
4	Ele	Poaceae	*Eleusine indica* (L.) Gaertn	9
5	Hou	Saururaceae	*Houttuynia cordata* Thunb.	9
6	Kyl	Cyperaceae	*Kyllingia nemoralis*	12
7	Lee	Poaceae	*Leersia hexandra* Sw.	9
8	Lyg	Lygodiaceae	*Lygodium flexuosum* (L.) Sw.	6
9	Nep	Lomariopsidaceae	*Nephrolepis cordifolia* (L.) Presl.	9
10	Pte	Pteridaceae	*Pteris vittata* L.	24
11	Sac	Poaceae	*Saccharum spontaneum* L.	9
12	Sci	Cyperaceae	*Scirpus juncoides* Roxb.	9
13	Sel	Selaginelaceae	*Sellaginella delicatula* (Desv.) Alst	15
14	The	Thelypteridaceae	*Thelypteris noveboracensis*	9
15	Thy	Poaceae	*Thysanolaena latifolia*	12

6.2.1 Bac Kan Province, Northern Vietnam

6.2.1.1 Site Description

The mine site is situated in Cho Don district, Bac Kan province, northern Vietnam (Fig. 6.1). This is one of the biggest Pb–Zn mines in Vietnam. Mining activities started from eighteenth century and still have been active currently [27]. Rainy season starts from April to September, and dry season from October to March. The average rainfall is around 100–600 and 8–22 mm month^{-1} in rainy and dry season, respectively. Humidity is 76–88% and 35–45% in rainy and dry season, respectively [27]. The highest and lowest average temperature is 31–36 °C and 10–11 °C, respectively [27]. The main ore minerals are galena (PbS), sphalerite (ZnS), pyrotin (FeS), pyrite (FeS_2), chalcopyrite ($CuFeS_2$), and arsenopyrite (FeAsS) [27]. In addition, high concentration of Mn was obtained in Pb–Zn ore and in sphalerite which was 9892–20,500 mg kg^{-1} and 0.09–0.23% [28, 29]. High concentrations of Pb, Zn, As, and Mn may result in the leaching of these heavy metals into the surrounding environments via mining activities.

6.2.2 Plant Accumulation and Translocation of Heavy Metals

High concentrations of heavy metals in the soil and water may result in high levels of these elements in the collected plant samples. The concentrations of all heavy metals varied greatly among sites and plant species [30]. The highest concentrations

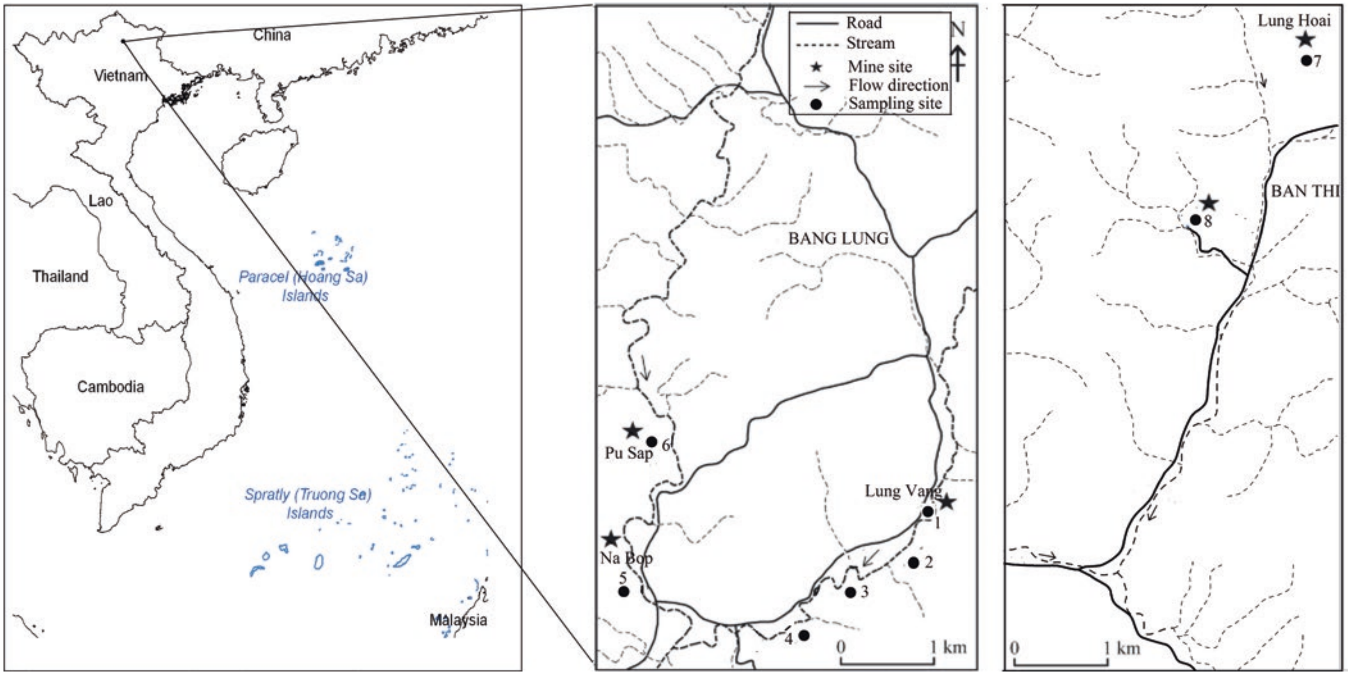

Fig. 6.1 Map showing the location of the Bac Kan sampling sites

of heavy metals (mg kg^{-1}-DW) in the plant root were found in *K. nemoralis* for Mn (2130), *N. cordifolia* for Zn (3780), *P. vittata* for As (861), *L. hexandra* for Cd (13.3), and *A. houstonianum* for Pb (2080); those in the shoot were found in *K. nemoralis* for Mn (1990), *N. cordifolia* for Zn (1710), *P. vittata* for As (2300), *A. houstonianum* for Cd (19.0), and *E. indica* for Pb (2010) (Tables 6.2 and 6.3). Among all plant species in the present study, highest concentrations of Mn, Zn, and As in the root and shoot were observed in *K. nemoralis, N. cordifolia*, and *P. vittata*, respectively.

Almost all collected plant species accumulated higher concentrations of Zn, As, and Pb than their toxicity threshold levels in plants. The concentrations of Mn, Zn, As, Cd, and Pb in the shoot of *A. houstonianum*, *E. indica*, and *H. cordata* were within and above the toxic levels for plant species (Tables 6.3 and 6.4). In addition, all plant species can adapt very well with the soil that highly contaminated with multiple heavy metals (Table 6.4). These results may indicate that plant species growing on the site contaminated with heavy metals were tolerant of these metals.

P. vittata showed great potential for accumulating As in the shoot (Table 6.2). The concentrations of As in the shoot of *P. vittata* L. were significantly higher than those in other plant species. Concentrations of As in the root were significantly higher than those in *B. pilosa*, *D. esculentum*, *K. nemoralis*, *L. flexuosum*, *S. spontaneum,* and *T. noveboracensis*. Mn concentrations in root of *P. vittata* were significantly higher than those in *B. pilosa*, *L. flexuosum*, and *S. spontaneum.* However, those in the shoot were significantly lower than those in *H. cordata* and *K. nemoralis*. Cd concentrations in the root of this species were lower than those in *H. cordata* and *S. juncoides*.

E. indica accumulated the highest concentration of Pb in the shoot among 15 collected plant species (Table 6.2). Pb concentration in *E. Indica* roots was higher than in roots of *B. pilosa* L., *K. nemoralis*, *L. flexuosum*, *N. cordifolia*, *S. spontaneum*, and *S. delicatula.* Concentrations of the heavy metals in the root and shoot of *E. indica* were significantly higher than those in *L. flexuosum*, those of As were significantly higher than those in *D. esculentum*, *K. nemoralis*, *L. flexuosum*, and *T. noveboracensis*. Cd concentrations in the root and shoot were significantly higher than those in *B. pilosa*, *L. flexuosum*, *S. spontaneum*, and *T. latifolia*.

6.2.3 *Potential Plant Species for Phytoremediation of Contaminated Soils*

The typical characteristics of an ideal plant species for phytoremediation are as follows: (1) a hyperaccumulator of metals which in aboveground tissues; (2) a high and fast-growing biomass and be repulsive to herbivores to avoid the escape of accumulated metals to the food chain; (3) BCF and TF values higher than 1; (4) a widely distributed, highly branched root system; (5) easy to cultivate and a wide geographic distribution; and (6) relatively easy to harvest [31, 32].

Table 6.2 Concentrations (mg kg^{-1}-DW) of Mn, Zn, and As in plant growing in and outside of the mine ($N = 6–24$)

		Mn		Zn		As	
No	Code name	Root	Shoot	Root	Shoot	Root	Shoot
1	Age	565 (85.2; 371–704)	555 (94.3; 238–1030)	514 (28.9; 197–1130)	563 (37.8; 152–1210)	272 (4.72; 162–350)	204 (15.9; 203–209)
2	Bid	130 (96; 115–155)	213 (142; 196–238)	191 (123; 149–265)	216 (112; 178–286)	183 (32.2; 156–213)	440 (34.5; 285–528)
3	Dip	1600 (221; 1190–2010)	215 (80.4; 81.7–349)	574 (56.2;544–604)	176 (26.3; 98–255)	82.5 (3.69; 73.3–91.7)	17.2 (19.0; 10.2–24.2)
4	Ele	385 (102; 142–628)	139 (90.4; 130–147)	1159 (57.6; 178–2140)	372 (41.9; 119–625)	339 (4.77; 230–448)	364 (1.55; 204–524)
5	Hou	987 (193; 941–1030)	710 (307; 434–985)	537 (53.8; 465–609)	319 (50.5; 234–404)	416 (2.99; 284–548)	240 (0.78; 197–283)
6	Kyl	1150 (210; 418–2130)	1440 (303; 1120–1990)	453 (76.0; 267–795)	239 (63.0; 173–284)	103 (0.99; 84.8–119)	57.2 (0.78; 29.6–97.5)
7	Lee	326 (277; 300–352)	147 (113; 118–176)	638 (138; 433–844)	170 (158; 142–197)	368 (43.5; 278–458)	191 (41.8; 182–199)
8	Lyg	119 (82.6; 97.2–149)	204 (84.7; 187–225)	38.1 (26.3; 31.3–43.8)	71.5 (21.7; 53.6–96.1)	20.1 (0.83; 16.3–23.1)	18.6 (13.4; 14.7–21.5)
9	Nep	614 (187; 566–662)	299 (156; 240–357)	2690 (183; 1590–3780)	1322 (158; 934–1710)	299 (52.0; 259–340)	176 (39.0; 158–193)
10	Pte	634 (254; 177–1200)	165 (98.1;65.0–358)	1150 (110; 247–3710)	388 (80.6; 155–1430)	464 (135; 291–861)	**1180** (149; 251–2300)
11	Sac	138 (134; 129–146)	112 (120; 75.0–150)	455 (123; 179–731)	167 (74.3; 106–237)	185 (54.0; 173–197)	174 (32.6; 166–182)
12	Sci	574 (183; 215–933)	301 (135; 225–376)	204 (126; 165–242)	73.0 (41.7; 59–87)	469 (0.83; 237–700)	190 (13.4; 183–197)
13	Sel	476 (153; 148–717)	278 (207; 117–430)	341 (89.3; 222–438)	246 (131; 206–278)	250 (87.7; 91–362)	89.3 (69.6; 78–164)
14	The	446 (131; 217–676)	212 (141; 159–365)	874 (93.4; 300–1450)	325 (112; 168–581)	48.2 (39.5; 42.1–54.3)	33.1 (25.5; 29.8–56.5)
15	Thy	304 (289; 110–532)	155 (118; 112–212)	233 (126; 196–288)	161 (94.6; 117–190)	333 (18.3; 172–480)	128 (16.9; 63.1–184)

Table 6.3 Concentrations (mg kg^{-1}-DW) of Cd and Pb in plant growing in and outside of the mine ($N = 6–24$)

No	Code name	Cd		Pb	
		Root	Shoot	Root	Shoot
1	Age	6.72 (0.50; 3.35–12.6)[a]	7.52 (0.56; 1.35–19.0)	1299 (11.8; 770–2080)	844 (4.87; 631–1070)
2	Bid	1.41 (0.75; 1.23–2.14)	0.60 (0.36; 0.52–0.75)	411 (74.1; 297–485)	505 (58.5; 378–563)
3	Dip	10.9 (0.71; 8.98–12.8)	0.71 (0.17; 0.70–0.72)	1307 (37.5; 949–1670)	200 (8.90; 154–246)
4	Ele	6.55 (0.75; 3.75–9.35)	5.14 (0.34; 2.93–7.34)	1840 (65.1;1810–1870)	**1300** (15.3; 595–2010)
5	Hou	12.3 (0.15; 11.1–135)	9.22 (0.08; 8.99–9.46)	843 (11.1; 606–1080)	**1060** (5.11; 999–1130)
6	Kyl	4.17 (0.15; 2.95–4.91)	2.18 (0.08; 1.70–2.53)	556 (21.0; 281–712)	386 (17.0; 116–702)
7	Lee	11.3 (0.54; 9.44–13.3)	0.77 (0.36; 0.56–0.97)	1910 (17.0; 1880–1940)	357 (8.79; 211–503)
8	Lyg	0.31 (0.12; 0.25–0.42)	0.18 (0.04; 0.12–0.24)	43.4 (8.29; 28.7–52.4)	9.42 (1.03; 8.44–10.1)
9	Nep	7.72 (0.64; 7.24–8.20)	8.68 (0.35; 7.95–9.41)	366 (63.7; 276–456)	501 (74.3; 492–510)
10	Pte	5.25 (0.48; 0.94–10.6)	0.72 (0.24; 0.37–0.99)	1070 (234; 453–1840)	544 (83.5; 92.0–781)
11	Sac	0.47 (0.38; 0.37–0.58)	0.25 (0.20; 0.25–0.38)	563 (42.0; 438–688)	807 (65.8; 781–832)
12	Sci	14.8 (0.12; 11.8–17.7)	5.27 (0.06; 4.58–5.97)	1650 (8.29; 1520–1780)	793 (1.03; 721–864)
13	Sel	2.59 (0.57; 2.40–2.79)	2.20 (0.35; 0.57–3.50)	359 (13.7; 234–613)	408 (6.86; 142–865)
14	The	2.22 (0.35; 2.16–2.28)	0.74 (0.27; 0.73–0.75)	865 (78.2; 842–888)	411 (89.9; 191–631)
15	Thy	0.70 (0.34; 0.47–0.85)	0.51 (0.15; 0.25–0.70)	667 (118; 474–886)	367 (23.5; 242–511)

[a]Average (reference, min–max)

Table 6.4 Concentrations (mg kg^{-1}) of heavy metals in the soil in and outside of the mine area

Site	Mn	Zn	As	Cd	Pb
1	**9270** ± 350	**7150** ± 1420	2290 ± 440	70.2 ± 15.1	8780 ± 790
2	4940 ± 290	5780 ± 1790	**5630** ± 2910	**93.7** ± 18.0	**9090** ± 1940
3	3620 ± 810	1720 ± 370	2450 ± 660	17.2 ± 5.24	4360 ± 1200
4	1730 ± 220	1570 ± 390	489 ± 307	3.71 ± 1.79	3360 ± 910
5	2410 ± 1010	1470 ± 130	2130 ± 810	4.72 ± 2.22	2470 ± 580
6	4010 ± 850	2010 ± 740	1550 ± 220	25.1 ± 9.7	3340 ± 720
7	3210 ± 770	4620 ± 840	538 ± 301	37.0 ± 25.3	4180 ± 960
8	4270 ± 260	6210 ± 300	858 ± 66	75.2 ± 5.7	8290 ± 710
Reference	817 ± 167	88.8 ± 2.5	4.77 ± 0.24	1.00 ± 0.05	70.8 ± 21.3

Values present means ± standard deviations ($N = 3–9$)

Table 6.5 Bioconcentration factors of plant growing around the mine

Code	Mn	Zn	As	Cd	Pb
Age	0.11 ± 0.08[a]	0.17 ± 0.08	0.09 ± 0.05	0.28 ± 0.12	0.12 ± 0.03
Bid	0.11 ± 0.08	0.11 ± 0.08	0.35 ± 0.15	0.12 ± 0.11	0.39 ± 0.22
Dip	0.12 ± 0.10	0.13 ± 0.07	0.05 ± 0.03	0.29 ± 0.19	0.06 ± 0.02
Ele	0.07 ± 0.04	0.08 ± 0.01	0.43 ± 0.39	0.63 ± 0.45	0.46 ± 0.28
Hou	0.76 ± 0.34	0.73 ± 0.59	0.12 ± 0.04	**2.00** ± 1.87	0.38 ± 0.05
Kyl	0.22 ± 0.01	0.11 ± 0.02	0.01 ± 0.01	0.14 ± 0.03	0.04 ± 0.02
Lee	0.45 ± 0.19	0.27 ± 0.16	0.52 ± 0.42	0.07 ± 0.08	0.19 ± 0.11
Lyg	0.21 ± 0.13	0.78 ± 0.30	0.36 ± 0.11	0.19 ± 0.07	0.02 ± 0.02
Nep	0.07 ± 0.03	0.26 ± 0.11	0.40 ± 0.26	0.61 ± 0.67	0.24 ± 0.02
Pte	0.10 ± 0.15	0.20 ± 0.15	**1.19** ± 0.50	0.10 ± 0.11	0.11 ± 0.06
Sac	0.04 ± 0.01	0.07 ± 0.07	0.12 ± 0.03	0.02 ± 0.01	0.26 ± 0.02
Sci	0.10 ± 0.03	0.04 ± 0.01	0.15 ± 0.01	0.58 ± 0.47	0.28 ± 0.13
Sel	0.18 ± 0.22	0.26 ± 0.27	0.23 ± 0.23	0.31 ± 0.30	0.09 ± 0.08
The	0.05 ± 0.03	0.08 ± 0.04	0.12 ± 0.13	0.13 ± 0.05	0.09 ± 0.05
Thy	0.06 ± 0.03	0.05 ± 0.03	0.11 ± 0.06	0.05 ± 0.08	0.08 ± 0.03
Average	0.18	0.22	0.28	0.37	0.19

[a] Mean ± standard deviation

Hyperaccumulators are defined as plants with leaves able to accumulate at least 100 mg $kg^{-1}$of Cd; 1000 mg $kg^{-1}$of As or Pb; or 10,000 mg kg^{-1} of Mn or Zn (dry weight) when grown in a metal-rich environment [33–35]. Among all plant species, hyperaccumulation levels (mg kg^{-1}-DW) were obtained in *P. vittata* (1180) for As (Table 6.2), in *E. indica* (1300) and *H. cordata* (1060) for Pb (Tables 6.2 and 6.3). Of which, *P. vittata* has been reported as a well-known hyperaccumulator of As [36]. *A. houstonianum* and *E. indica* have been reported to hyperaccumulate Pb [32, 37]. *H. cordata* is a hyperaccumulator of As (1140 mg kg^{-1}-DW); however, to the best of our knowledge, no previous study has reported the hyperaccumulation of Pb in *H. cordata*.

Bioconcentration factor (BCF) values of Mn, Zn, As, Cd, and Pb of 15 plant species varied within 0.04–0.76, 0.04–0.78, 0.01–1.19, 0.02–2.00, and 0.02–0.46, respectively (Table 6.5). BCF values of plants for Mn, Zn, and As at the uncontaminated site were significantly higher than those at the mine site. This is possibly due to low concentrations of heavy metals in associated soils outside of mining area (Table 6.4). BCF values of Mn and Zn were correlated ($p < 0.05$). BCF values higher than 1 were only observed in *H. cordata* (2.00) and *P. vittata* (1.19) for Cd and As, respectively. This result reflected high accumulation capacity of heavy metals by these species. Most BCF values were found to be lower than 1. This is possibly due to the existence of heavy metals in various geochemical forms in soils (water-soluble, exchangeable, bound to carbonate, bound to Fe-Mn oxide, bound to organic matter, and residual forms) [38–40]. In addition, the possible source of heavy metals was derived from a sulfide deposit, consequently, these heavy metals are assumed to partially exist as sulfides. The occurrence of heavy metals in sulfides,

Table 6.6 Translocation factors of plant growing around the mine

Code	Mn	Zn	As	Cd	Pb
Age	0.97 ± 0.08[a]	**1.15** ± 0.08	0.84 ± 0.05	0.81 ± 0.12	0.75 ± 0.23
Bid	**1.64** ± 0.08	**1.14** ± 0.08	**2.41** ± 0.15	0.43 ± 0.11	**1.23** ± 0.22
Dip	0.12 ± 0.10	0.31 ± 0.07	0.22 ± 0.03	0.07 ± 0.09	0.18 ± 0.02
Ele	0.57 ± 0.04	0.48 ± 0.01	**1.03** ± 0.39	0.78 ± 0.75	0.70 ± 0.28
Hou	0.73 ± 0.34	0.58 ± 0.59	0.61 ± 0.04	0.76 ± 0.87	**1.35** ± 0.35
Kyl	**1.65** ± 0.01	0.63 ± 0.02	0.54 ± 0.11	0.53 ± 0.03	0.63 ± 0.22
Lee	0.46 ± 0.19	0.28 ± 0.16	0.54 ± 0.42	0.07 ± 0.08	0.19 ± 0.11
Lyg	**1.72** ± 0.13	**1.88** ± 0.30	0.78 ± 0.11	0.58 ± 0.07	0.22 ± 0.09
Nep	0.50 ± 0.03	0.52 ± 0.11	0.59 ± 0.26	**1.12** ± 0.67	**3.46** ± 0.62
Pte	0.27 ± 0.15	0.45 ± 0.25	**2.98** ± 0.50	0.29 ± 0.11	0.43 ± 0.16
Sac	0.80 ± 0.01	0.43 ± 0.07	0.94 ± 0.23	0.51 ± 0.11	**1.50** ± 0.42
Sci	0.72 ± 0.03	0.39 ± 0.12	0.54 ± 0.11	0.36 ± 0.27	0.48 ± 0.13
Sel	0.63 ± 0.22	0.75 ± 0.37	0.43 ± 0.23	0.85 ± 0.40	0.99 ± 0.28
The	0.41 ± 0.03	0.31 ± 0.04	0.64 ± 0.13	0.33 ± 0.15	0.48 ± 0.15
Thy	0.65 ± 0.03	0.70 ± 0.03	0.39 ± 0.16	0.70 ± 0.28	0.54 ± 0.23
Aver	0.18	0.22	0.28	0.37	0.19

[a] Mean ± standard deviation

combined with the fact that the poor structure of soil developed in mine tailings may reduce metal availability to root over short periods of time [41].

Translocation factor (TF) values of Mn, Zn, As, Cd, and Pb in 15 plant species varied within 0.12–1.72, 0.28–1.88, 0.22–2.98, 0.07–1.12, and 0.18–3.46, respectively (Table 6.6). It is noted that most TF values of heavy metals in this study were lower than 1. This is in line with the result reported by Stoltz and Greger [42] that most of the plant species growing on mine tailings have a restricted translocation of metals and As to the shoot. The restriction of upward movement from root to shoot can be considered as one of the tolerance mechanisms [43]. The average TF values of Mn, Zn, As, Cd, and Pb of plants growing at the uncontaminated site were 0.28, 0.50, 3.53, 0.30, and 0.41, respectively. The TF value of Cd was significantly lower than that of Mn, As, and Pb. TF values of plants for Mn, Zn, and As at the uncontaminated site were significantly higher than those at the mine site. The translocation of Mn from root to shoot in *D. esculentum*, *P. vittata*, and *T. noveboracensis* was significantly lower than that in other species. TF values of As in *B. pilosa*, *E. indica*, and *P. vittata* were significantly higher than those in other plants. Significantly higher TF values of Pb in *B. pilosa*, *H. cordata*, *N. cordifolia*, and *S. spontaneum* than those in other species were also observed. *B. pilosa* showed the high capacity to translocate multiple heavy metals from the root to the shoot (Table 6.6).

Among all plants collected in the present study, *P. vittata* is the most widely distributed species. The results of the present study were in agreement with the previous study that *P. vittata* L. is an efficient As hyperaccumulator [36]. The highest concentrations of As, Pb, Zn, Mn, and Cd in shoot of *P. vittata* L. were 358, 1430, 2300, 0.99, and 784 mg kg^{-1}-DW, respectively. TF values exceeded 1 were obtained for As. In addition, *P. vittata* L. has considerable biomass, grows fast, and propagate easily [36, 44, 45]. Therefore, this plant has high potential for phytoremediation of multi-metals, especially for As [44, 45], Zn and As [46], Cd and As [47], and multiple heavy metal-contaminated soils [32].

Of the three Pb hyperaccumulators identified in this study, *E. indica* (L.) accumulated highest concentrations of Pb in the shoot. *H. cordata* had the highest translocating factor of Pb from root to shoot (TF = 1.35). TF values of *E. indica* and *A. houstonianum* and BCF values of all Pb hyperaccumulators were lower than 1. This study was conducted to assess the phytoremediation potential of plants growing on a site contaminated with heavy metals. Results of this research indicated that among 15 plant species being collected, *P. vittata* L. is a good candidate for phytoremediation of As; *A. houstonianum*, *E. indica*, and *H. cordata* are potential species for phytoremediation of Pb. Further studies are required to confirm the phytoremediation potential of those plant species through greenhouse and field experiments as well as to establish the agronomic requirements and management practices in order to investigate their whole phytoremediation possibilities.

6.3 Selection of Indigenous Plants Suitable for Phytoremediation in Thai Nguyen Province

The study was performed at four mining sites located at two districts of Thai Nguyen province, northern Vietnam: Tan Long (Zn/Pb mine) and Trai Cau (Fe mine) site in Dong Hy district, Ha Thuong (Ti/Sn mine) and Yen Lang (coal mine) in Dai Tu district. Soil samples were collected at the same place with plant samples (Figs. 6.2 and 6.3)

This research was conducted to determine soil concentrations of As, Pb, Cd, and Zn at four mining sites of Thai Nguyen province as well as to identify indigenous potential plants for phytoremediation. Total 33 indigenous plants and 12 soil in situ plant samples in these areas were collected for heavy metal analysis. The soils of surveyed mining areas contained 181.2–6754.3 mg kg^{-1} As, 235.5–4337.2 mg kg^{-1} Pb, 0.8–419 mg kg^{-1} Cd, and 361.8–17565.1 mg kg^{-1} Zn depending on the characteristics of each mining site. As compared to the upper limit of As (15 mg kg^{-1}), Cd (1.5 mg kg^{-1}), Pb (70 mg kg^{-1}), and Zn (200 mg kg^{-1}) for industrial soil in Vietnam [48], these soils are much higher than standard values.

The collected 33 plant species can grow at the mine tailings or in the soils affected by mining waste. The heavy metal concentrations in their roots and shoots of these plant species were evaluated. In the total of these selected plants, only six potential indigenous plant species of Thai Nguyen province was presented in the Table 6.7. The results showed that two ferns, *Pteris vittata* and *Pityrogramma calomelanos* were capable of accumulating high arsenic concentrations. As concentrations in shoot and root of *P. vittata* were 5877 and 2643 mg kg^{-1}, respectively, while these values of *P. calomelanos* were 2426 and 2256 mg kg^{-1}. Remarkably, a large

Fig. 6.2 Location of survey areas in Thai Nguyen province

Fig. 6.3 Some sampling sites in Bac Kan and Thai Nguyen

Table 6.7 Heavy metal concentration in shoots and roots of six potential indigenous plant species of Thai Nguyen province

	As (mg kg^{-1})		Pb (mg kg^{-1})		Cd (mg kg^{-1})		Zn (mg kg^{-1})	
Plant species	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Pteris vittata L.	**5876.5 ± 99.6**	**2642.5 ± 72.3**	9.4 ± 1.4	10.2 ± 1.7	0.4 ± 0.1	1.3 ± 0.3	152.3 ± 12.7	220.5 ± 23.5
Cynodondactylon L.	44.1 ± 3.5	765.5 ± 23.2	538.5 ± 25.2	**4579.6 ± 88.5**	4.4 ± 0.7	34.6 ± 4.8	**912.8 ± 42.5**	15.6 ± 3.8
Eleusine indica L.	25.2 ± 2.8	236.0 ± 15.1	664.5 ± 45	**4638.2 ± 210.4**	9.3 ± 1.3	26.5 ± 2.5	**4346.8 ± 157.9**	**3108.7 ± 213.5**
Equisetum ramosissimum (Vauch)	28.2 ± 2.6	34.3 ± 4.1	455.2 ± 32.6	**1025.7 ± 65.8**	9.2 ± 1.5	29.0 ± 3.6	**1346.2 ± 130.2**	**3756.9 ± 145.7**
Cyperus rotundus L.	19.9 ± 1.7	37.7 ± 3.6	**941.3 ± 35.2**	**1560.2 ± 113.5**	7.2 ± 2.1	9.5 ± 2.4	**1201.4 ± 147.3**	**2194.4 ± 155.7**
Pityrogramma calomelanos L.	**2426.3 ± 104.5**	**2256.0 ± 123.4**	49.9 ± 5.6	85.4 ± 7.4	1.0 ± 0.3	1.1 ± 0.5	368.6 ± 15.7	230.8 ± 24.6

Values are mean ± standard deviation of three replicates amount of As from roots of these ferns transported to shoot, facilitating its removal from the soil. Many research results have reported that they are two As hyperaccumulating ferns [28, 29, 32, 36, 49–51]. None of the collected plant species had high Cd accumulating ability.

Zn accumulating ability in some investigated plant species was quite high. *E. ramosissimum*, *C. rotundus*, and *E. indica* can accumulate Zn in their shoots with 1346, 1201, and 4347 mg kg^{-1}, respectively, and in their roots with 3757, 2194, and 3109 mg kg^{-1} Zn, respectively. As indigent plants, they can easily adapt to the local conditions being also potential for phytoremediation.

Our findings in Thai Nguyen province indicate that two ferns *P. vittata* and *P. calomelanos* are suitable for As treatment in the mining soil of Ha Thuong, Dai Tu, district (Table 6.7). Four grasses, *E. indica*, *C. dactylon*, *C. rotundus*, and *E. ramosissimum* are potential for Pb, Zn removal from soils. Some research results reported that *E. indica* is Pb hyperaccumulator [32, 37].

6.4 Some Research Results in Greenhouse Experiment of Potential Plant Species

Based on the screening results, three species, namely, *P. vittata*, *P. calomelanos*, and *E. indica* were selected with an introduced plant *Vetiveria zizanioides* and a crop plant *Brassica juncea* for evaluation under greenhouse conditions.

6.4.1 Pteris vittata *and* Pityrogramma calomelanos

The obtained results from greenhouse experiments showed that *Pteris vittata* and *Pityrogramma calomelanos* can grow in the mining soil containing 15,146 ppm As. Although they are As hyperaccumulators, the plants still also have the ability to accumulate Cd, Pb, and Zn. *Pteris vittata* and *Pityrogramma calomelanos* can tolerate 5000 and 4000 mg kg^{-1} Pb (concentration of Pb was established by adding $Pb(NO_3)_2$ in the garden soil); 1200 and 300 mg kg^{-1} Cd (concentration of Cd was established by adding $Cd(NO_3)_2$ in the garden soil), respectively. The highest level of As accumulation in *Pteris vittata* and *Pityrogramma calomelanos* are 6042 and 4034 mg kg^{-1} (in the fronds); 3756 and 2256 (in the roots), respectively. Concentration of As, Cd, Pb, and Zn in *Pteris vittata* were comparable to those found by An et al. [46] and Ha et al. [32]. From 3 to 4 months after growing there is appropriate time for harvesting plant biomass if applied in practical processing (Fig. 6.4).

Fig. 6.4 Pot experiments of potential plant species

6.4.2 Eleusine indica

Eleusine indica can be used for remediating the soil contaminated with Pb and Zn. The results of the survey showed that this plant can grow in the waste area of lead, zinc processing factory. Analyzing Pb and Zn concentration in soil and plants showed that if soil contained 4316.9 mg kg^{-1} Pb, there would be 664.5 and 4638.2 mg kg^{-1} Pb in shoots and roots of the plant, respectively; if soil contained 1000 mg kg^{-1} Zn, there would be 761.6 and 2011.3 mg kg^{-1} in shoots and roots, respectively. *Eleusine indica* could grow well at the concentration of Pb and Zn (in the form of $Pb(NO_3)_2$ and $Zn(NO_3)_2$), respectively. Other studies have found *Eleusine indica* (L.) higher accumulating Pb in the shoots [32, 37].

6.4.3 Vetiveria zizanioides

In mining soil contaminated with Pb from 1400.5 to 2530.1 mg kg^{-1}, *Vetiveria zizanioides* still grew well after 90-days treatment. Some characteristics of plant growing on Pb-contaminated soil such as height, root length, biomass, and the chlorophyll concentration increased more than those on control soil (soil without Pb). Pb concentration analysis in soil after this experiment showed that the Pb extraction effect from the contaminated soil by *Vetiveria zizanioides* could reach from 87% to 92.6%. However, the average Pb accumulation in its shoots and roots were not high being only 24 and 349 mg kg^{-1}, respectively. This species also can accumulate As and Cd taken from soil. Many of our further experimental results confirmed feasibility of using *Vetiveria zizanioides* as phytostabilization agent for Pb, Cd, and As in contaminated soils. Some research results also reported that Vetiver grass has the ability to accumulate wide range of heavy metals [52–55].

6.4.4 Brassica juncea

As, Pb, and Cd accumulations of *Brassica juncea* were quite high. All three heavy metals can be accumulated in roots more than in the shoots. In trace concentration, heavy metals can stimulate plant growth, but at higher concentrations (Cd > 25 mg kg^{-1}, As > 200 mg kg^{-1}, and Pb > 2000 mg kg^{-1}) they inhibited plant growth. Pb accumulation in shoots and roots of *Brassica juncea* grown on 2000 mg kg^{-1}Pb soils were 1325 and 2546.2 mg kg^{-1}, respectively. The concentration of Pb accumulated in *Brassica juncea* shoots in this study was similar to that reported in study of Lombi et al. [56] and Jae et al. [57]. When cultivated on soils containing 25 mg kg^{-1} As and Cd, concentration in shoots and roots were 185.6 and 228.9 mg kg^{-1} for As, 185.6 and 228.9 mg kg^{-1} for Cd, respectively. *Brassica juncea* can be used to remove As, Pb, and Cd concentration in contaminated soil but it should be noted that this plant is also a popular green vegetable. Therefore, the use of this plant species for phytoremediation is limited due to the risk of poisoning human through consumption of its heavy metal-contaminated leaves.

6.5 Field Evaluation of Heavy Metal Accumulating Potential of the Selected Terrestrial Plants

6.5.1 Study at Ha Thuong and Tan Long Mines, Thai Nguyen Province

The field study was performed at Ha Thuong and Tan Long mine site. Selection of the experimental sites was based on three criteria: (1) areas affected by mining activities, containing high concentration of heavy metals As, Pb, Cd, and Zn; (2) potential of indigenous plants for phytoremediation; (3) local conditions suitable for operation model.

6.5.2 Ha Thuong Field Experimental Site

The analysis of soils collected at Ha Thuong Ti/Sn mine site showed very high concentration of As (4521 mg kg^{-1}), moderate concentration of Pb and Zn (235 and 463 mg kg^{-1}, respectively), low concentration of Cd (4.5 mg kg^{-1}), and low pH (2.3). Concentration of As, Cd, Pb, and Zn in the polluted soils was301.4, 3, 3.4, and 2.3 times higher than the permitted standards for agricultural soils, respectively (Table 6.8). At this site, there is no plant species survived, except *Pityrogramma calomelanos*. The source of soil contamination is from tin mining wastewater discharged daily into the drain near this site. Spreading of contaminants has often occurred in rainy season, when the whole area is totally submerged in water for several hours or longer with frequency of 3–5 times per year.

Table 6.8 Soil characteristics of Ha Thuong area before and after growing *P. vittata, P. calomelanos*, and *V. zizanioides*

Times	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	pH (KCl)	OM (%)	CEC (cmolkg^{-1})
S0 (0 month)	4521 ± 324	4.5 ± 0.9	235 ± 67	463 ± 85	2.3 ± 0.8	0.21 ± 0.13	3.1 ± 0.6
S1 (8 months)	2317 ± 389	2.3 ± 0.7	115 ± 51	216 ± 37	6.7 ± 0.8	1.8 ± 0.5	11.1 ± 1.3
S2 (12 months)	2011 ± 215	1.8 ± 0.8	70 ± 12	191 ± 21	7.2 ± 0.6	2.3 ± 0.6	10.3 ± 0.8
S3 (24 months)	1360 ± 180	0.8 ± 0.5	25 ± 8	112 ± 34	7.3 ± 0.5	3.6 ± 0.7	12.1 ± 2.1
S4 (36 months)	956 ± 87	0.3 ± 0.1	9 ± 2	60 ± 12	7.2 ± 0.4	4.1 ± 0.5	11.8 ± 3.2

Note: *OM* organic matter, *CEC* cation exchangeable capacity; $n = 5$, values are presented in mean ± standard deviation. Allowable limits of As, Cd, Pb, and Zn in agricultural soil recommended by the Vietnam National Technical Regulation are 15, 1.5, 70, and 200 mg kg^{-1}dw, respectively

The experimental area at Ha Thuong mine site is 700 m^2. Before experiment started, several plant species (*Sesbania sesban, Reynoutria japonica, Senna alata*) had been planted in this area for creating a favorable environment, and CaO had been added to raise soil pH to 7. Three plant species (*Pteris vittata, Pityrogramma calomelanos*, and *Vetiveria zizanioides*) were tested at this site. Concentration of heavy metals and As over 3-year period is presented in Table 6.8 (Fig. 6.5).

6.5.3 Tan Long Field Experimental Site

Soils at Tan Long experimental site contained very high concentration of Pb and Zn (3470 and 3191 mg kg^{-1}, respectively), moderate concentration of As (213 mg kg^{-1}), low concentration of Cd (52 mg kg^{-1}), and high pH value of 8.2. At this site, *Pteris vittata* was found the most popular, while other species, such as *Pityrogramma calomelanos,* was also detected but with less number as compared to Ha Thuong site.

Tan Long experimental site has an area of 740 m^2. Vetiver and elephant grass were cultivated around the experimental site to control erosion and leaching. Three plant species were used at this site, including *Pteris vittata, Vetiveria zizanioides*, and *Eleusine indica*.

Concentrations of As, Cd, Pb, and Zn (mg kg^{-1} dw) in the soils at Ha Thuong and Tan Long experimental site were determined at 0, 8, 12, 24, and 36 months after cultivation of selected plants (Tables 6.8 and 6.9). To increase the efficiency of phytoremediation, mycorrhiza fungi, EDTA, phosphorous, and organic fertilizers were applied at two experimental sites. In general, soil concentrations of heavy metals and As were markedly reduced over 3 years at both experimental sites. Particularly, soils contaminated with low or moderate concentration of heavy metal and As (Cd, Pb, and Zn at Ha Thuong site, As and Cd at Tan Long site) were effectively remediated to contain the level of heavy metals that are below the limits of Vietnam National Technical Regulation. It should be noted that the removal effectiveness of the heavy metals from the soil depends on the plant species; plant biomass; the added of mycorrhiza fungi, EDTA, P, organic fertilizers; plant–microorganisms relationship and soil leaching (Fig. 6.6).

6.6 The Uptake Capacity for Heavy Metals of *Vetiveria zizanioides* at Field Conditions

Khanh Son landfill site is located above hill area of Lien Chieu District, Da Nang City. This area was the municipal solid wastes dumping site of Da Nang City since 1992, and closed in 2006. The studied site was selected at a dumping area inside the landfill where the solid wastes were kept for 2 years, covered with 0.5 m of surface soil. The solid wastes were already decomposed and mixed. The second experimental site was selected at waste disposal point with an area of 1500 m^2

Fig. 6.5 Ha Thuong experimental site before and after growing *Pteris vittata, Pityrogramma calomelanos*, *and Vetiveria zizanioides*

located in residential area of Hoa Minh ward, Lien Chieu district, Danang city. The studied site was the place used for holding and recycling the second-hand cars. Vetiver grass was cultivated at a density of 20 seedlings.m^{-2} for both experimental sites. Heavy metal concentrations in shoot (stem and leaves) of vetiver were determined at 3, 6, and 12 months after cultivation of vetiver.

Heavy metal concentrations accumulated in vetiver shoot were gradually diminished with time (Table 6.10). The highest concentration of Zn, Cu, and Pb in stems and leaves of Vetiver grown at Khanh Son landfill were 342.4, 30.3, and 5.6 mg kg^{-1}, respectively. At Hoa Minh waste landfill, vetiver accumulated the highest Zn and Pb concentration of 36.4 and 6.4 mg kg^{-1}, respectively. The concentrations of Zn, Cu, and Pb in shoot of vetiver grown at field condition were higher than those of vetiver grown under greenhouse condition. The concentration of heavy metals in stem and leaf of Vetiver was highest after 3 months of transplanting at both Khanh Son and Hoa Minh areas. After 12 months of growth, the amount of Zn accumulated by vetiver was 0.9 gm^{-2} year^{-1} and 1.5 gm^{-2} year^{-1} at Khanh Son landfill and Hoa Minh waste disposal site, respectively (Table 6.11).

In terms of physical–chemical characteristics and the concentration of heavy metals in soil, the obtained results showed that the contents of organic matter (OM) and total nitrogen (N_{ts}) increased at both experimental sites after the experiment was completed. The amount of organic matter increased from 9% to 13% and the total nitrogen increased from 23% to 68% at the end of experiment compared with those

Table 6.9 Soil characteristics of Tan Long area before after growing *P. vittata*, *V. zizanioides* and *E. indica*

Times	As (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	pH (KCl)	OM (%)[a]	CEC (cmol$_c$kg^{-1})[b]
S0 (0 month)	213 ± 54	52 ± 11	3470 ± 123	3191 ± 231	8.2 ± 1.3	0.6 ± 0.2	4.3 ± 0.5
S1 (8 months)	127 ± 23	31.2 ± 1.9	2135 ± 121	2365 ± 237	7.6 ± 0.7	3.8 ± 0.7	15.7 ± 2.4
S2 (12 months)	93 ± 16	22.5 ± 2.8	1924 ± 202	2132 ± 131	7.8 ± 0.5	3.3 ± 0.6	16.5 ± 2.8
S3 (24 months)	47 ± 9	10.2 ± 1.5	1367 ± 185	1512 ± 214	7.9 ± 0.4	4.6 ± 0.8	17.1 ± 3.1
S4 (36 months)	16 ± 7	5.7 ± 1.3	954 ± 96	1034 ± 123	7.7 ± 0.6	5.1 ± 0.6	16.8 ± 5.3

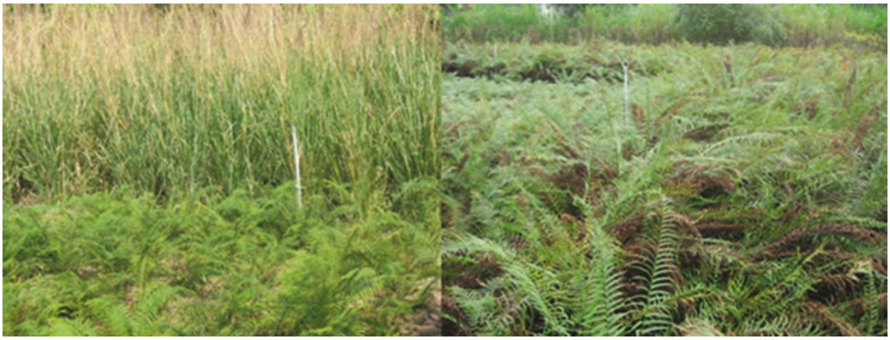

Fig. 6.6 Phytoremediation in Tan Long mining site

Table 6.10 Concentrations of heavy metals (ppm) in aerial parts (stems and leaves) of Vetiver

		Periods of experiment		
Places	Heavy metal	3 months	6 months	12 months
Khanh Son Landfill	Zn	342.4 ± 3.4	305.4 ± 6.5	287.5 ± 7.1
	Cu	30.2 ± 0.9	27.37 ± 1.8	23.2 ± 2.8
	Pb	5.6 ± 0.5	5.76 ± 0.3	4.1 ± 0.1
Hoa Minh waste disposal site	Zn	336.4 ± 3.9	321.6 ± 0.9	310.5 ± 3.7
	Pb	6.4 ± 0.1	6.3 ± 0.1	5.6 ± 0.2

Table 6.11 The amount of studied-heavy metals per 1 m^2 after 12 months at the field conditions

	Amount of heavy metals (g/m^2)		
Places	Zn	Cu	Pb
Khanh Son Landfill	0.931	0.075	0.013
Waste disposal site at Hoa Minh	1.469	–	0.026

at the beginning of experiment. In contrast, the contents of bioavailable phosphorus in both areas reduced from 12% to 23%. In addition, the amount of bioavailable potassium in the soil at Khanh Son increased 31%, whereas this amount at Hoa Minh reduced 5%. These results were also consistent with the results reported in the study of Phien and Tam [58]. Significantly, the amount of heavy metals in soils at the end of experiment was lower than that at the beginning of experiment. The reduction for Zn, Pb, and Cu were 13–16%, 7–12%, and 17%, respectively.

6.7 Conclusion

Mining activities in Vietnam have resulted in large areas of land contaminated with high concentrations of heavy metals and As. The contaminated soils require immediate remediation to control adverse effect of contaminants on human and environment.

Among several available remedial technologies, phytoremediation is the most appropriate because the technology is simple, cost-effective, and environmentally friendly. Several research programs have been conducted since the last decade in order to search for indigenous hyperaccumulators of As, Cd, Pb, and Zn and evaluate the selected plant species for phytoremediation purpose under greenhouse and field conditions.

Among plant species being collected in Bac Kan and Thai Nguyen, *P. vittata* and *P. calomelanos* are good candidates for phytoremediation of As. *A. houstonianum*, *E. indica, H. cordata*, *C. dactylon*, *C. rotundus*, and *E. ramosissimum* are potential species for phytoremediation of Pb and Zn. The mixed cultivation of *P. vittata, V. zizanioides*, and *E. indica* at Tan Long mine site, and *P. vittata, P. calomelanos*, and *V. zizanioides* at Ha Thuong mine site together with application of mycorrhiza fungi, EDTA, phosphorous, and organic fertilizers, showed very promising results. Concentrations of As, Pb, and Zn were significantly reduced over 3-year period. It can be concluded that the mixed cultivation of the selected plants can be used to remediate As-, Pb-, and Zn-contaminated soils.

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Chapter 7
Essential Elements and Toxic Metals in Some Crops, Medicinal Plants, and Trees

Elena Masarovičová and Katarína Kráľová

Abstract Plants play an ever-increasing role not only for providing safe and healthy food for a growing world population but also for new biotechnologies including phytoremediation of areas contaminated by toxic metals, phytofortification used in functional foods preparation and nanoagrochemicals application in agriculture. Since species of genus *Brassica* are not only important crops but they have use for technical purposes, we evaluated these important crops from the aspect of nutrition or toxic metal responses. Medicinal plants are presented as a source of natural substances widely used in pharmaceutical, food and cosmetics industries and potentially also in phytoremediation technology. Therefore, we analyzed the effect of bioelements and toxic metals on growth and physiological processes of this important group of the plants. Trees (both forest and fast growing trees) as one of the world's most abundant raw materials for industrial products and renewable energy as well as their non-production functions (reducing erosion, moderating the negative climatic changes, and phytoremediation procedures) are outlined. We have emphasized that plant responses to different nutrient and toxic metal conditions are expressed through structural composition and physiological processes. Results from experiments with above-mentioned plants treated with bioelements and toxic metals are shortly presented. Here, we used ion form of elements (Cd, Cr, Cu, Hg, Ni, Pb, Zn) and elements as complexes (Cu, Cd, Fe, Se, Zn). Finally, we stressed that both scientists and politicians will have to accept fundamental bioethical principles to ensure the sustainable development of human society as well as essential protection of the environment and nature.

Keywords Bioelements • Crops • Growth • Physiological processes • Phytoremediation • Phytofortification • Medicinal plants • Nanoagrochemicals • Toxic metals • Trees

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7.1 Introduction

Plants play an ever-increasing role for providing safe and healthy food for a growing world population and for replacing limited, and expensive, fossil resources as feedstock for the production of energy and industrial materials. The strategic agenda for the plant research outlines the approach that can contribute to fulfil consumer demand for safe, sustainable, and healthy food. Novel plants aim at delivering non-allergic foods and foods with longer shelf lives, better nutritional composition, and more varied tastes. These plants may need less input in terms of water, fertilizer or pesticides and will be more stress-resistant, mainly against drought or seasonal instabilities caused by climate change. Farmers should increase agricultural productivity while decreasing its environmental footprint. Plants (both crops and trees) or plant waste will in the future be an important source for the production of energy, biofuels, and biopolymers, replacing the use of fossil fuels as feedstock. However, *new technologies* must be applied within systems that are both *economically and environmentally sustainable.*

One reason for interest in plant–metal interaction has been the recent attention on the use of plants either to remediate toxic metal-contaminated soils or increasing the bioavailable concentrations of essential nutrients in edible portions of food crops through agronomic intervention or genetic selection. In addition, since plants are known to interact with different metals, they have been used for the "green biosynthesis" of metal nanoparticles. Such bioinspired methods are dependable, environmentally friendly, and benign. In general, phytoremediation, phytofortification, and metal nanoparticle biosynthesis are thus natural green biotechnology with using crops, medicinal plants as well as trees.

7.2 Crops

In general, there are top five crops produced in the world (sugar cane, maize, wheat, rice, and potatoes) and ten crops that feed the world (maize, potatoes, sweet potatoes, yams, cassawa, soybeans, sorghum, plantain, wheat, and rice) [1]. Thus it is very difficult to discuss all these most important crops from the aspect of nutrition or toxic metal responses. Therefore, we decided to evaluate the species of genus *Brassica* that is not only an important crop but it has use for technical purposes. Moreover, some of rapeseed genotypes can be used in both phytoremediation and phytofortification technologies. Authors of this chapter have also some experiences with rapeseed. Many experiments with this species have been done in our laboratory or under field conditions and many papers we published, too (see "References").

7.2.1 General Characteristic of Genus Brassica

Brassica is a genus from family *Brassicaceae* that species are informally known as cruciferous vegetables, cabbages or mustard plant and commonly used for food (e.g. cabbage, cauliflower, broccoli or Brussels sprouts) and include a number of weeds, both wild taxa and escapees from cultivation. It counts over 30 wild species and hybrids as well as many cultivars and hybrids of cultivated origin. However, it should be stressed that there is some disagreement among botanists on the classification and status of *Brassica* species and subspecies. More of them are seasonal plants (annuals or biennials) and some are even small shrubs. Species of genus *Brassica* have been the subject of much scientific interest for their agricultural and food importance (e.g., [2, 3]). Six species, *B. carinata*, *B. juncea*, *B. oleracea*, *B. napus*, *B. nigra*, and *B. rapa,* evolved by the combination of chromosomes from three earlier species, as described by the "Triangle of U theory"—theory about the evolution and relationships between members of the plant genus *Brassica* (e.g., [4]). This genus is native in the wild in Western Europe, the Mediterranean, and temperate regions of Asia and many wild species grow as weeds, especially in North America, South America, and Australia. Almost all parts of plants are used for food, including the roots, stems, leaves, flowers, buds, and seeds. Some forms with white or purple foliage or flowerheads are also grown for ornamental intention. There is also very important use of rapeseed oil for technical purposes, especially as a biofuel. It seems very promising the use of some rapeseed varieties for phytoremediation of soils contaminated by toxic metals (see Sect. 7.5 of this chapter).

7.2.2 Effect of Cadmium and Some Bioelements on Brassica Species

Brassica juncea L. (Indian mustard) together with *Vigna radiata* (L.) Wilczek (mung bean) are important crops in the poorer countries—mainly of Asia, whereby the leaves, the seeds, as well as the stem of Indian mustard are edible. For their high nutritive values they are source of proteins, Ca, P, certain vitamins and some cultivars possess excellent aroma (cf. Betal et al. [5]). Šimonová et al. [6] determined the effect of different Cd concentrations (6–120 μmol dm^{-3}) on Hill reaction activity (HRA) of isolated chloroplasts, content of chlorophylls (Chls) and carotenoids (Cars) as well as both Cd uptake and accumulation in plant organs. Seeds of studied crops were grown in thermostat on wet cellulose wadding at 80% of relative air humidity and air temperature of 25 ± 1 °C for 4 days. Then the seedlings were transferred into the plastic containers filled with 2 dm^3 of Hoagland solution (control variant) and cultivated in growth chamber under relative air humidity 60–70%, day/night temperature 25/20 ± 1 °C, and 12 h photoperiod with irradiance 100 μmol m^{-2} s^{-1} PAR. The solution was continuously aerated. In the growth stage of primary true leaves in both species, the seedlings were transferred to the Hoagland solution with above-mentioned Cd concentration. The response of plants to the Cd effect was evaluated 5 days after Cd application. It was found that Cd stress inhibited HRA of both species; the mung bean showed a higher sensitivity to Cd treatment than Indian mustard. The leaves of Cd-treated plants possessed lower content of Chls and Cars, whereby negative effect increased with Cd concentration. A difference between studied crops was also found in Cd uptake and Cd accumulation. In both species, Cd was accumulated more in the roots than in the shoots, with higher accumulation in *B. juncea* than *in V. radiata*.

Rapeseed (*Brassica napus* L. var. *napus*; syn: *Brassica napus* L. ssp. *oleifera*) is other species from the genus *Brassica* that belongs in structure of actual agriculture to the perspective and economically interesting crops also under Slovakian climatic condition (cf. Tóth and Hudec [7]). Following cereals and maize it is the third most important crop in Slovakia. Rapeseed has large-scale utilization, mainly as a food, further in pharmaceutical and chemical industry and oil extrusions are important nourishing component in animal fodder. Moreover, this species is used as a technical plant for production of renewable biofuels (FAME—fatty acid methyl ester) or PPO (pure plant oils) (in detail see Masarovičová et al. [8]). It should be stressed that rapeseed is not only an essential crop, technical and melliferous beneficial plant, but this species will become a perspective functional crop in the near future. From such plants it is possible (using phytofortification biotechnology) to prepare functional food fortified by substances with high nutritional value [8–10]. Because of higher accumulation of some toxic metals (mainly Cd) into the root and shoot, rapeseed was assigned to the plant species potentially used also in phytoremediation technology [11, 12]. In our earlier paper [13] we investigated production potential of chosen rapeseed cultivars from the aspect of soil quality (applied agrotechnology and plant protection), whereby were tested cultivars represented three production regions—maize production region (MPR), rapeseed production region (RPR), and potato production region (PPR) of Slovakia. Following six rapeseed cultivars were tested: ES ASTRID (medium-early and low type cultivar, France, PPR), ATLANTIC (medium-early and high type cultivar, France, PPR), CALIFORNIUM (medium-early to early and medium-high type of cultivar, France, MPR), LABRADOR (late and low to medium-high type of cultivar, France, RPR), MANITOBA (late and medium-high type of cultivar, France, RPR), and OPONENT (late and high type of cultivar, Czech Republic, MPR). Field experiment was realized at the experiment area of Centre for Research of Crop Production, Research Institute of Crop Production in Borovce near Piešťany, West Slovakia. Since agrotechnology as well as plant protection of rapeseed is complicated, we recommend to read it in detail in the above-mentioned paper. However, it is important to stress that the soil on experimental area is Phaeozem formed from loess, pH of the soil was 5.5–7.2; humus content was 1.8–2%; potassium content was good; phosphorus content was medium; and magnesium content was high (Melich II). From production parameters were estimated seed production (yield), from qualitative parameters were determined oil content (in %) and oil production (in t/ha). It was found that the lowest seed yield, oil content as well as oil production had genotype Californium. High seed yield was estimated for genotypes Oponent, Atlantic and ES Astrid, whereby Oponent had both high oil content and oil production. Content of the most important fatty acid from the aspect of the FAME quality—oleic acid ranged from 60.02 (Labrador) to 65.80% (Atlantic). Oponent had also higher content of oleic acid (62.12%). Seeds of all studied genotypes had low content of linolenic acid (8.33–9.59%), which is important for oxidation stability of fatty acid methyl ester (biocomponent for biodiesel). These analyses were realized according to actual EU standards in the accredited institution, State Veterinary and Food Institute in Bratislava, Slovakia. From this aspect the most suitable genotype seems to be Atlantic. In the case of all genotypes content of erucic acid ranged from <0.01 to 0.05%, which is in agreement with corresponding EU standards. Considering all studied quantitative and qualitative parameters, it should be concluded that for analyzed soils and under Slovakian climatic condition suitable genotype seems to be Czech genotype Oponent.

Later [14, 15] we evaluated production potential of the same six rapeseed cultivars from the point of various climatic condition. From production parameters was estimated seed production, from qualitative parameters were determined oil content, oil production, and content of the substantial fatty acids in the seed. Air temperature and atmospheric precipitation were chosen as the most important climatic factors for rapeseed production. Mean month values of these factors in the 2007/2008 years were compared with the values found for long-term period 1961–1990 years. Based on these data, it could be concluded that in general weather course was favorable for all important stages of the growth (ontogenetical development): sowing, flowering as well as seed maturing. In August 2007, when rapeseed was sown, both air temperature and precipitation were supernormal, September and December were substandard cold with supernormal precipitation. Winter months (January and February) were supernormal warm and with partially limited precipitation. Spring months till harvest were 1.5–2.5 °C over month average of air temperature, and precipitation were 75–164% of normal values. High seed production was estimated for cultivars Oponent, Atlantic and ES Astrid, whereby Oponent had both high oil content and oil production. Content of the most important fatty acid, oleic acid, ranged from 60.02 (Labrador) to 65.80% (Atlantic). Oponent had also higher content of oleic acid (62.12%). Seeds of all studied cultivars had low content of linolenic acid (8.33–9.59%) and content of erucic acid ranged from <0.01 to 0.05%, which is in agreement with corresponding EU standards. Considering all studied quantitative and qualitative parameters, it was concluded that for Slovakian climatic condition suitable cultivar seems to be Czech cultivar Oponent. Tatarková et al. [16] determined in homogenized soil samples coming from the experimental area contents of phosphorus and inorganic-, nitrate- and ammonium nitrogen. The experimental plot was evaluated in terms of basic nutrients content. The highest sensitive response to the applied fertilization and N, P soil content in spring and consequently the highest yield and seed production was found for cultivar Labrador: yield 4.68 t ha^{-1} and seed production 2.28 dkg $plant^{-1}$.

7.2.3 Response of Some Crops to Toxic Metal Application

Roots (especially different types of roots) are important for plants in terrestrial ecosystems because these have different functions. Most of plants form one or more orders of lateral root branches that vary in their thickness, branching patters, growth rates, capacity for secondary growth and structural features, as well. Higher orders of lateral roots are generally thinner, shorter, and do not live as long as of lower orders. Young roots with living epidermal cells and root hairs are often considered to be responsible for the most direct nutrient uptake (e.g., Nyambane and Mwea [17]). Therefore, roots of some species (e.g., cucumber and lettuce) are recommended for toxicity testing and environmental assessment [18].

In connection with uptake of elements (ions) from the soil into the root and transport of them from the root in the shoot, there were established two important factors: bioaccumulation factor (BAF) and translocation factor (TF). BAF is defined as the ratio of metal content (concentrations) in plant dry mass (μg g^{-1} d.m.) to those in soils (μg g^{-1} soil) or in solution (μg mL^{-1}). TF is the ratio of metal content in the shoot (μg g^{-1} d.m.) and in the root (μg g^{-1} d.m.). TF has been used to determine the effectiveness of plants in translocating metal ions from the root to the shoot. Both above-mentioned factors have to be considered for categorization (classification) of metallophyte (in detail see Masarovičová et al. [9]).

As mentioned above phytotoxicity testing of toxic metals based on evaluation of reduction of root and shoot growth of plants is widely used (e.g., [19, 20]). We investigated the effects of seven metal ions (Cd(II), Cr(VI), Cu(II), Hg(II), Ni(II), Pb(II), and Zn(II)) on length of roots of five rapeseed (*Brassica napus* L. *subsp. napus*) cultivars registered in Slovakia (Atlantic, Baldur, Californium, Oponent, and Verona) and evaluated their phytotoxic effect using IC_{50} values [21]. From rapeseed cultivars Atlantic is a medium-early and high type genotype suitable for potato production region; Californium is a medium-early to early and medium-high type of genotype suitable for maize production region; Oponent is a late and high type of genotype suitable for potato production region and Verona is a late and medium-high genotype suitable for maize and potato production regions and Baldur is a medium-early and medium-high type of genotype suitable for all production regions. In general, the toxicity of metal ions decreased in the following order Cu > Cr >Hg > Cd > Pb > Ni > Zn. Atlantic, Baldur, and Californium were more sensitive to Cd than to Ni, for Oponent and Verona higher toxicity exhibited Ni. The sensitivity of studied cultivars treated with toxic metals decreased as follows: for Cd: Atlantic > Californium > Verona > Baldur > Oponent; for Cr: Atlantic = Californium = Verona > Baldur = Oponent; for Pb: Atlantic > Verona > Californium > Baldur > Oponent, for Zn: Atlantic > Californium > Oponent > Verona > Baldur, for Cu: Atlantic > Californium = Verona > Baldur > Oponent, for Hg: Oponent > Californium > Atlantic = Verona > Baldur and for Ni: Oponent = Atlantic > Verona > Baldur > Californium.

From the studied rapeseed cultivars, Atlantic and Californium were found to be most sensitive to tested metals. On the other hand, high tolerance to metal treatment was determined for Baldur. Czech cultivar Opponent showed high tolerance to Cd, Cr, Cu, and Pb, but it was sensitive to Hg and Ni. The obtained results showed that root tolerance index can serve as good biomarker for evaluating the relative toxicity of toxic metals to different rapeseed cultivars.

Our results are in agreement with findings of [22] who estimated following toxicity rank for root growth inhibition of maize plants: $Cu^{2+} > Hg^{2+} > Cd^{2+} > Zn^{2+}$, whereby the toxicity showed correlation with the affinity of metal ions to –SH groups. The tested metal ions are known as mitotic poisons. In the concentration range 0.1–10 mmol dm^{-3} Cu^{2+} exhibited toxic effect on chromosomal morphology in maize and induced c-mitosis, anaphase bridges, and chromosome stickiness [23]. According to Doncheva [24], treatment with copper results in the interruption of progression of nuclei at the crucial G_1/S transition point of the cell cycle, when Cu prevents their entry into mitosis and the effect of copper on root meristem cell proliferation is reflected in the decreased root growth. Cu^{2+} decreased mitotic index which reflects the frequency of cell division in *Vicia faba* root meristematic cells and numerous micronuclei, chromatid bridges and lagging/lost chromosomes found in the meristematic cells of *V. faba* indicated the clastogenic effect of Cu^{2+} [25]. Copper-induced root growth inhibition of *Allium cepa* L. involved disturbances in cell division and DNA damage, whereby microtubules were one of the target sites of Cu toxicity in root tip meristematic cells, and Cu exposure substantially impaired microtubule arrangements [26]. Genotoxic effect of copper was determined also in *Triticum aestivum* L. ([27] and rye (*Secale cereale*) roots [28]. Ni [29] and Zn [30] were also found to decrease mitotic index in *Zea mays* L. and cytotoxic effects of Hg on root tip cells of *Cicer arietinum* L. were estimated by Cavusoglu et al. [31], while short-term exposure to Cr (VI) caused cytogenetic damage in root tip meristems of barley seedlings [32]. Adverse effects of Cr(VI) on mitotic index were described also by Chidambaram et al. [33] and Eleftheriou et al. [34]. Inhibitory effect of Cd^{2+} ions on mitotic index as well as on active mitotic index for *Hordeum vulgare* and *Setaria italica* was estimated by Yadav and Srivastava [35] and Amirthalingam et al. [36] who reported that mitotic divisions in root of *Vigna unguiculata* have been withheld when the Cd stress increased and DNA damage in cells manifested in strand breakage, removal of nucleotides and variety of modifications in organic bases of nucleotides due to ROS formation was observed.

In plants copper is an essential element, usually bound to proteins and involved in numerous processes where it participates on catalyzing redox reactions, whereby only 2% of plant Cu occurs in its free form. Cu in plastocyanin is indispensable in the electron transport chain and it is also a component of the active cytochrome c oxidase complex in the mitochondrial electron transport chain. It is involved also in the light reaction of photosynthesis as well as in cell detoxification by Cu-containing enzymes glutathione-*S*-transferase or Cu-Zn superoxide dismutase. However, at high concentrations it is toxic and inhibits plant growth, photosynthesis and respiration, impairs protein synthesis, induces ROS formation, causes water loss and inactivates key metabolic enzymes [37–39]. Thus, as the site of inhibitory action of Cu the donor and acceptor side of photosystem (PS)II have been suggested (e.g., [40–44]). Copper also damages chloroplasts either by inducing iron deficiency or by replacing Mg in the chlorophyll by Cu [45–47]. Application of 50 and 100 μmol dm^{-3}

Cu resulted in significantly decreased *B. napus* plant growth, biomass, photosynthetic pigments, and gas exchange characteristics and reduction of activities of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) along with protein contents was observed as well accompanied with the increased malondialdehyde (MDA) and H_2O_2 in both leaves and roots [48]. On the other hand, it was found that selenium can protect rapeseed seedlings not only from Cd-induced oxidative stress [49] but it can also alleviate Cu toxicity in rape [50]. Yurela [51] overviewed main features concerning copper function, acquisition and trafficking network as well as interactions between copper and other elements. Review focused on the adverse effects of Cu excess on growth and yield of essential food crops was presented by Adrees et al. [52]. On the other hand, *Brassica* sp. have ability to bioaccumulate heavy metals and can be used to reduce the level of contaminants in the soil (phytoremediation), and thus to clean up and prepare soils for cultivation [53–55].

Cu translocation within *B. napus* plants from external solution was found to be low and excess Cu significantly decreased other microelement content, such as Fe and Mn in plants, caused reduction of photosynthetic pigments and inhibition of plant growth, whereby longer exposure to Cu resulted also in accumulation of highly reactive oxygen species, whereby *B. napus* was found to be more sensitive to Cu-induced stress than *B. juncea* [56, 57]. Copper was considerably more toxic against *B. napus* plants than Zn, it was retained in the roots and was poorly transported to shoots, while Zn proved to be highly mobile, it was concentrated in the upper leaves and actively transported. While high Cu concentrations slowed strongly Zn uptake by the roots but practically did not change its movement over the plant, Zn concentrations facilitated Cu uptake by the roots and its transfer to shoots [58]. External Zn stress resulted in increased Zn content in rapeseed plants, while significant lower concentrations of P, Cu, Fe, Mn, and Mg were estimated, especially in roots, and also increased lipid peroxidation was determined [59]. In *B. napus* plants excess Cu (200 μmol dm^{-3}) induced chlorosis on young leaves similar to Fe deficiency symptoms [60].

In an experiment, we evaluated the effect of copper on some production and biochemical characteristics of 3 weeks old *B. napus* plants (cv. Verona) which were for 7 days hydroponically cultivated in the presence of $CuSO_4$ (0.5–60 μmol dm^{-3}). At lower Cu concentrations (0.5–3 μmol dm^{-3}) a significant increase of biomass (both plant organs), with highest stimulation at application of 0.5 μmol dm^{-3} Cu(II) was observed [61]. Similarly, treatment with low Cu(II) concentrations resulted in increase of fresh weight of *Helianthus annuus* L. seedlings and *Zea mays* L. root growth [23, 62] and increased rape growth grown on Cu-polluted paddy soil [63]. However, in the concentration range 6–60 μmol dm^{-3} $CuSO_4$ we estimated notable reduction of biomass. This is in agreement with findings of Zaheer et al. [64] who observed that exposure to 50 and 100 μmol dm^{-3} Cu significantly reduced the growth, biomass production, chlorophyll content and soluble proteins of *B. napus* seedlings, causing also enhanced production of H_2O_2, MDA and electrolyte leakage in leaf and root tissues of rapeseed plants.

In our experiment Cu-induced stress manifested by chlorosis was observable already at treatment with 12 and 24 μmol dm^{-3} and leaves of plants treated with 60 μmol dm^{-3} were wilted and desiccated. Ali et al. [65] estimated leaf chlorosis and lesser biomass yield in hydroponically cultivated *Brassica oleracea* var. *capitata* plants already at concentrations ≥2 μmol dm^{-3} Cu. Due to Cu(II) treatment we observed also significant decrease in the content of Chl*a*, Ch*b*, and Cars already at concentration 6 μmol dm^{-3}. Cu is known to interfere with the biosynthesis of the photosynthetic machinery by modifying the pigment and protein composition of photosynthetic membranes [66] and to modify nutrient uptake. Feigl et al. [57] observed that excess Cu significantly decreased other microelement content, such as Fe and Mn in the shoots of *B. napus* what resulted in decreased concentrations of photosynthetic pigments in *B. napus* leaves and was accompanied with more intense growth inhibition. The reduction in *Phaseolus vulgaris* leaf Chl concentration due to the Cu-mediated Fe deficiency was explained also by Pätsikkaä et al. [45]. According to Purakayastha et al. [67], root length emerged as the powerful parameter to dictate the uptake of metals by *Brassica* spp.

With increasing Cu concentration from 0.5 to 60 μmol dm^{-3}, Cu concentration in rapeseed plant organs, cv. Verona linearly increased, in roots from 222.7 to 9249 mg kg^{-1} and in shoots from 6.2 to 47.9 mg kg^{-1} [61]. Hence, the amount of Cu accumulated in roots was 36 to 193 times higher than in shoots and TF values showed a decrease from 0.028 (0.5 μmol dm^{-3}) to 0.005 (60 μmol dm^{-3}). While the portion of Cu allocated in shoots from the total Cu amount accumulated by plant was 27.6% at application of 0.5 μmol dm^{-3}, at treatment with 60 μmol dm^{-3} it represented only 8.4%. These results are in agreement with several researchers. For example, compared to control plants treatment with 50, 100, and 150 μmol dm^{-3} resulted in 5.1, 6.3, and 7.6 times higher Cu concentration accumulated in the leaves of *Sinapis alba* after 10 days exposure, whereby for *B. napus* plants it was 3, 5, and 7 times higher [68]. Significantly higher accumulated Cu amount in roots of *B. napus* compared to shoots was observed previously also by Rossi et al. [69]. Greater Cu content in root than in shoot indicates adoption of exclusion mechanism to tolerate the toxicity in which the roots accumulate the metals preventing its subsequent transport to the shoots [70, 71].

The oxidation state of chromium significantly affects its toxicity. The oxidation state of Cr is important because the common triplet oxidation state (Cr^{III}) is not toxic as compared to the hexavalent form [72]. However, Cr(III) which is thought essential for animals in trace amounts, is toxic to plants even at low concentration: it is reported to cause severe oxidative damage and exhibit adverse effects on plant growth, water balance, and pigment content [73–75]. Cr can inhibit δ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, and Cr mostly in the form of Cr(VI) can replace Mg ions from the active sites of many enzymes and deplete Chl content [76]. It is believed that Cr(III) enters the Fenton reaction, whereby the catalytic activity of Cr(III) is much higher in a Fenton reaction system compared to other metals like Co(II), Cd(II), Zn(II), Mn(II) and Fe(III) but lower than Cu(II) [77].

Cr(VI) stress inhibited growth of *B. juncea* L. plants and was directly interrelated with its accumulation, whereby treatment with Cr(VI) resulted in the modulation in activities of various stress markers (SOD, POD, APX, glutathione reductase (GR), dehydroascorbate reductase (DHAR), and lipid peroxidation) [78]. Pandey et al. [79] observed significant increases in lipid peroxidation and tissue concentration of H_2O_2 in *B. juncea* plants exposed to 2 and 20 μmol dm^{-3} Cr(VI). Significant reduction of *B. napus* plant growth, biomass, Chl contents, and Cars as well as soluble protein concentrations, while considerable increase of SOD, guaiacol peroxidase, CAT, and APX activity and MDA levels was estimated as a result of Cr(VI)-induced stress [80, 81]. H_2O_2 may act as a signal that triggers defense mechanisms which in turn protects canola seedlings from Cr(VI)-induced oxidative damage [82]. Zaimoglu et al. [83] estimated that due to Cr(VI) application after a significant increase, a sharp decrease in the activity of APX and GR in *Brassica juncea* L. and *Brassica oleracea* L. plants occurred and the researchers suggested that a coordinated increase in APX and glutathione reductase activities in both *Brassica* species under Cr stress play a role as signals to protect the plants from Cr-induced stress. Induction of phytochelatins along with antioxidant defense system in response to Cr stress suggested the cumulative role of phytochelatins and antioxidants in conferring tolerance against accumulated Cr in *B. juncea* [84]. Increased concentrations of some of antioxidant enzyme activities in leaves and roots of four *B. napus* cultivars exposed to Cr was observed by Gill et al. [85] who also estimated that application below 400 μmol dm^{-3} Cr caused changes in leaf ultrastructures like broken cell wall, immature nucleus, a number of mitochondria, ruptured thylakoid membranes and large size of vacuole and starch grains, while at concentrations exceeding 400 μmol dm^{-3} Cr damage of roots in the form of disruption of Golgi bodies and diffused cell wall was estimated. At treatment of eight canola cultivars with 100 μmol dm^{-3} Cr(VI) the estimated Cr concentrations in aerial parts of plants ranged from 255.0 to 705.8 μg Cr g^{-1} d.w., whereby the cultivar with the highest accumulated Cr amount showed the lowest levels of chlorophyll content and highest levels of lipid peroxidation [86]. The Cr contents in stem, leaf, and root of *B. juncea* plants usually were heightened with increased concentrations of Cr(VI) in soil and average Cr concentration in the leaves of Laifengjiecai and Sichuanhuangzi cultivars growing 70 days in the soil spiked with 300 mg kg^{-1} Cr(VI) reached 167.30 and 197.60 mg kg^{-1}, while the maximum Cr contents in plant shoots were 1.71 and 2.81 mg/plant, indicating that portions of Cr removed by plant shoots were 0.23 and 0.38% of Cr content in treated soil [87]. Significant translocation of Cr from the roots into the above-ground parts was estimated in soybean treated with Cr(VI) [88]. High activities of antioxidant enzymes supported by high Cr concentrations in roots and aerial parts established the Indian mustard as a potential hyperaccumulator and a hypertolerant species to Cr stress [89]. Karuppanapandian and Manoharan [90] found that uptake and translocation of Cr in *Vigna mungo* L. plants was relatively higher during first 12 h of treatment with 100 μmol dm^{-3} Cr(III) as well as Cr(VI) and Cr-treated roots of *Vigna mungo* L. retained 15 times more Cr than the shoots. Bluskov et al. [91] found that *B. juncea* plants grown on soils supplemented with 100 mg kg^{-1} of Cr (III) or Cr(VI) concentrated Cr mainly in the roots and removed about 48 and 58 μg Cr/plant. Cr was localized, and probably sequestered, in epidermal and cortical cells in the roots and epidermal and spongy mesophyll cells in the leaves and Cr (III) was detected, primarily as acetate in the roots and oxalate in the leaves. Thus, *B. juncea* was found to be able to detoxify more toxic Cr (VI). Similarly, in mesquite which could be classified as a hyperaccumulator of Cr, supplied Cr(VI) was uptaken by the mesquite roots, however, the data analyses of the plant tissues demonstrated that it was fully reduced to Cr(III) in the leaf tissues [92]. *Eichhornia crassipes* (water hyacinth), supplied with Cr(VI) in nutrient culture, also accumulated non-toxic Cr(III) in root and shoot tissues, whereby the reduction of Cr(VI) to Cr(III) appeared to occur in the fine lateral roots and Cr(III) was subsequently translocated to leaf tissues where it was bound to oxalate ligands [93]. Conversion of CrO_4^{2-} in the root to Cr(III) by several plants was reported by Zayed et al. [94], whereby translocation of both Cr forms from roots to shoots was extremely limited and accumulation of Cr by roots was 100-fold higher than that by shoots and did not depend on the applied Cr species. Chelates and organic acids were found to enhance Cr(III) accumulation, but toxic effects were not avoided and Cr (III) complexes were as toxic to plants as Cr(VI) [95]. Han et al. [74] found that chromium from Cr(VI)-contaminated soils was more phytotoxic to *B. juncea* plants than that from Cr(III)-contaminated soils and caused growth retardation, reduced the number of palisade and spongy parenchyma cells in leaves, clotted depositions in the vascular bundles of stems and roots, and increased number of vacuoles and electron dense materials along the walls of xylem and phloem vessels. Studies on Cr(III) and Cr(VI) speciation in the xylem sap of maize plants showed that Cr(III) and Cr(VI) were present as mobile and soluble anionic organic complexes, probably Cr (III)-citrate in the xylem sap [96].

Gong et al. [97] found that Cr(VI) inhibited PS II in *Chlamydomonas reinhardtii* mainly through damaging the oxygen evolving complex (OEC) and blocking the electron transfer from Q_A to Q_B. On the other hand, Gupta et al. [98] observed in 7-day-old seedlings of *Brassica juncea* that Cr (VI) promoted PS II-mediated photoreactions and found that Cr enhanced tolerance of PS II to alkaline pH. Deterioration of oil quality by Cr(VI) application was manifested by reduction of oleic, linoleic, and linolenic acid contents of lipids and increased erucic acid content in *B. napus* due to increasing Cr(VI) concentration [99].

Physiological changes induced by Cr stress in plants were overviewed by Hayat et al. [100] and chromium interactions in plants were summarized by Shanker et al. [101].

In our experiment we investigated the response of hydroponically cultivated 3 weeks old rapeseed (*Brassica napus* L., cv. Verona) plants to Cr(III)-nitrate applied in the concentration range from 6 to 480 μmol dm^{-3} [102]. Application of Cr(III) resulted in reduced length, fresh mass as well as dry mass of rapeseed plant organs, whereby the inhibitory effect increased with increasing metal concentration in the external solution, the roots being more sensitive to Cr(III) treatment than the shoots. Significant reduction of the concentration of assimilation pigments (Chl *a*, Chl *b*, Cars), proteins and reduced thiol groups in rapeseed leaves was also estimated after treatment with Cr(III), while the concentration of thiobarbituric acid reactive substances (TBARS) increased with increasing Cr(III) concentration. Even though the reduction in root growth could be due to inhibition of root cell division or elongation, or due to the extension of the cell cycle [103], damage to the root apparatus due to Cr(III) treatment might cause an unbalanced supply of nutrients and/or an alteration of their role in anabolic pathways, which ultimately arrest normal physiological and developmental processes [104] and it can be at high Cr concentrations connected also with the collapse and subsequent inability of the roots to absorb water from the medium [105]. Changes in plant water relations resulting in decrease in physiological availability of water due to Cr(III) treatment were reported by Pandey and Sharma [106]. The reduction in shoot height might be mainly due to the reduced root growth and consequently lesser nutrients and water transport to the above parts of the plant. Decrease in chlorophyll concentration in leaves of Cr(III)-treated *B. napus* plants could be explained by reduced leaf tissue concentration of Fe because Cr(III) impairs the Fe requiring steps of chlorophyll and heme biosynthesis [107]. The decrease in protein contents in Cr(III)-treated plant at higher concentrations of Cr was probably due to adverse effects of ROS, which may cause degradation of a number of proteins [80, 108]. Cr(III) ions at increased concentrations can interfere with several metabolic processes and decrease of concentration of reduced thiol groups in the leaves of rapeseed plants due to increasing supply of Cr(III) indicated that thiol-rich peptides were consumed and detoxification mechanisms in plants failed to eliminate toxic effects of chromium [102]. Cr(III) can be endogenously reduced to Cr(II) by biological reductants such as cysteine and NADPH and in turn, the newly formed Cr(II) reacts with H_2O_2 producing hydroxyl radicals and causes tissue damage [109]. Cr(III)-induced oxidative stress was reflected in the rise of TBARS levels [102] what is in agreement with findings of Karuppanapandian and Manoharan [90]. The BAF values determined for rapeseed (cv. Verona) roots varied in close range from 1241 to 913 and they were by one till three orders higher than those determined for shoots which increased with increasing Cr(III) concentration in hydroponics from 1.92 (6 μmol dm^{-3}) to 30.18 (480 μmol dm^{-3}). Low mobility of Cr(III) within the plants was reflected by low values of translocation factors ranging from 0.0015 to 0.0330 [102].

Cd^{2+} and Hg^{2+} ions are known to inhibit photosynthetic electron transport (PET) and they were found to interact with the intermediates Z^+/D^+, i.e., with the tyrosine radicals on the donor side of PS II situated in the 161st position in D_1 and D_2 proteins, with the primary donor of PS I (P700), whereby the oxidation of Chl*a* dimer in the reaction center of PS I occurred yet in the dark and with the manganese cluster which is situated in the oxygen evolving complex. These ions damaged also all mechanisms, i.e., direct, cyclic and non-cyclic reductions of $P700^+$ and formed complexes with amino acid residues constituting photosynthetic peptides what was suggested as possible mechanism of their inhibitory action [14, 110]. Singh and Singh [111] and Fodor et al. [112] situated the site of Cd^{2+} inhibitory action in the site of Q_A or Q_B on the acceptor side of PS II. Inactivation of PS II activity by Ni^{2+} compounds at donor side of PS II due to interaction with $Z^\bullet/D^\bullet$ intermediates and manganese cluster in OEC was estimated as well [113–115] and inhibitory effects of Ni^{2+} ions on photosynthetic apparatus could be also connected with their ability to form complexes with amino acid residues in photosynthetic proteins [116].

In contrast to Cd^{2+} and Hg^{2+} which bind readily to SH-groups, Ni would rather bind to aromatic nitrogen [117].

Application of 10 mg Cd kg^{-1} d.m. of soil caused visible symptoms of chlorosis on *B. napus* leaves and a statistically significant decrease in aerial biomass, whereby biomass decrease depending on the soil type decreased in the following order: mineral neutral > organic neutral > mineral acidic > organic acidic and addition of Cd to soil increased the $Cd^{2+}:Ca^{2+}$ and $Cd^{2+}:Mg^{2+}$ ionic ratios [118]. Armas et al. [119] observed that low Cd concentrations stimulated growth of *B. juncea* plants, while application of large Cd concentrations resulted in growth inhibition and increase in lipid peroxidation due to Cd treatment, which was always greater in shoots than in roots, whereby an increase in guaiacol peroxidase, ascorbate peroxidase and catalase activities was estimated. Sikka and Nayyar [120] denoted that significant reduction in the dry matter yield of *B. juncea* occurred with application of ≥5 mg Cd kg^{-1} soil. Application of Cd to soil decreased the content of micronutrients in plants, but significant reduction occurred only for Fe at rates beyond 50 mg Cd kg^{-1} soil. Since no visual toxic symptoms were observed on the leaves of *B. juncea* grown in a sandy loam soil polluted with Cd (5–80 mg kg^{-1} soil), it could be concluded that Cd may accumulate in this vegetable without visual evidence of its presence. However, accumulated Cd content by plant organs increased with increasing concentration of applied Cd, being much higher in roots than in shoots. While the relationship of Cd with Zn and Fe was synergistic in both roots and shoots at the lower rates but antagonistic at higher Cd application rates, in the case of Mn and Cu, the relationship was negative and antagonistic.

Study of *in vitro* grown callus and seedlings of *B. juncea* treated with equimolar Cd concentrations showed that the overall activity of antioxidative enzymes (SOD, CAT, and APX) was found to be higher in callus in comparison to seedlings of *B. juncea* indicating that calli were more tolerant toward Cd-induced oxidative stress [121]. Verma et al. [122] found that treatment with Cd resulted in an increase in ionically bound cell wall peroxidase activity in roots of seedlings which showed direct correlation with increased level of H_2O_2 in roots.

B. juncea was found to accumulate more Cd in the shoots compared to *B. napus*, whereby excess Cd increased the lipid content of *B. juncea* leaves grown in the presence of Cd, but did not affect fatty acids composition, while in *B. napus* leaves an alteration in the lipid composition as well as a decrease in the lipid contents was estimated [123, 124]. Higher level of Cd caused significant accumulation of proline, gradual increases in activities of antioxidant enzymes such as catalase and peroxidase along with increased lipid peroxidation and decreased the concentrations of soluble proteins and chlorophylls [125].

Cd induced alteration in lipid profile of developing *B. juncea* L. seed: total and non-polar lipids decreased regularly with increasing Cd doses; a positive correlation was found between saturated fatty acid (palmitic acid, stearic acid) and Cd concentration, while unsaturated fatty acids (oleic acid, linoleic acid, linolenic acid) were found to be decreasing with increasing Cd concentration. Based on increased ratio in the saturated/unsaturated acids due to Cd treatment it can be assumed that the synthesis or activity of olelyl-CoA desaturase enzyme was affected significantly [126].

Goswami and Das [127] investigated Cd phytoremediation ability of Indian mustard and found that the highest shoot and root Cd accumulation (10,791 and 9602 μg g^{-1} d.w., respectively) was achieved with application of 200 mg kg^{-1} Cd and the maximum leaf Cd accumulation was 10,071.6 μg g^{-1} d.w. at 100 mg kg^{-1} Cd, after 21 days of treatment. Harmful Cd-induced effects on growth, photosynthesis, and oxidative stress in *Brassicacae* sp. could be alleviated by application of Se [49], salicylic acid, and Ca [128, 129].

Cd treatment of *B. juncea* plants was associated with a rapid accumulation of phytochelatins in the root, where the majority of the Cd was coordinated with sulfur ligands, probably as a Cd-S_4 complex, while Cd moving in the xylem sap was coordinated predominantly with *O*- or *N*-ligands and Cd translocation to the shoot appeared to be driven by transpiration [130].

Nickel is essential for higher plants in low concentrations but becomes toxic to plants when applied in excess causing plant growth inhibition, chlorosis, necrosis, and wilting. Its entry in root system can occur via passive diffusion (cation transport system) and active transport, using the magnesium ion transport system, or by high-affinity Ni transport proteins [131] and toxic effects of Ni are reflected in disturbance of mineral nutrition, photosynthesis, water relations, respiration and nitrogen metabolism of plants [132–134]. Due to Ni-induced oxidative stress which is connected with ROS formation, oxidation of macromolecules in plant tissues [133] and impairment of membrane function resulting from lipid peroxidation occurs [135, 136]. Krupa et al. [137] indicated an indirect effect of Ni on photosystems related to the disturbances caused by the metal in the Calvin cycle reactions and downregulation or even feedback inhibition of electron transport by the excessive amounts of ATP and NADPH accumulated due to non efficient dark reactions.

Amari et al. [138] showed that xylem transport rate of Ni in *B. juncea* increased with increasing Ni supply and a positive correlation was established between Ni and citrate concentrations in the xylem sap and shoots of plant accumulated also significant concentrations of malic acid and histidine. Khan et al. [139] reported that H_2O_2 alleviated Ni-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in *B. juncea* plants. Due to application of Ni proline and MDA in the leaves of *B. juncea* increased with increasing metal concentration, while soluble protein content was decreased [140]. However, genotypic variation in phytoremediation potential of *B. juncea* plants exposed to Ni stress was estimated [141, 142]. Wang et al. [143] found that at the exposure of *Brassica juncea* L. var. megarrhiza to the metal concentration of 300 μmol dm^{-3} as much as 98% of the Cu and 79% of Cd were retained in the roots, while Ni was relatively uniformly distributed between leaves (32%), stems (29%), and roots (39%), whereby the dominant storage compartments for Cd and Cu in the stems and leaves were the cell wall and soluble fractions and the soluble fraction was the dominant storage compartment for Ni in stems and leaves.

An overview focused on the Ni uptake, essentiality and toxicity in plants was presented by Yusuf et al. [144]. Screening of five Brassica species (*B. juncea*, *B. campestris*, *B. carinata*, *B. napus*, and *B. nigra*) for hyperaccumulation of Zn, Cu, Ni, Pb, and Cd showed that *B. napus* accumulated highest amount of Pb, Ni, and

Cd. Succinic acid was characterized and quantified as one of the dominant organic acids in root exudates of promising *Brassica* species indicating probable role of this acid in metal acquisition through complexation [145].

Marchiol et al. [146] evaluated bioconcentration factors related to roots (BCF_R) and shoots (BCF_S) of *B. napus*, *B. juncea*, *Raphanus sativus*, and *B. carinata* grown on a substrate contaminated by several heavy metals caused by the use of contaminated irrigation water and found that BCF_R was >1 for all the species for Cd, Cu, Ni, and Zn without significant differences among species, while BCF_S were lower than 0.5.

Mercury is toxic metal which may directly inhibit enzymes by interacting with protein –SH groups causing protein conformation changes and subsequent enzyme inactivation [117] and binding of Hg to small biothiols such as glutathione and cysteine was confirmed, as well [147, 148]. Excess Hg results in visible symptoms of phytotoxicity, such as reduced growth, chlorosis, etc. [149, 150], and physiological disorders in plants [151], PET inhibition [110, 152, 153], closure of leaf stomata and physical obstruction of water flow in plants [154, 155], inhibition of nutrient uptake [156], and induction of oxidative stress in plants [157, 158]. According to Kopittke et al. [159], the toxicity of Hg(II) with median toxic concentration 0.47 μmol dm^{-3} Hg against plants grown in solution was higher than the toxicity of Cu, Cd, As, Co, Ni, Zn, and Mn. The mobility of Hg within the plant is very low [149, 160, 161], it remains predominantly immobilized in roots where it can be non-specifically absorbed to cell walls or sequestered in complex form with phytochelatins in the vacuoles of the root cells [162] and it was reported that even 95–99% of the Hg taken up by the roots did not reach the shoots [160, 163]. Chen et al. [148] reported that Hg in plant organs of 10-day-old seedlings of *Brassica chinensis* which were exposed for 3 days to 200 μmol dm^{-3} $HgCl_2$ were estimated 26,089 μg g^{-1} d.m. in roots and 2839 μg g^{-1} d.m. in shoots.

B. juncea plants which were grown hydroponically in an Hg-spiked solution effectively generated an enzymatic antioxidant defense system, especially CAT to scavenge H_2O_2, resulting in lower H_2O_2 levels in shoots with higher mercury concentrations and tested cultivars demonstrated an efficient metabolic defense and adaptation system to Hg-induced oxidative stress. A majority of Hg was accumulated in the roots and low translocation of Hg from roots to shoots was observed [157]. Elevated Hg concentrations (≥2 mg dm^{-3}) resulted in significant reduction in both biomass and leaf relative water content and caused significantly changed leaf cellular structure represented by thickly stained areas surrounding the vascular bundles, reductions in the number of palisade and spongy parenchyma cells and reduced cell size and clotted depositions [164]. Up to hundreds of ppm Hg accumulated in the roots of Indian mustard plants grown with soil contaminated by HgS were observed by Su et al. [165]. In *Brassica juncea* L. Czern & Coss, cv. Pusa Jai Kisan reduction in growth, activities of antioxidant enzymes such as SOD, CAT, APX, and GR were enhanced with increase of applied Hg concentration. The Hg-induced alterations in growth were connected with increase in lipid peroxidation (MDA and H_2O_2), while the enhanced activities of antioxidant enzymes secured protection of plants from Hg-induced oxidative stress [166]. In plants of *B. juncea* co-treated with Hg and Se high molecular-weight Se/Hg-containing compounds were found primarily in the plant root extract which may be protein-associated [167]. Tolerance of *Brassica juncea* to mercury can be enhanced also by carbon monoxide [168].

We compared the response of 3 weeks old hydroponically cultivated rapeseed (*Brassica napus* L., cv. Verona) plants which were exposed for 7 days to Cd, Ni (6−120 μmol dm^{-3}), and Hg (6−60 μmol dm^{-3}) [169]. While local symptoms of chlorosis were observed at treatment with 6 μmol dm^{-3} Ni, 12 μmol dm^{-3} Hg, and 60 μmol dm^{-3} Cd, plants exposed to 60 μmol dm^{-3} Ni or Hg showed considerable chlorosis, their leaves were wilted and roots were brownish. Reduction of root and shoot length due to metal treatment was only moderate and application of 120 μmol dm^{-3} Cd and Ni caused 19% reduction of root length and 13% (Cd) or 6% (Ni) reduction of shoot length compared to the corresponding control plants. On the other hand, the same reduction (22%) of both plant organs was obtained with application of 60 μmol dm^{-3} Hg. In general, reduction of dry mass of plant organs increased with increasing metal concentration in external solution; nevertheless, more significant reduction showed root dry mass. The toxicity of metals which was reflected in reduced root dry mass decreased as follows: Hg (IC$_{50}$: 26.1 μmol dm^{-3}) > Cd (IC$_{50}$: 78.3 μmol dm^{-3}) > Ni (IC$_{50}$: 84.1 dm^{-3}) and the same rank of metals toxicity was obtained for shoot dry mass. Applied metals reduced shoot water content, whereby water content in the above-ground part of plants after application of 60 μmol dm^{-3} Cd, Ni, and Hg represented 76.9%, 94.9%, and 74.4% of the control and due to treatment with 120 μmol dm^{-3} Cd and Ni it declined to 63.0% and 87.3%, respectively. The concentrations of Chl*a* and Chl*b* as well as Cars in leaves of young rapeseed plants decreased as the metal supply was increasing, whereby at treatment with 60 μmol dm^{-3} the mean reduction of pigment concentrations compared to the control was about 50% for Cd and Ni and >60% for Hg.

Protein concentration in rapeseed leaves showed exponential decay with increasing metal concentration in hydroponics and at the highest applied metal concentration the estimated reduction of protein concentrations compared to control plants was 52.7% (Cd), 39.1% (Ni), and 43.6% (Hg). Concentration of TBARS also rose with increasing metal concentration and at the treatment with 120 μmol dm^{-3} it was 1.64 (Cd) and 2.28 (Ni) times higher than that of the control, at application of 60 μmol dm^{-3} Hg it was 2.91 times higher.

In the studied concentration range 0−120 μmol dm^{-3} Cd or Ni and 0−60 μmol dm^{-3} Hg, metal concentration accumulated in the roots showed linear increase with increasing external metal concentration. The most effective metal accumulation in roots showed Hg, the lowest one Ni. While linear increase of metal concentration in shoots in the whole investigated concentration range was estimated for Ni and Hg, gradual saturation of shoot tissue with Cd was observed at concentrations higher than 24 μmol dm^{-3}. The levels of Hg in rapeseed shoots were the lowest in the whole studied concentration range.

BAFs determined for roots ranged from 1436 to 952 for Cd and from 410 to 225 for Ni, showing a decrease with increasing external Cd and Ni concentration, while the corresponding BAFs determined for Hg varied only slightly (2227–2567). BAF values related to shoots ranged from 68.6 to 29.1 for Cd, from 29.7 to 40.4 for Ni, and from 7.4 to 12.7 for Hg. The effectiveness of metal translocation from root to shoot increased as follows: Hg < Cd <Ni. The TF values estimated for Hg (0.0033−0.0057) were by one to two orders lower than TFs determined for Cd (0.0478−0.0306) and Ni (0.0724−0.1795), respectively.

Reduction of dry mass of plant organs due to application of studied metals (Cd, Ni, Hg) estimated in the present experiment which was observed previously also by many researchers in a wide spectrum of model plants is connected mainly with inhibition of root cell division (e.g., [170]), inhibition of chlorophyll synthesis [171, 172], lower absorption of macronutrients and microelements from cultivation medium [173, 174], inhibition of photosynthesis [110, 175, 176] and replacement of Mg in chlorophyll by the studied metals [46]. Kopittke et al. [159] reported that across a range of plant species and experimental conditions, the phytotoxicity of the trace metals followed the trend Hg > Cd > Ni and median toxic concentrations were 0.47 μmol dm^{-3} for Hg, 5.0 μmol dm^{-3} for Cd, and 19 μmol dm^{-3} for Ni.

Gradual decline in shoot water content of rapeseed plants, cv. Verona with increasing metal concentration, which was considerably higher at Hg or Cd treatment compared to Ni application, could be connected with changes in plant roots that inhibit water uptake what results in reduced physiological availability of water [177, 178].

Changes in plant water relations causing physiological drought in *B. juncea* L. plants due to exposure to Cd connected with changes in plant water relations were observed by Singh and Tewari [125] and Gajewska et al. [179] who observed decline in relative water content due to treatment with higher Ni concentrations. Hg treatment was found to inhibit water uptake through aquaporins in plasma membranes in higher plants and decrease in transpiration and water use efficiency in plants occurred due to inhibition of water channels in wheat root cells [154]. Hg rapidly and significantly decreased the pressure-induced root water flux in tomato plants exposed to Hg [155]. Similarly as in our experiment, the strong decrease of the concentration of assimilation pigments with increasing metal (Cd, Ni or Hg) concentration was observed by several researchers (e.g., [76, 180–186]).

This decrease can be caused for example by toxic effect of Cd on photosynthetic pigments causing degradation of Chl and Cars as well as inhibition of their biosynthesis [187, 188] and by inhibition of biosynthesis of photosynthetic pigments resulting in disturbances in electron transport rates of PS I and PS II and subsequent generation of oxygen free radicals [189]. The reduction in pigment contents due to Ni toxicity could be attributed to δ-aminolevulinic acid utilization due to inhibition of Chl biosynthesis by creating nutrient imbalances [76] and due to replacement of Mg^{2+} ions by toxic metals [186].

The decrease in protein contents in metal-treated plants is connected with effects of ROS, which may be due to degradation of a number of proteins [108] as a result of increased protease activity [190] as well as with the effect of these metals on nitrate reductase activity [76] and it may also be a consequence of an inhibition of cell division in young cells which are characterized with particularly intensive protein synthesis [191]. Reduction in soluble protein content in Cd-treated plants was also reported by Costa and Spitz [192] and Mohan and Hosetti [193], while Maleva et al. [194], Duman and Ozturk [195], and Ali et al. [196] published similar results concerning Ni-treated plants and protein degradation due to Hg treatment was observed in wheat chloroplasts by Panda and Panda [197].

Presented results concerning increase of TBARS levels due to treatment with Cd, Ni, and Hg are in agreement with findings of several researchers, e.g., Ansari et al. [166] who informed about Hg-induced increase in lipid peroxidation causing alterations in growth of *B. juncea*, Maheswari and Dubey [198] who observed elevated levels of H_2O_2 and TBARS in rice (*Oryza sativa* L.) seedlings treated with 200 and 400 μmol dm^{-3} Ni or with results concerning increased TBARS levels due to treatment with Cd in *B. napus* [180], *Brassica juncea* [199], *Brassica campestris* [200], and *Pisum sativum* plants [201].

Brassica varieties grown in hydroponics which were tested for Cd accumulation in shoots accumulated 200–600 mg Cd kg^{-1} dry mass after treatment with 10 μmol dm^{-3}. Rapeseed plants, cv. Verona used in our study, which were exposed to 12 and 24 μmol dm^{-3} Cd accumulated in the shoots 115.2 and 191.7 mg Cd kg^{-1} d.m., respectively. Consequently, it can be concluded that for Verona cultivar lower levels of toxic metals in the shoots are characteristic, indicating its tolerance against Cd. In this experiment Hg accumulation in the roots of Verona cv. was more than by two orders higher than that in the shoots what is in agreement with previous findings that the translocation of Hg from root to shoot is appreciably limited and predominant amount of mercury uptaken by plants remains immobilized in roots [149]. TF values estimated by Marchiol et al. [146] for *B. napus* plants (cv. Kabel) grown 60 days on multi-contaminated soil containing 38.6 mg kg^{-1} Cd and 46.9 mg kg^{-1} Ni were 0.0053 for Cd and 0.0009 for Ni, while TF values evaluated for considerably younger rapeseed plants of Verona cultivar grown in hydroponics in the presence of 120 μmol dm^{-3} Cd and Ni factors were considerably higher, 0.0306 and 0.1795, respectively [169], what could be connected with better bioavailability of tested metals in plants cultivated in hydroponics as well as with appropriate levels of all essential nutrients required for growth of rapeseed plants.

7.2.4 Effect of Metal Complexes on Crops

Chelators can significantly affect biological activity of metal ions. The chemical form of the metal is a very important determinant in understanding the quantitative aspects of metal toxicity and it significantly affects uptake of metal by plants. Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum* was reported by Taylor and Foy [202] and positive effect of chelating agent application for more effective Cu, Zn, and Pb uptake by several plants was described in [203]. Cysteine, histidine and aspartate or glutamate are major cellular ligands of Zn that form tetrahedral coordinations [204] and these ligands bind to Zn with a greater affinity and with more stability than to Fe, thereby protecting the sulfhydryl groups against oxidation [205]. Vacuolar sequestration of Zn by high levels of vacuolar citrate may be a central mechanism in the accumulation of Zn in plants, exposed to either low or high levels of this metal [206] and the high root zinc concentration and decreased zinc translocation from root into the shoot in pea plants treated with zinc and succinate suggested that succinate facilitated the formation of metal-succinate complexes in the roots [207]. The application of Zn complexes also significantly increased Zn uptake by maize plants [208]. In leaves mainly Zn-citrate complexes exist even though malate is more abundant and in xylem sap citrate and histidine are the prevalent ligands of Zn [209]. Organic acids, most notably nicotianamine, and specialized proteins bind iron before it can be inserted into target molecules for biological function and inside the cells and generation of highly toxic hydroxyl radicals by iron redox reactions is avoided by intricate chelation mechanisms [210]. In our research we focused our attention on the effects of different metal complexes and metal chelates on PET, growth of alga *Chlorella vulgaris* and growth of vascular plants (maize and mustard) as well as on metal accumulation in plant organs of *Zea mays* L.

In pyruvideneglycinatocopper(II) complexes the chelate-forming ligand is the dianion of pyruvideneglycine (condensation product of pyruvic acid and glycine). Three donor atoms of Schiff base form with the Cu(II) ion two five-membered chelate rings affording a structure which is sufficiently stable also in aqueous solutions. The planar arrangement of donor atoms around the Cu(II) ion of the complexes with Schiff bases results in strong stability increase of Cu(II) oxidation state. PET-inhibiting activity of pyruvideneglycinatocopper(II) complexes with *S*-donor ligands thiourea (tu), ethylenethiourea (ettu) and chlorophenylthiourea (cphtu) expressed by IC_{50} value was lower than that of $CuCl_2 \cdot 2H_2O$ (11.8 μmol dm^{-3}): 18.4 μmol dm^{-3} for Cu(pyrgly)(tu)(H_2O), 23.3 μmol dm^{-3} for Cu(pyrgly)(ettu)(H_2O), 35.1 μmol dm^{-3} for Cu(pyrgly)(cphtu) [211]. Increasing lipophilicity of the additional molecular ligand led to activity decrease. Toxic effect of these Cu(II) compounds applied in the concentration 100 μmol dm^{-3} on maize growth was reflected in reduction of dry mass of roots, stems and leaves related to the corresponding control plants, whereby the most inhibited parameter was dry mass of leaves, while dry mass of roots and stems was only slightly affected. The smallest toxic effect on reduction of leaf dry mass exhibited Cu(II) chelate with most lipophilic ligand, i.e., L = *N*-(2-chlorophenyl) thiourea and the treatment with 100 μmol dm^{-3} of this compound resulted in increased accumulation of Cu in the above-ground part of the plant representing approximately 10% from the total uptaken Cu amount. The toxic effects of these Cu(II) chelate are probably due to the substitution of their additional ligands with *N*-, *S*- or *O*-donor ligands present in proteins of plant cells.

Using EPR spectroscopy as the site of action of Cu chelates in the photosynthetic apparatus the Z^+/D^+ intermediate and oxygen evolving complex, both situated on the donor side of PS II was estimated and also interaction of Cu(II) complexes with aromatic amino acids residues of proteins was confirmed by fluorescence measurements [40, 41]. The IC_{50} values related to PET inhibition in spinach chloroplasts estimated for a set of aqua(aryloxyacetato)copper(II) compounds (aryl = substituted phenyl) varied in the range from 4.58 to 22.59 μmol dm^{-3}, whereby the most effective compounds contained Cl substituents in their molecule [40]. On the other hand, great difference between IC_{50} values of simple carboxylate copper compound

$[Cu(sal)_2(H_2O)_2]\cdot 2H_2O$ (IC_{50} = 12.6 μmol dm^{-3}) [212] and chelates potassium [aqua-(*N*-salicylidene-L-glutamato)cuprate, $K^+[Cu(sal\text{-}L\text{-}glu)(H_2O)]\cdot 2H_2O$ (IC_{50} = 1967 μmol dm^{-3}), diaqua(*N*-pyruvidene-β-alaninato)copper(II) monohydrate, $[Cu(pyr\text{-}\beta\text{-}ala)\cdot H_2O]\cdot H_2O$ (IC_{50} = 261 μmol dm^{-3}) and potassium [(isothiocyanato)-(*N*-salicylidene-β-alaninato)cuprate(II)], $K^+[Cu(sal\text{-}\beta\text{-}ala)NCS]$ (IC_{50} = 1007 μmol dm^{-3}) [213] could be connected with higher stability of chelates in aqueous solution. Similar results were obtained in the study of antialgal activity of above-mentioned Cu(II) compounds [214]. In comparison with $CuCl_2\cdot 2H_2O$ and $Cu(sal)_2\cdot 4H_2O$, the lower inhibitory effect of $K[Cu(sal\text{-}L\text{-}glu)]\cdot 2H_2O$ on growth of maize was confirmed, too [212]. In the concentration range of higher metal concentrations the most sensitive parameter to Cu(II) toxicity was the primary root growth, whereby the formation of lateral roots and root hairs was also pronouncedly suppressed. The IC_{50} values obtained for the inhibition of root and shoot dry mass were higher than those obtained for the inhibition of root and shoot growth. While the effects of $CuCl_2\cdot 2H_2O$ and $Cu(sal)_2\cdot 4H_2O$ were comparable, $K^+[Cu(sal\text{-}L\text{-}glu)]\cdot 2H_2O$ exhibited significantly lower toxic effect on investigated production characteristics, with the exception of primary root inhibition. Because the stability constants for complexes of copper with salicylic and glutamic acid are relatively high ($\log K_1$ = 10.6 and 7.85 respectively), it could be assumed that the biological activity of the studied Cu(II) complexes could correlate with the ability of these complexes to exchange their additional ligands (H_2O molecules) with *N*-, *S*- or *O*-donor ligands occurring in proteins of *Zea mays* L. Organic ligands can also contribute to better transport of metal ions through the lipophilic regions of cell membranes. The lower inhibitory effect of $K^+[Cu(sal\text{-}L\text{-}glu)(H_2O)]\cdot 2H_2O$ probably results from higher stability of this anionic chelate complex in aqueous solutions and it could be also assumed that due to sterical conditions the access of copper bound in this complex compound with polydentate ligands to its site of action is more intensively limited than that of copper with monodentate ligands. These findings are in accordance with our previous findings [40, 41, 213] that the different coordinating modes of acidoligands pronouncedly affect the biological activity of Cu(II) compounds.

A set of anti-inflammatory Cu(II) complexes with biologically active ligands of the type $CuX_2\cdot H_2O$ and CuX_2L_y, where X = flufenamate (*N*-(α,α,α-trifluoro-*m*-tolyl) anthranilate), mefenamate (2-((2,3-dimethylphenyl)amino)benzoate)), niflumate (2-(α,α,α-trifluoro-*m*-toluidino) nicotinate), naproxenate (6-methoxy-α-methyl-2-naphthaleneacetate); L = nicotinamide, *N,N*-diethylnicotinamide, ronicol (3-hydroxymethylpyridine), caffeine, methyl-3-pyridylcarbamate; and *y* = 1 or 2 was investigated related to inhibition of oxygen evolution rate (OER) in the suspensions of *Chlorella vulgaris* [214] and spinach chloroplasts [215]. The anionic X ligands increased the inhibitory effect while the effect of the L ligands was not significant. Taking into account the X ligands, the inhibitory activity decreased in the order flufenamate ~ niflumate > mefenamate > naproxenate, i.e., the most active inhibitors were compounds containing fluorine atoms in their molecules. The PET inhibiting activities of these compounds in spinach chloroplasts were approximately two to three orders higher (IC_{50}: 6.6–14.2 μmol dm^{-3}) than those determined for OER inhibition in *C. vulgaris* (IC_{50}: 0.976–2.291 mmol dm^{-3}) what could be connected with the fact that in *C. vulgaris* for reaching the site of action the inhibitor must penetrate through the outer and inner algal membranes, while in partially broken spinach chloroplasts used in this study the corresponding inhibitor could directly interact with the thylakoid membranes.

Six chelate cuprates of the composition $M^+[Cu(TSB)(X)]^-$ containing tridentate Schiff base dianion ligands (TSB^{2-}) of *N*-salicylideneaminoacidato type (derived from α-alanine or β-alanine, valine, phenylalanine), additional pseudohalogeno ligands (NCS^-or NCO^-), and M (K, NH_4 or Na) as well as six molecular (*N*-salicylidene-β-alaninato)copper(II) complexes of the composition [Cu(sal-β-ala)(L)] with additional organic molecular ligands (L = imidazole (im), pyrazole (pz), pyridine (py), quinoline (quin), urea (ur) or thiourea (tu)) were investigated on their effects on reduction of chlorophyll content in statically cultivated green alga *Chlorella vulgaris* and PET inhibition in spinach chloroplasts [216]. The differences in immediate toxic effects of all studied Cu(II) complexes on the PET inhibition in spinach chloroplasts were relatively small and more significant effect of individual ligands on the biological activity was not observed. The inhibitory effectiveness of the majority of the tested compounds (with the exception of compounds Cu(sal-β-ala)(im) and Cu(sal-β-ala)(tu)) was approximately by two orders lower than that of $CuCl_2{\cdot}2H_2O$ and $Cu(CH_3COO)_2{\cdot}H_2O$. As mentioned above, the lower inhibitory effect of both types of Cu(II)-chelate complexes probably resulted from their higher stability in aqueous solutions.

The IC_{50} values of six molecular Cu(II) complexes with additional organic ligands and *N*-salicylidene-β-alaninato(2-) ligand related to reduction of chlorophyll content in *C. vulgaris* varied in the range from 21.0 μmol dm^{-3} (Cu(sal-β-ala)(quin)$\cdot H_2O$) to 58.7 μmol dm^{-3} (Cu(sal-β-ala)(tu)) and the antialgal activity decreased in the following order: Cu(sal-β ala)(quin)$\cdot H_2O$ > Cu(sal-β-ala)(im) > Cu(sal-β-ala)(pz)$\cdot 2H_2O$ > Cu(sal-β-ala)(py) > Cu(sal-β-ala)(ur) > Cu(sal-β-ala)(tu). The applied amino acid strongly affected the antialgal activity of Cu(II) cuprates against *C. vulgaris* and it decreased in the following order: β-alanine, α-alanine, phenylalanine, valine. The IC_{50} values determined for compounds NH_4[Cu(sal-β-ala)(NCS)] and $Na_4[Cu_2$(sal-β-ala)$_2(NCS)_2](SCN)_2{\cdot}4H_2O$ (40.8 and 37.4 μmol dm^{-3}, respectively) were about two times lower than for K[Cu(sal-DL-α-ala)(NCS)] and five times lower than the corresponding IC_{50} value estimated for K[Cu(sal-DL-α-ala)(NCO)] (82.5 and 198.3 μmol dm^{-3}, respectively) [216].

In M(II) nicotinamide complexes $M(L)_2(nia)_2$ (M=Cd or Zn) which contain coordinated molecules of nicotinamide and anionic ligands L, where L is CH_3COO^-(ac) and NCS^-, nicotinamide molecule is coordinated to M(II) atom through the ring nitrogen atom as a monodentate ligand and anionic ligands are also monodentate coordinated to M(II) atom, whereby acetate anion acts as *O*-donor ligand and NCS^-anion as *N*-donor ligand [217]. The IC_{50} values related to PET inhibition in spinach chloroplasts determined for $Zn(NCS)_2(nia)_2$, $Cd(NCS)_2(nia)_2$, and $Cd(ac)_2(nia)_2$ (4.82, 4.44, and 6.64 mmol dm^{-3}, respectively) were greater than that of $CdCl_2{\cdot}2.5H_2O$ (IC_{50} = 1 mmol dm^{-3}). The IC_{50} value for $Zn(ac)_2(nia)_2$ could not be determined and treatment with 6.64 mmol dm^{-3} of $Zn(ac)_2(nia)_2$ resulted only in

25% PET inhibition with respect to the control (application of 5.26 mmol dm^{-3} of $ZnCl_2$ led to 75% PET inhibition). It is evident that in comparison with acetate ligands the NCS^- ligands contributed to enhanced toxicity of $M(L)_2(nia)_2$ complexes and compounds with Cd as the central atom were more toxic than those with Zn compounds with the same ligands. Moreover, it could be assumed that during the experiment the ligands L and nicotinamide probably were not replaced by the "biological ligands" (amino acid residues) occurring in the proteins of spinach chloroplasts. Similar results related to toxicity of above-mentioned Cd and Zn compounds were obtained also in experiment focused on reduction of chlorophyll content in *C. vulgaris* [217]. Application of 100 µmol dm^{-3} of $M(L)_2(nia)_2$ resulted in reduced dry mass of roots and shoots of hydroponically cultivated maize plants exposed to metal compounds for 7 days related to the untreated control plants, whereby the root dry mass was inhibited to a greater extent than the shoot dry mass with the exception of $Zn(ac)_2(nia)_2$ which did not affect shoot dry mass. The presence of NCS^- ligands caused enhanced toxicity of $Zn(NCS)_2(nia)_2$ complex comparing to the effect of $Zn(ac)_2(nia)_2$. The toxicity of similar $Cd(L)_2(nia)_2$ complexes was greater than that of complexes with Zn as central metal atom. In general, treatment with $M(ac)_2(nia)_2$ resulted in better accumulation of metals in the individual plant organs. However, while the structure of L ligands in $Cd(L)_2(nia)_2$ did not affect Cd content in leaves, application of $Zn(ac)_2(nia)_2$ led to approximately four times higher Zn content in maize leaves than application of $Zn(NCS)_2(nia)_2$. In maize plants treated with $Zn(L)_2(nia)_2$ complexes besides estimation of Zn content in the plants also the content of two essential metals Mn and Cu was determined and the more toxic effect of NCS^-ligands related to that of acetate ligands was reflected also in significantly lowered amounts of Mn and Cu in roots, stems, and leaves of maize plants treated with $Zn(NCS)_2(nia)_2$ compared with $Zn(ac)_2(nia)_2$ [217].

The IC_{50} values related to OER inhibition in *C. vulg*aris determined for $ZnCl_2{\cdot}H_2O$ and a set of carboxylato and halogenocarboxylato zinc(II) compounds ranged from 0.112 to 1.362 mmol dm^{-3} and the inhibitory activity decreased in the following order: $Zn(BrCH_2COO)_2 > ZnCl_2{\cdot}H_2O > Zn(ICH_2COO)_2 > Zn(ClCH_2CH_2COO)_2 > Zn(ClCH_2COO)_2{\cdot}2H_2O > Zn(CH_3CH_2COO)_2 > Zn(CH_3CH_2CH_2COO)_2 > Zn(CH_3COO)_2{\cdot}\ 2.5H_2O > Zn((CH_3)_2CHCOO)_2 > Zn(HCOO)_2$ indicating a quasi-parabolic course of OER-inhibiting activity on the lipophilicity of carboxylato zinc(II) complexes [218]. It could be noted that the branching of the alkyl chain was connected with decreased solubility of the compound and resulted in decreased biological activity, while the introduction of halogene substituent (Cl or I) into ligand led to activity increase. The above-mentioned Zn(II) compounds also inhibited growth of *Sinapis alba* roots, whereby the inhibitory activity of the Zn(II) compounds containing $RCOO^-$ ligands was higher ($IC_{50} = 0.033$–2.147 mmol dm^{-3}) than that of $ZnCl_2{\cdot}H_2O$ ($IC_{50} = 2.971$ mmol dm^{-3}) and the halogenocarboxylato zinc(II) complexes exhibited higher inhibitory activity than the corresponding carboxylato zinc(II) compounds [218]. At application of 1 mmol dm^{-3} of $ZnCl_2{\cdot}H_2O$ and Zn(II) complexes $Zn(CH_3COO)_2{\cdot}2.5H_2O$, $Zn(CH_3CH_2COO)_2$, $Zn(ClCH_2CH_2COO)_2$ and $Zn(CH_3CH(Cl)COO)_2$ on hydroponically cultivated *Zea* mays L. (c.v. Karolina) shoot length reduction was observed and two compounds, $Zn(ClCH_2CH_2COO)_2$ (with Cl substituent bonded on β-carbon) and $ZnCl_2 \cdot H_2O$, caused also strong reduction of root dry mass. The presence of Cl-substituent on α-carbon in $Zn(CH_3CH(Cl)COO)_2$ led to significant activity decrease. On the other hand, treatment with studied compounds at concentration 1 mmol dm^{-3} practically did not affect root dry mass (with the exception of $Zn(CH_3CH(Cl)COO)_2$, which caused an increase of root dry mass, comparing to the effect of equimolar $ZnCl_2 \cdot H_2O$). The bioaccumulated metal content in roots, stems, and leaves of maize plants depended on the applied carboxylato and halogenocarboxylato zinc(II) compounds, it increased with their increasing concentrations and the effectiveness of metal translocation from roots into the shoots was connected with the structure, as well as the applied external concentration of Zn(II) compounds. In general, BAF values related to roots, stems, and leaves determined for $ZnCl_2 \cdot H_2O$ were higher than those determined for Zn(II) complexes $Zn(CH_3COO)_2 \cdot 2.5H_2O$, $Zn(CH_3CH_2COO)_2$, $Zn(ClCH_2CH_2COO)_2$ and $Zn(CH_3CH(Cl)COO)_2$ and it could be concluded that ionic compound $ZnCl_2 \cdot H_2O$ more easily penetrated into the roots and its translocation in maize plants is also effective. Moreover, it could be assumed that free Zn(II) ions form complexes with organic acids occurring in the plant, and Zn will be translocated into the above-ground part of the plant in the form of Zn-malate, Zn-citrate, etc. [209].On the other hand, TF values corresponding to the ratio of accumulated metal amount in shoots and roots (which sharply decreased with increasing concentration of Zn(II) compounds) estimated for the concentration range 10^{-5}–10^{-3} mol dm^{-3} were as follows: 0.679–0.484 for $ZnCl_2 \cdot H_2O$, 0.606–0.102 for $Zn(CH_3COO)_2 \cdot 2.5H_2O$, 0.954–0.134 for $Zn(CH_3CH_2COO)_2$, 1.195–0.311 for $Zn(ClCH_2CH_2COO)_2$ and 1.193–0.069 for $Zn(CH_3CH(Cl)COO)_2$ [218].

The IC_{50} values of the Ni(II) compounds of the type NiX_2L_4 (where X = Cl, NCS or bromoacetate (Brac); L = nicotinamide (nia), 3-hydroxymethylpyridine (ron), imidazole (iz) and 4-methylpyridine (4-pic)) concerning inhibition of mustard (*Sinapis alba* L.) roots were estimated in the range from 0.414 to 0.944 mmol dm^{-3} and increased in the following order: $NiCl_2 \cdot 6H_2O < Ni(NCS)_2(\text{ nia})_4 < Ni(NCS)_2(iz)_4 < Ni(BrCH_2COO)_2(nia)_4 < Ni(NCS)_2(ron)_4 < NiCl_2(ron)_4 < Ni(NCS)_2(\gamma\text{-pic})_4$. The inhibitory activity of Ni(II) complexes markedly depended on the structure of *N*-donor ligands L (ron, iz, nia, γ-pic), whereby the lowest inhibitory activity of $Ni(NCS)_2(\gamma\text{-pic})_4$ could be connected with the fact that the ligand 4-methylpyridine do not form supplementary H-bonds with suitable "biological" ligand in the cell. On the other hand, the formation of H-bonds between $–CONH_2$ group of nicotinamide ligand or NH group of imidazole ligand and suitable target sites of proteins is highly probable and these interactions result in plant growth reduction. The root growth-inhibiting activities of $NiCl_2(ron)_4$ and $Ni(NCS)_2(ron)_4$ were similar indicating minor effect of X ligand on the biological activity. Lowered inhibitory activity of $Ni(BrCH_2COO)_2(nia)_4$ with respect to that of $Ni(NCS)_2(nia)_4$ could be connected with more lipophilic X ligands ($BrCH_2COO^-$) causing decrease of aqueous solubility of this Ni(II) compound as well as its limited transport through hydrophilic regions of the cell membranes [219]. Using EPR spectroscopy it was found that above-mentioned Ni(II) compounds inhibited PET in spinach chloroplasts due to interactions with Z^+/D^+ intermediates, i.e., with tyrosine cation-radicals Tyr_Z and Tyr_D situated in D_1 and D_2 proteins on the donor side of PS II and with the manganese cluster in the oxygen evolving complex, as well [113]. The Ni(II) complexes applied at higher concentration inhibited shoot growth of maize (cv. Karolina) more than root growth; the highest reduction of dry mass of stems and leaves of maize was observed for the treatment with two compounds with NCS^- ligands, namely $Ni(NCS)_2(nia)_4$ and $Ni(NCS)_2(iz)_4$. Based on the comparison of the effects of tested Ni(II) compounds applied in concentrations 250 and 10 μmol dm^{-3} on dry mass of maize leaves of hydroponically cultivated plants, it could be concluded that the most toxic effects were exhibited by $Ni(NCS)_2(nia)_4$ and $Ni(NCS)_2(iz)_4$ which reduced dry mass of leaves already at relatively low concentration (10 μmol dm^{-3}). On the other hand, application of 10 and 100 μmol dm^{-3} $NiBr_2(nia)_2$ had stimulating effect on growth of primary root of maize plants [220].

Using EPR spectroscopy effect of three iron compounds, $FeCl_3{\cdot}6H_2O$, $[Fe(nia)_3Cl_3]$ and $[Fe(nia)_3(H_2O)_2](ClO_4)_3$ on PET in spinach chloroplasts was studied and it was found that due to the interaction of these compounds with tyrosine radicals Tyr_Z and Tyr_D located at the donor side of PS II, electron transport between the photosynthetic centers PS II and PS I was interrupted and the treatment with $[Fe(nia)_3(H_2O)_2](ClO_4)_3$ resulted also in a release of Mn(II) ions from the oxygen-evolving complex situated on the donor side of PS II [221]. An adverse effect of iron stress on the photosynthetic electron transport was observed previously by several researchers (e.g., [222, 223]). Application of 1 mmol dm^{-3} of Fe(III) compounds inhibited the primary root growth of maize much more than shoot growth and the growth of adventitious roots as well as root hairs was suppressed [221]. However, at this concentration expressive reduction of leaf dry mass was estimated, $[Fe(nia)_3(H_2O)_2](ClO_4)_3$ being the most toxic. In this compound the ClO_4^{2-} anions are not bound to the Fe atom by a coordination bond and it can be assumed that they exhibit toxic effects, whereby it could be supposed that water molecules in the coordination sphere of this complex can easily be substituted by another ligands such as residues of amino acids in proteins. Treatment with $[Fe(nia)_3Cl_3]$ resulted also in significantly higher Fe concentration in plant roots than application of $[Fe(nia)_3(H_2O)_2](ClO_4)_3$ and $FeCl_3{\cdot}6H_2O$.

Previously, it was reported that also alga *Scenedesmus quadricaud*a cells accumulated 2.7–19.6 times higher Fe amounts in cells after treatment with iron complexes compared to the inorganic salt $FeCl_3{\cdot}6H_2O$ [224]. On the other hand, leaf Fe concentration of all three above-mentioned Fe(III) compounds was higher than that in stems, therefore it could be assumed that they substitute their H_2O ligands and form complexes with organic amino acids occurring in the cell and these complexes secure the mobility of Fe within the plant. The TF values estimated for the treatment with 0.25, 0.5, and 1.0 mmol dm^{-3} decreased in the following order: $FeCl_3{\cdot}6H_2O$ (0.277, 0.265, 0.143) > $[Fe(nia)_3(H_2O)_2](ClO_4)_3$ (0.142, 0.189, 0.137) > $[Fe(nia)_3Cl_3]$ (0.046, 0.038, 0.066) [221].

7.3 Medicinal Plants

There are many hundreds of medicinal plants that can be grown in temperate climates and there are probably a great deal more with properties as yet undiscovered. Medicinal plants are thus potential plant factories for new natural drugs. Much more research needs to be carried out on a whole range of medicinal plants in order to find safer, more holistic alternatives to the synthetic drugs so often used nowadays. Moreover, medicinal species are widely used not only in pharmaceutical but also in food and cosmetics industries, then higher content of toxic metals in their organs is undesirable. Thus, it is inevitable to have sufficiency information concerning the effect of both bioelements and toxic substances on this important group of the plants.

7.3.1 Effect of Toxic Metals and Bioelements on Medicinal Plants

Because heavy metals may be introduced into medicinal plant products through contaminated agricultural resources and/or poor production practices, it is necessary to document levels of toxic metals in herbs extensively used in preparation of products and standardized extracts and investigate whether some species can accumulate toxic elements exceeding permissible levels proposed by the World Health Organization and European Pharmacopoeia (e.g., [225–228]).

Some regions in Eastern Slovakia regions are traditionally used for commercial chamomile cultivation in field conditions, therefore continuous control of heavy metals content in their shoots utilized for therapeutic purposes is indispensable. Therefore, we collected and analyzed data concerning Cd accumulation in chamomile plants cultivated in Eastern Slovakia regions in field conditions [229]. From data related to the period 1999−2001 that were obtained in four investigated localities (Streda nad Bodrogom, Košice, Michalovce, Nová Lubovňa), it was found that the highest Cd level in anthodia (0.168 ± 0.078 mg kg^{-1}) was estimated in plants growing in the locality Streda nad Bodrogom where the lowest Cd content in the soil was estimated (0.111 ± 0.042 mg kg^{-1}) and the resulting BAF was 1.514. Because for further three localities in which Cd concentration in soil was in the range from 0.222 to 0.335 mg kg^{-1} and the BAF values ranged from 0.676 to 0.234 it is evident that Cd translocation was more effective from less contaminated soil. However, Cd content in anthodium of chamomile plants cultivated and collected in different localities of Eastern Slovakia in the period 1995–2002 showed significant fluctuations in individual years with extreme values in years 2001 (0.003 mg Cd kg^{-1}) and 2000 (0.505 mg Cd kg^{-1}) suggesting considerable impact of actual climatic relations on the metal uptake and accumulation. This was confirmed by the linear increase of Cd content in chamomile anthodium dry mass with the increase of mean hydrothermic coefficient of Seljaninov (HC; an integrated index of hydrothermic parameters taking into account the total amount of precipitations and thermal sum of the mean daily temperatures exceeding 10 °C in the investigated period) [230] for the period April–June evaluated from the data measured in the meteorological stations Streda nad Bodrogom and Michalovce in years 1999−2002. In our pot experiments using Cd concentration range in the soil 0–20 mg kg^{-1}, the Cd content in shoots and roots of chamomile plants was higher than that in anthodia; however, in both cases it increased linearly with increasing Cd concentration in the soil. The decreased bioavailability of Cd bound to soil particles was reflected in lower BAF values compared to those determined in experiments in which plants were cultivated hydroponically in the presence of soluble Cd salt (e.g., [231]). Moreover, Grejtovský et al. [232] reported that Cd accumulation in anthodia of chamomile plants cultivated on naturally contaminated soil is lower than in those which were cultivated in artificially contaminated substrates: anthodia of plants cultivated on naturally contaminated soil contained only 17% Cd from the total accumulated Cd amount in the plant, while anthodia of plants cultivated in pot experiments using additional Cd contamination accumulated till 33.25%, whereby not even the highest applied Cd dose (20.0 mg kg^{-1}) exhibited toxic effect on chamomile biomass or any visible damage of plants. In general, it should be stressed that Cd bioavailability does not depend only on plant species but it is influenced by many factors, such as pH, organic substances in soil, mechanical composition of soil, redox potential, cation exchange capacity, etc. (in detail see [233, 234]).

Pavlovič et al. [231] investigated the effect of Cd on physiological and production characteristics in two tetraploid cultivars of *Matricaria recutita* (cv. Goral and cv. Lutea) in response to the uptake and accumulation of Cd under different cultivation conditions. The concentration gradient that was used in the experiments reflects Cd content in the soil from non-contaminated to highly contaminated sites [235]. Seeds were germinated and grown in soil in growth chamber under standard conditions: 25 °C, 80% relative humidity and 100 μmol m^{-2} s^{-1} PAR irradiance with day/night photoperiod of 16 h light/8 h dark. Fourteen days after germination, 20 seedlings of each variant were washed to remove the soil adhering to the roots and primary root length was measured. The filter papers with the plants were coiled, put into flask and 2 cm submerged in Hoagland solution with the following Cd concentrations: 3, 6, 12, 24, 60 μmol dm^{-3} $Cd(NO_3)_2 \times 4H_2O$ p.a. The plants growing in Hoagland solution without Cd served as control variant. The plants were cultivated at 25 °C, 80% relative humidity and 200 μmol m^{-2} s^{-1} PAR for 7 days. Primary root length, root and shoot dry mass, as well as Cd content in plant organs were determined after 7 days of treatment. In another experiments the older plants were grown in the greenhouse conditions in the soil for 7 weeks after germination. Then, the plants were washed in water and transferred to hydroponic Hoagland solutions (control) and Hoagland solution containing above-described Cd concentrations as well as cultivation conditions. Root and shoot dry weight and Cd content in plants organs were determined after 7 days of exposure. Significant inhibition of root growth was observed in both chamomile cultivars after Cd treatment. Fragility, browning, and twisting of roots were also observed. In shoots leaf roll, chlorosis and leaf growth inhibition occurred. During the root test chamomile plants cv. Goral formed the anthodia in all concentrations except control, despite the fact that the plants were only 3 weeks old. From 4 to 5 weeks earlier blossoming under Cd administration was also recorded for Cd hyperaccumulator *Arabidopsis halleri* [236]. The plants growing in paper rolls exceeded the limit of Cd hyperaccumulator (100 μg g^{-1}) sensu Baker [237] at 60 μmol dm^{-3} Cd in solution. Grejtovský and Pirč [234] also found over 100 μg g^{-1} d.w. in shoots of plants growing in contaminated substrates. Experimental plants growing in hydroponic solution exceeded the threshold for Cd hyperaccumulator at 6 μmol dm^{-3} Cd and accumulated up to five times more Cd in the shoots than the plants growing in paper rolls. It should be stressed that 12 μmol dm^{-3} cadmium concentration in hydroponic solution represents strong contaminated soil [235]; however, the Cd effect on plant was stronger in comparison to the soil, because Cd ions are not bound to the soil particles and so all ions are available for plant uptake. The measurements confirmed higher inhibition of photosynthesis in cv. Lutea (70% of control, $P = 0.01$) although these plants accumulated less Cd than the plants of cv. Goral (75% of control, $P = 0.05$). Similar decrease of shoot dry weight (72% of control) in both cultivars was also detected. Decrease of net photosynthetic rate could be due to structural and functional disorders in many different levels. In both chamomile cultivars shoot and root respiration rates were not changed significantly. Considering all found results it was concluded that this plant species exhibited high tolerance to Cd treatment. Since Cd application induced higher production of specific secondary metabolites in chamomile plants such as α-bisabolol [238], polyacetylenes ene-yne-dicycloethers and sesquiterpene (E)-β-farnesene [239], herniarine, umbelliferone [240], these substances could play a supplementary role in detoxification mechanism induced by Cd. This additive defensive mechanism was also confirmed by Kráľová and Masarovičová [241] for cadmium and *Hypericum perforatum* L. and secondary metabolites hypericin, pseudohypericin (naphthodianthrone derivates) and quercetin that are produced by this medicinal plant. Similarly, Palivan et al. [242] observed formation of copper complexes with hypericin.

Within investigation of metal impact on *M. recutita* plants, we focused our attention also on the effect of the stage of ontogenetical development on growth of chamomile plants and accumulation of supplied cadmium and copper in plant organs [243]. Seeds of *M. recutita* L., cv. Bona were sown on the soil in two agrotechnical date: March 30 (experiment A) and May 28 (experiment B). Plants from experiment A were grown 12 weeks in the test room at daylight, while those of experiment B were cultivated only 8 weeks. Then the plants were placed into hydroponics where they were cultivated 7 days at controlled conditions: control and variants 6, 12, and 24 μmol dm^{-3} $CdSO_4$ or $CuSO_4$, all in Hoagland solution and thereafter some production characteristics and metal content in plant organs were estimated. Plants of cv. Bona were found to be tolerant against application of $CdSO_4$ and $CuSO_4$ because more expressive toxic effects were observed until at application of the highest metal concentration (24 μmol dm^{-3}). However, it could be noted that while the shoot dry mass of plants cultivated in both experiments was comparable, root dry mass of plants (control as well as Cd (Cu) variants) from experiment A was approximately twofold than that from experiment B. However, in this context it could be mentioned that plants grown in soil in experiment B were exposed to higher levels of irradiation.

Cd and Cu accumulation in both plant organs increased with increasing metal concentration in hydroponics, whereby in plant roots it was considerably higher than in the shoots. Elder, app. 3-month-old chamomile plants from experiment A, originating from sowing in agrotechnical date, showed higher physiological activity and accumulated in plant tissues higher Cd amounts than 2-month-old plants from experiment B, which were sown later (May), thus after the agrotechnical date.

The BAF values related to Cd accumulation in roots were in the range 2177–2752 for plants from experiment A and 1831–2171 for plants from experiment B, whereas the BAF values related to Cu accumulation in roots ranged from 1124 to 1836 (experiment A) and from 1273 to 1578 (experiment B), respectively. The BAF values related to Cd accumulation in shoots showed exponential decrease with increasing metal (Cd or Cu) concentration.

In contrast to BAF values related to metal accumulation in roots which were only slightly higher for Cd compared to Cu, the BAF values related to metal accumulation in shoots estimated for Cd were approximately by one order higher than those determined for Cu. In the case of Cd they ranged from 266.9 to 441.7 (experiment A) and from 246.3 to 355.3 (experiment B), while the corresponding BAF ranges for Cu were 13.2–45.6 (experiment A) and 13.6–35.8, respectively. These results confirmed our previous findings [244] concerning markedly higher Cd mobility in chamomile plants. The values of translocation factor evaluated for Cd were in the range 1.154–0.68 (experiment A) and 0.568–0.353 (experiment B), while for Cu the TF values were estimated in the range 0.134–0.067 (experiment A) and 0.079–0.048 (experiment B), respectively. While at plants exposed to the lowest metal concentration (6 μmol dm^{-3}) within experiment A the fraction of accumulated metal allocated in shoots related to the total amount of metal accumulated by plants was >50% Cd and 11.9% Cu, in plants from experiment B the corresponding values were lower, namely 36.2% Cd and 4.5% Cu, respectively. Significantly higher Cd fraction in shoots of plant cultivated within experiment A was observed also at application of higher $CdSO_4$ concentrations, while at application of higher $CuSO_4$ concentrations the differences were lesser. Thus, it can be concluded that physiologically more active shoots exhibited higher attraction power due to more intensive transpiration flow (e.g., [245]).

For plants from experiment B the ratio of root dry mass to shoot dry mass ranged from 0.323 to 0.352, while the corresponding range for plants cultivated in experiment A was only 0.142–0.171. This was reflected probably in lower tolerance of plants from experiment A against Cd-induced stress what led to enhanced extent of Cd accumulation in both plant organs. High Cd fraction allocated in shoots related to the total Cd amount accumulated by plants was significantly affected by the fact that in plants from experiment A shoot dry mass of Cd-treated plants was 5.86–7.06-fold higher compared with root dry mass, while for plants from experiment B the ratio of shoot dry mass to root dry mass was only 2.84–3.10.

Based on the above-discussed results it could be concluded that *M. recutita*, cv. Bona is relatively tolerant against Cd-induced stress, however the translocation of this metal from roots to shoots is significantly affected by intrinsic factors (ontogenetic development, redistribution of assimilates between root and shoot) as well as external factors such as date of outplanting or cultivation conditions (especially temperature affecting the transpiration flow).

Interesting results were found by Masarovičová et al. [246] who investigated the effect of cadmium (6–240 μmol Cd dm^{-3}) on root and shoot dry mass, length of root and shoot and root respiration rate of 6 weeks old plants of *Chamomilla recutita* and *Hypericum perforatum.* It was found that root dry mass of both studied medicinal plants was the most inhibited parameter, whereas the length of main root was not strongly affected. This fact could be explained with significant reduction of lateral roots and root hairs by cadmium treatment. Stress-induced higher respiration rate of metal-treated plants correlated with root growth inhibition what was reflected in lower value of root dry mass. Relatively high Cd uptake into the root required increased energy costs coming from root respiration rate. Both studied species accumulated in the shoot high Cd concentrations without evident negative effect on their growth and dry mass production. Based on found results it seems that both medicinal plants could have application in rehabilitation and recovering of cadmium contaminated sites. Similar findings were earlier published by Kráľová et al. [247] for older (6 months old) plant of *Hypericum perforatum* cultivated hydroponically: control variant in 0.05% $Ca(NO_3)_2$ and Cd treated variant in 0.05% $Ca(NO_3)_2$ with 12 μmol dm^{-3} $Cd(NO_3)_2$ (pH = 5.5). The root dark respiration rate of the Cd-treated plants was faster than in control plants. The highest Cd concentration was determined in the root (1792 μg g^{-1} d.w.) compared with the leaves (290 μg g^{-1} d.w.) or stem (220 μg g^{-1} d.w.). Moreover, Cd supported the release of some bioelements (Mn, Fe, and Cu) from the membranes in both, the stem as well as root. Consequently, ions of these bioelements were transported into the leaves where their higher content was estimated.

Kummerová et al. [248] studied effects of zinc (12–180 μmol dm^{-3}) alone and in mixtures with 12 μmol dm^{-3} Cd on metal accumulation, dry masses of roots and shoots, root respiration rate, variable to maximum fluorescence ratio (F_V/F_M), and content of photosynthetic pigments in hydroponically cultivated chamomile (*Matricaria recutita*) plants. The content of Zn in roots and shoots increased with increasing external Zn concentration and its accumulation in the roots was higher than that in the shoots. While at lower Zn concentrations (12 and 60 μmol dm^{-3}) the presence of 12 μmol dm^{-3} Cd decreased Zn accumulation in the roots, treatment with 120 and 180 μmol dm^{-3} Zn together with 12 μmol dm^{-3} Cd caused enhancement of Zn content in the root. Presence of Zn (12–120 μmol dm^{-3}) decreased Cd accumulation in roots. On the other hand, Cd content in the shoots of plants treated with Zn + Cd exceeded than in the plants treated only with 12 μmol dm^{-3} Cd. Only higher Zn concentrations (120 and 180 μmol dm^{-3}) and Zn + Cd mixtures negatively influenced dry mass, Chls and Cars content, F_V/F_M and root respiration rate. Chl *b* was reduced to a higher extent than Chl *a*.

In the paper of Owen et al. [249], an “expected” range for 16 elements (Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pt, Sr, Y, and Zn) in *Hypericum perforatum* dry herb and processed preparations (tablets and capsules) was determined. The major elemental constituents in the analyzed samples were Ca (300–199,000 μg g^{-1}), Mg (410–3530 μg g^{-1}), Al (4.4–900 μg g^{-1}), Fe (1.154–760 μg g^{-1}), Mn (2.4–261 μg g^{-1}), Sr (0.88–83.6 μg g^{-1}), and Zn (7–64 μg g^{-1}). The application of PCA (Principal Component Analysis) to the elemental profiles for the analyzed samples clearly differentiated the dry herb samples from the processed samples with additional differentiation between tablets and capsules. A reduction in the average concentration of B, Ba, Cd, Ni, and Mn occurred post formulation and it has been postulated that this could be due to factors such as the extraction process and/or powder dilution. Higher levels of Ca and Mg found in processed products were identified as expected, but higher levels of Cr, Y, and Sr were also found, which could be due to contamination from metal alloys used in the manufacturing process. PCA model identified a 7-element fingerprint (Ba, Ca, Cd, Fe, Ni, Sr, and Y) capable of differentiating between the three categories of investigated products of *H. perforatum*. Results indicating sample forms (i.e., herb, tablet, and capsule) were differentiated by a change in the elemental profile contributed by excipient addition, dilution, and/or the extraction process.

7.3.2 Effect of Metal Chelating Agents on Medicinal Plants

Mobilizing amendments such as chelating and desorbing agents increase the bioavailability and mobility of metal(loid)s, whereby mobilizing agents can be used to enhance the removal of heavy metal(loid)s through plant uptake and soil washing [250] and thus chelator-enhanced phytoextraction of heavy metals is an emerging technological approach for a non-destructive remediation of contaminated soils. The presence of ethylenediaminotetraacetic acid (EDTA) in soil can alter the mobility and transport of toxic metals such as Zn, Cd, and Ni due to the formation of water soluble chelates, thus increasing the potential for metal pollution of natural waters. However, due to environmental persistence of EDTA in combination with its strong chelating abilities in phytoextraction increasingly less aggressive alternative strategies such as the use of organic acids or more degradable aminopolycarboxylic acids are preferred [251, 252].

We investigated the effect of EDTA on bioaccumulation of Cu, Zn, and Cd with 2-month-old *Matricaria recutita* L. plants (cv. Goral) cultivated in hydroponics and exposed for 7 days to 12, 24, and 60 μmol dm^{-3} of individual metals alone or with addition equimolar EDTA concentration [244]. The application of tested compounds without and with EDTA practically did affect neither the length nor the dry matter of roots and shoots of *M. recutita.* The metal content in plant organs increased with increasing metal concentration in the hydroponic solution, whereby accumulation of all tested metals in roots was higher than in the shoots. Addition of equimolar EDTA concentration resulted in significant decrease of bioaccumulated Cu and Zn amount in plant roots, whereas sharp increase of Cu shoot concentration was observed, while Cd shoot concentration was elevated only slightly and Zn concentration showed even a moderate decrease. For treatment with the lowest metal concentration (12 μmol dm^{-3}) the fraction of metal accumulated in the shoots from the total metal amount accumulated by plant without and with EDTA addition increased from 16.9 to 33.5% for Cd, from 44.06 to 65.04% for Zn, and from 12.46 to 34.41%

for Cu. Independently on the concentration of applied metal, the addition of equimolar EDTA concentration caused that more than 60% Zn and more than 30% Cd accumulated by chamomile plants was allocated in the shoots, while the enhancement of shoot metal contents in plants treated with higher Cu concentration due to application of EDTA was dramatic, from 5.2 to 45.2% at 24 μmol dm^{-3} and from 4.8 to 59.2% at 60 μmol dm^{-3}. The above-mentioned results indicate that the effect of EDTA on metal bioaccumulation in roots and shoots of chamomile plants depends on the applied metal (Cd, Zn, or Cu) and is closely connected with values of stability constants of corresponding metal chelates with EDTA. Very efficient translocation of Cu into the shoots observed at the presence of EDTA could be connected with the largest value of stability constant of Cu-EDTA chelate ($\log K_1 = 18.8$) which is more than 100 times higher than the corresponding stability constants for Cd-EDTA ($\log K_1 = 16.36$) and Zn-EDTA ($\log K_1 = 16.5$) chelates [253].

In an another experiment we compared the effect of $ZnSO_4$ without and in the presence of equimolar EDTA as well as zinc acetate applied in a wide concentration range (12–120 μmol dm^{-3}) on zinc accumulation in 7-week-old chamomile plants (cv. Goral) exposed for 7 days to Zn compounds. Within this concentration range the fraction of Zn allocated in the shoots from total metal amount accumulated by plant depended on the applied Zn form and was as follows: 30–45% for $ZnSO_4$, 35.2–54.4% for $Zn(CH_3COO)_2$ and 62–70% for $ZnSO_4$-EDTA application. Significantly lower effect of zinc acetate on Zn accumulation in the shoot is connected with very low stability constant of Zn with acetic acid ($\log K_1 = 1.03$) [254]. Reduced Zn accumulation in roots at EDTA application and at a much lower extent also at $Zn(CH_3COO)_2$ application can be explained by suppressed transport of the formed complexes through plasmalemma of root cells, whereby specific binding of these complexes on cell walls does not occur like to binding of $ZnSO_4$.

A further set of our experiments was focused on the study of effects of $CuSO_4$, copper salicylate tetrahydrate ($[Cu(sal)_2\cdot(H_2O)_2]_2\cdot H_2O$) and seven copper(II) chelates: copper *N*-pyruvidene-*β*-alaninate trihydrate ($[Cu(pyr\text{-}\beta\text{-}ala)(H_2O)_2]H_2O$), potassium salicylidene-L-glutamatecuprate(II) dehydrate ($K^+[Cu(sal\text{-}L\text{-}glu)]\cdot 2H_2O$) as well as pyruvideneglycinatocopper(II) complexes with additional molecular *S*-donor ligands, urea and ethylenethiourea ($Cu(pyrgly)(urea)(H_2O)$; $Cu(pyrgly)(ettu)(H_2O)$), *N*-donor ligands, pyridine and aniline ($Cu(pyrgly)(py)(H_2O)_2$; $Cu(pyrgly)(anil)(H_2O)$), and *O*-donor ligands, H_2O ($[Cu(pyrgly)(H_2O)_3]$) applied at concentration 24 μmol dm^{-3} on dry mass and Cu accumulation in plant organs of 2 months old plants of *Matricaria recutita* (var. Goral) which were exposed to metal compounds for 7 days [255]. At the applied concentration 24 μmol dm^{-3} the studied Cu compounds did not affect significantly the length of roots and shoots of chamomile plants. In general, Cu was allocated predominantly in chamomile roots, however application of Cu in the form of chelate led to more effective Cu translocation into the shoots in comparison to $CuSO_4$ treatment. The BAF values for $CuSO_4$ related to roots and shoots were 3141 and 18.0, respectively, while for Cu chelates the BAF values related to roots ranged from 737 ($Cu(pyrgly)(py)(H_2O)_2$) to 2524 ($[Cu(pyr\text{-}\beta\text{-}ala)(H_2O)_2]\cdot H_2O$) and those related to shoots from 14.4 ($Cu(pyrgly)(urea)(H_2O)$) to 21.6 ($[Cu(pyrgly)(H_2O)_3]$). The fraction of Cu allocated in the shoots from total metal amount accumulated by chamomile plant decreased as follows: Cu(pyrgly)(py)$(H_2O)_2$ (7.3%) > [Cu(pyrgly)$(H_2O)_3$](6.4%) > Cu(pyrgly)(urea)(H_2O)(5.3%) > Cu(pyrgly)(ettu)(H_2O)(4.7%) > Cu(pyrgly)(anil)(H_2O)(4.3%) > [Cu(sal)$_2$·$(H_2O)_2$]·$2H_2O$(4.0%) > K^+[Cu(sal-L-glu)]·$2H_2O$(3.8%) > $CuSO_4 \cdot 5H_2O$(2.0%) > [Cu(pyr-β-ala)$(H_2O)_2$]·H_2O (1.2%). Hence, with the exception of [Cu(pyr-β-ala)$(H_2O)_2$]·H_2O the studied chelates accumulated in shoots more Cu than $CuSO_4 \cdot 5H_2O$, even though the stability constants (log K_1) related to Cu chelates with some amino acids, namely 19.2 for cysteine, 10.6 for histidine, 8.22 for glycine, 7.85 for glutamic acid, 7.13 for β-alanine and the corresponding log K_1 value for salicylic, citric acid and pyruvic acid (10.6, 6.1, and 2.2, respectively) are significantly lower than log K_1 for Cu-EDTA chelate (18.8) [253].

According to Nowack et al. [252], metal chelates with EDTA are taken up via the apoplastic pathway and disruption of the Casparian band is necessary to achieve the high metal concentrations in shoots. Therefore, adding chelators to a soil increases not only the total dissolved metal concentration but also changes the primary route of plant metal-uptake from the symplastic to the apoplastic pathway. The other synthetic chelators and low molecular weight organic acids were also found to be suitable for improving phytoremediation of metal-polluted soils (e.g., [256]). For example, application of chelator ethylenediaminetriacetic acid on the Cd-contaminated soil resulted in more than twofold increase of total Cd in *Calendula officinalis* [257], while the combinative treatment using Cd (30 mg kg^{-1}) + humic acid (2 g kg^{-1}) + EDTA (5 mmol kg^{-1}) caused maximum Cd-accumulation in root, shoot and flower of *C. officinalis* up to the extent of 115.96, 56.65 and 13.85 mg kg^{-1} and similar Cd contents in plant organs were achieved also with the treatment consisting of Cd (15 mg kg^{-1}) + humic acid (2 g kg^{-1}) + ethylenediaminedisuccinic acid (EDDS; 5 mmol kg^{-1}) [258].

7.3.3 Effects of Cadmium and Zinc Compounds Containing Se in Different Oxidation States on Crops and Medicinal Plants

Selenium is an essential nutrient for animals, microorganisms and some other eukaryotes and it has beneficial effects on vascular plants. The ability of some plants to accumulate and transform Se bioactive compounds has important implications for human nutrition and health, and for the environment [259]. However, high Se concentrations are phytotoxic [260–262]. The major selenocompound estimated in cereal grains, grassland legumes, and soybeans was found be selenomethionine [263].

The role of Se in stimulation and inhibition of plant growth in various agricultural crops as well as biofortification of some crops with Se using agronomic and genetic approaches is summarized in the review paper of Kaur et al. [264], while Sieprawska et al. [265] comprehensively reported about involvement of Se in protective mechanisms of plants under environmental stress conditions. Feng et al. [266] summarized the findings concerning implication of Se in the regulation of ROS and antioxidants, the inhibition of uptake and translocation of heavy metals, changes in the speciation of heavy metals, in rebuilding of the cell membrane and chloroplast structures and recovery of the photosynthetic system and the researchers suggested that Se could be involved also in regulation of the uptake and redistribution of elements essential in the antioxidative systems or in maintaining the ion balance and structural integrity of the cell and it may interfere with electron transport by affecting the assembly of the photosynthesis complexes. Increased total respiratory activity in leaves and flowers of Se-treated *Brassica* plants resulted in higher seed production [267].

The increase in growth of hydroponically cultivated mungbean (*Phaseolus aureus* Roxb.) plants due to treatment with Na_2SeO_4 resulted in significant stimulation of the activity of starch hydrolyzing enzymes-amylases and sucrose hydrolyzing enzyme-invertase which was associated with elevation of activities of sucrose synthesizing enzymes-sucrose synthase and sucrose phosphate synthase indicating that upregulation of enzymes of carbohydrate metabolism provided energy substrates for enhanced growth [268]. Owusu-Sekyere et al. [269] reported that carbohydrate metabolism upregulated by Se via altered redox potential may have some stimulatory effects on nodulation of alfalfa (*Medicago sativa* L.), a N_2-fixing plant of high nutritive value, which is an important forage legume for sustainable agriculture.

Although chemical properties of Se are similar to sulfur, and in plants they share common metabolic pathways and compete in biochemical processes affecting uptake, translocation, and assimilation pathways in plants, incorporation of Se instead of S can result in altered tertiary structure and dysfunction of proteins and enzymes, for example if selenocysteine is incorporated into proteins in place of cysteine [270, 271]. Lyi et al. [272] reported that due to reduction of selenate and selenite to selenide and subsequent coupling with *O*-acetylserine, selenoaminoacids (Se-cysteine and Se-methionine) are formed which can be non-specifically incorporated into proteins in place of cysteine and methionine and contribute to Se toxicity in Se non-accumulator plants.

Higher plants take up Se preferentially as selenate via the high-affinity sulfate permease [273]. Selenite is passively taken up into plants, while selenate enters plant cells through a process of active transport mediated by sulfate transporters and directly competes with uptake of sulfate [274–276]. Li et al. [277] found that the phosphate transporter competitively carries selenite in wheat. Sulfate was found to be involved in the root-to-shoot translocation of Se in *B. napus* supplied with selenate, but not selenite [278].

Selenate is the predominant form of bioavailable Se in oxic soils and selenite is more abundant in anoxic wetland conditions. The reduction of selenate to selenite appears to be a rate-limiting step in the Se assimilation pathway, since most plants supplied with selenate accumulate predominantly selenate, while plants supplied with selenite accumulate organic Se [279]. Plants can also volatilize methylated Se. While the enzyme ATP sulfurylase appears to be rate-limiting for the assimilation of selenate to organic Se, cystathionine-γ-synthase is rate-limiting for dimethylselenide volatilization [280]. Foliar application of selenite showed approximately 50%

less efficient accumulation of Se in shoots compared with selenate and both treatments exhibited a positive effect in particular on the level of reduced glutathione, whereby selenate-treated plants exhibited higher content of phytochelatin 3 in red clover (*Trifolium pratense* L.) [281].

In *Triticum aestivum* and Indian mustard (*Brassica juncea*) plants grown on seleniferous area of Punjab in India, the highest Se enrichment was estimated in the upper plant parts what corresponds to the high uptake rate and mobility of selenate within plants. Occurrence of dimethylselenide and methylselenocysteine in different plant parts indicated that active detoxication takes place via methylation and/or volatilization [282]. The organic Se raised Se concentrations in *Brassica napus* plants much less effectively than the inorganic selenite [283].

Morlon et al. [284] reported that the inhibition of growth of *Chlamydomonas reinhardtii* alga by selenite was linked to impairments observed at the subcellular level, whereby chloroplasts were the first target of SeO_3^{2-} cytotoxicity, with effects on the stroma, thylakoids and pyrenoids and Geoffroy et al. [285] demonstrated that selenate disrupts the photosynthetic electron chain in *C. reinhardtii* alga causing also ultrastructural damage (chloroplast alterations, loss of appressed domains).

Se can alleviate phytotoxic effects of heavy metals. Barrientos et al. [286] found that it is possible to counterbalance negative effects of Cd concerning growth inhibition, decreased concentration levels of essential micronutrients and oxidative damage by addition of Se. Addition of Se improved the dry weight of root and shoots, photosynthesis and stomatal conductance in Cd-treated hydroponically cultivated cucumber (*Cucumis sativus*) at early growth stage [287]. Treatment with Se or S alleviated Cd-induced oxidative stress by increasing proline accumulation as a result of increased activity of glutamyl kinase and decreased activity of proline oxidase and reduced ethylene level, increased the activity of glutathione reductase and glutathione peroxidase, reduced oxidative stress and improved photosynthesis and growth of wheat [288]. Application of Se alleviated Cd toxicity in pepper (*Capsicum annuum* L.) plants at the reproductive stage by restricting Cd accumulation in fruits and enhancing their antioxidant activity what resulted in improvement of the reproductive and stress tolerance parameters [289]. Beneficial effects of Se on different plants under Cd stress were also described by several other researchers (e.g., [290–292]). Arsenic-induced oxidative stress in roots and shoots of *Oryza sativa* L. was significantly ameliorated by Se supplementation through modulation of antioxidant enzymes and thiols [293] and exogenous Se application alleviated chromium toxicity by preventing oxidative stress in cabbage (*Brassica campestris* L. ssp Pekinensis) leaves [294].

Root elongation test is suitable to evaluate hazardous waste sites and to assess toxicity of metals (e.g., [295–297]), including metal nanoparticles [298, 299]. Chen et al. [300] who investigated roots of *Brassica rapa* under Se(IV) stress found that Se inhibits root elongation by repressing the generation of endogenous hydrogen sulfide in root tips. Application of H_2S donor NaHS resulted in the increase in endogenous H_2S and significantly alleviated Se(IV)-induced ROS over-accumulation, oxidative impairment, and cell death in root tips, which further resulted in the recovery of root growth under Se(IV) stress.

Lepidium sativum L. and *Sinapis alba* L. are sensitive test species widely used in the phytotoxicity testing of toxic metals because they are rapidly growing species and are cheap and easy to analyze (e.g., [19, 20]). Gomez-Ojeda et al. [301] investigated the effect of $CdCl_2$ and Na_2SO_3 treatment on *Lepidium sativum* L. and found that after exposure to both elements the changes in glyoxal and methylglyoxal concentrations were clearly attenuated as compared to a single stress or treatment and possible *in vivo* formation of CdSe quantum dots was also suggested.

To compare phytotoxic effects of Se(IV) and Se(VI), the effect of selenium oxoacids and some of their salts of the type $MSeO_3$ and $MSeO_4$ (where X = Cd, Zn, and Na_2) on length of roots and shoots of rapeseed (*Brassica napus* L.) after 72 h exposure of seeds in the dark at mean air temperature (25 ± 0.5 °C) was evaluated and the set of tested compounds was completed with three other Se-containing compounds, H_2SeO_3, an adduct of H_2SeO_4 with nicotinamide (H_2SeO_4.nia), and $Cd(NCSe_2)(nia)_2$ (in which oxidation state of Se is (–II), as well as with $CdSO_4$, $ZnSO_4$, and $Cd(NCS)_2(nia)_2$) [261]. With the exception of H_2SeO_3 and Na_2SeO_3 root growth of rapeseed seedlings was inhibited by tested compounds to a greater extent than the shoot growth. The inhibitory activity of the compounds containing Se in their molecules expressed by IC_{50} values varied in the range from 77 to 270 μmol dm^{-3} for root growth inhibition and from 134 to 710 μmol dm^{-3} for shoot growth inhibition, while that of their sulfur analogs ($CdSO_4$, $ZnSO_4$, and $Cd(NCS)_2(nia)_2$) was several times lower. As the most effective inhibitor $Cd(NCSe)_2(nia)_2$ was estimated with IC_{50} values 77 μmol dm^{-3} for root and 134 μmol dm^{-3} for shoot growth inhibition. This complex contains nicotinamide ligands which are unidentate coordinated to Cd(II) atom and NCSe ligands (acting as *N*-donor ligands) which are also unidentate coordinated to Cd(II) atom [302]. It could be noted that $CdSeO_4$ exhibited comparable root growth inhibiting toxicity with $Cd(NCSe)_2(nia)_2$ (IC_{50}: 78 μmol dm^{-3}) but with lower effectiveness in shoot growth inhibition (IC_{50}: 256 μmol dm^{-3}).

The phytotoxicity of tested compounds related to root growth inhibition decreased in the following order: $Cd(NCSe)_2(nia)_2$ ~ $CdSeO_4$ > $CdSeO_3$ > $ZnSeO_4$ > H_2SeO_4. nia > Na_2SeO_3 > H_2SeO_3 > Na_2SeO_4 > $ZnSeO_3$ > $CdSO_4$ > $ZnSO_4$ > $Cd(NCS)_2(nia)_2$ and for shoot growth inhibition the estimated rank was similar: $Cd(NCSe)_2(nia)_2$ > Na_2SeO_3 > H_2SeO_3 > H_2SeO_4 . nia > $CdSeO_4$ > $ZnSeO_4$ ~ CdSe O_3 > Na_2SeO_4 > $ZnSeO_3$ > $CdSO_4$ > $ZnSO_4$ > $Cd(NCS)_2(nia)_2$. In general, the phytotoxicity of Se(IV) compounds in shoots was higher than that of Se(VI) compounds, but the shoot growth inhibition by $CdSeO_4$ (IC_{50}: 0.256 μmol dm^{-3}) was more effective than with $CdSeO_3$ (IC_{50}: 364 μmol dm^{-3}). This could be explained as follows: in contrast to selenate which enters plant cells through a process of active transport and treatment with $CdSeO_4$ results in higher mobility, selenite is passively taken up and translocation of Cd into the shoots at application of $CdSeO_3$ is lower. Consequently, $CdSeO_3$ exhibit less toxic effect in shoots than the more mobile Cd selenate.

In an another study in which at the same experimental conditions the root and shoot growth of cress (*Lepidium sativum* L.) seedlings in the presence of some Se compounds was estimated the IC_{50} values related to root inhibition varied in the range from 8.1 to 91 μmol dm^{-3} and those for shoot inhibition from 19.5 to 130 μmol dm^{-3}, whereby the most toxic compound was $Cd(NCSe)_2(nia)_2$ and the lowest toxicity was exhibited by Na_2SeO_4 [260]. Thus, sensitivity of cress seedlings to the treatment with Se containing compounds was significantly higher than that of *Brassica napus* [261]. However, it could be noted that in cress seedlings comparable phytotoxic effect to that of $Cd(NCSe)_2(nia)_2$ exhibited ionic compound with $SeCN^-$ anion (KSeCN) as well as Na_2SeO_3 with IC_{50} values 10 and 13.3 μmol dm^{-3} for root inhibition and 24.5 and 27.2 μmol dm^{-3} for shoot inhibition.

Tested compounds $Cd(NCS)_2(nia)_2$ and $Cd(NCSe)_2(nia)_2$ contain biologically active isothiocyanate and selenocynate ligands.

Linear triatomic thiocyanate and selenocyanate groups with 16 valence electrons possess a great variety of bonding possibilities [303]. In general, isothiocyanates (R–N=C=S) were found to be quite reactive, although less than the related isocyanates (R–N=C=O). The isothiocyanate anion is a resonance hybrid with greater charge on the S [304], although charge can be localized on either the sulfur ($^-$S–C≡N) or the nitrogen (S=C=N$^-$), depending on the environment [305]. Thiocyanate (R–S–C≡N) is sometimes produced, particularly in members of the *Alyssum*, *Lepidium*, and *Thlaspi* families [306]. Thiocyanate ion was found to inhibit shoot and root growth of several plants [271, 307, 308] and at concentrations exceeding 2000 ppm NH_4SCN inhibited PET in isolated chloroplasts and inhibited the conversion of glycine to sugars, while it had no effect on conversion of glycine to organic acids in leaf tissue of cotton (*Gossypium hirsutum* L.) [309]. The relative toxicity of $SeCN^-$ was comparable to that of selenate and selenite using the metalloid-resistant bacterium LHVE as the test organism, whereby the reduction and methylation of $SeCN^-$ was similar to that of selenate and selenite by other metalloid-resistant bacteria [310]. Moreover, cultures of LHVE amended with $SeCN^-$ on agar plates produced red, elemental selenium after 3 days [311].

In plants sulfur from thiocyanate may enter the sulfur assimilation pathway what results in the production of other volatile sulfur gases, e.g., dimethylsulfide [312] and the same assimilation pathway was reported for assimilation of SeO_4^{2-} in *Brassica juncea* [313, 314]. Therefore, a pathway of $SeCN^-$ metabolism was proposed in analogy to thiocyanate metabolism in plants by de Souza et al. [315].

In the phytotoxicity test in which mustard was used $Cd(NCSe)_2(nia)_2$ (IC_{50} for root/shoot: 0.077/0.134 mmol dm^{-3}) showed much higher toxicity than its sulfur analog $Cd(NCS)_2(nia)_2$ (IC_{50} for root/shoot: 1.292/1.031 mmol dm^{-3}). The great differences in the toxicity (of about one order) could be connected with the differences in overall stability constants of both compounds which is 602.56 for complex compound $Cd(NCS)_2$ and only 199.53 for $Cd(NCSe)_2$ [316]. Due to three times lower stability of the compound with $NCSe^-$ ligands, after their release from the complex Cd could interact with suitable target groups on biomolecules and $NCSe^-$ anion may exert its harmful effect, as well.

In further experiments, we investigated the effects of some Cd and Zn compounds containing Se in their molecules on production and biochemical characteristics as well as on accumulation of Cd(Zn) and Se in three crops *Vigna radiate* [317], *Pisum sativum* [318] and *Brassica juncea* [319] as well as in three medicinal plants, *Hypericum perforatum* [178], *M. recutita*, cv. Goral [320] and cv. Lutea [302], and *Salvia officinalis*, cv. Primorska [321].

In experiment with *Vigna radiata* L. 3 days old seedlings were exposed to $CdSO_4$, $CdSeO_4$, and $CdSeO_3$ (0.5, 1.0, 2.0, 3.0 a 4.0 μmol dm^{-3}) in hydroponic solution and cultivated for 7 days [317]. Due to treatment with $CdSO_4$ and $CdSeO_4$ reduction of root dry mass was comparable (approximately 25% at 4.0 μmol dm^{-3}), while reduction of shoot dry mass of young seedling was greater with $CdSO_4$ (approximately 42%) as with $CdSeO_4$ (approximately 27%). For all tested compounds Cd concentration in shoots increased linearly with increasing metal concentration in hydroponics, while the dependence of Cd concentration in roots showed polynomic course indicating gradual saturation of root tissue with Cd. Substitution of sulfur with Se resulted in significant reduction of Cd accumulation in both plant organs. The lowest bioaccumulation of Cd was observed after $CdSeO_3$ treatment. The TF significantly depended on the applied compounds and they decreased as follows: $CdSO_4 > CdSeO_4 > CdSeO_3$. Whereas after treatment with higher $CdSO_4$ concentrations (3 and 4 μmol dm^{-3}, respectively) the fraction of Cd from total amount of metal accumulated by the plant found in its shoots represented more than 20%, for $CdSeO_4$ and $CdSeO_3$ it was only 14.5% and 7–8%, respectively. These results indicate that *V. radiata* plants in the early ontogenetic stage are sensitive to Cd-induced stress what is in agreement with previous findings of Šimonová et al. [6] and Wahid and Ghani [322, 323]. The reduced toxicity of Cd selenate and Cd selenite compared to that of $CdSO_4$ is caused probably by formation of insoluble Cd-Se compounds in roots [324, 325], whereby this effect was more pronounced with selenite. According to Wahid and Ghani [322, 323] accumulated Cd exhibits toxic effects mainly in mesophyll, presumably due to interference with essential nutrient uptake what results in the reduction of growth in different phenological stages of *V. radiata*. Cultivars of *V. radiata* which were tolerant to Cd induced stress exhibited higher peroxidase and catalase activity than the sensitive cultivars [326]. Dhir et al. [327] observed gradual increase of proline concentration with increasing Cd^{2+} concentration in *V. radiata* plants and Anjum et al. [328] demonstrated that Cd tolerant genotype of *V. radiata* has powerful antioxidant defense system securing sufficient protection against Cd-induced oxidative stress.

In experiment with *Pisum sativum* L. 3 days old seedlings were exposed to $CdSeO_4$, $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$(3–60 μmol dm^{-3}) for 14 days in controlled conditions [318]. Dry mass of roots and shoots of pea plants decreased with increasing concentration of the studied compounds and treatment with 60 and 120 μmol dm^{-3} resulted in desiccation of the shoots as well as in the damage of root cells by Cd resulting in uncontrolled ion uptake what was documented with significant increase of the corresponding BAF values. Cd concentration in the roots reached higher levels than in the shoots and accumulated Cd amount in plant organs increased with increasing Cd concentration. In general, the BAF compounds related to Cd accumulation in roots decreased in following order: $Cd(NCSe)_2(nia)_2 > CdSeO_3 > CdSeO_4$, while BAF values related to Se accumulation in roots estimated for $CdSeO_4$ were significantly lower than those for $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$, respectively. The corresponding BAF values related to Cd bioaccumulation in pea shoots ranged from 7.3 to 50.0 for $CdSeO_4$, from 5.6 to 35.6 for $CdSeO_3$, and from 11.9 to 35.9 for $Cd(NCSe)_2(nia)_2$, while the TF ranges related to Se bioaccumulation in shoots were

49.4–173.8 for $CdSeO_4$, 8.7–25.9 for $CdSeO_3$, and 5.6–16.9 for $Cd(NCSe)_2(nia)_2$. Application of 3 μmol dm^{-3} resulted in reduced Cd uptake for treatment with $CdSeO_3$ and reduced Se uptake for treatment with $CdSeO_4$ indicating interactive effects of Cd and Se. The results confirmed higher mobility of $CdSeO_4$ within the pea plants when 38% of Cd and 89% of Se from the total accumulated metal amount by the plant was allocated in the shoots, while the corresponding fraction estimated for $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$ reached only 18% for both Cd and Se. Similar findings were presented by Arvy [274], Shanker et al. [324, 329], Kráľová et al. [302], and Lešíková et al. [320]. According to Whanger [325], the presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in their binding from low-molecular-mass proteins to higher-molecular-mass ones.

Indian mustard (*Brassica juncea* L.) belongs to plants which are able to accumulate considerable concentrations of both Cd and Se in their shoots [130, 330] what could be explained with rapid accumulation of phytochelatins in the roots where the majority of the Cd is coordinated with sulfur ligands, probably as a $Cd\text{-}S_4$ complex, while in the xylem sap Cd is coordinated predominantly with *O*- or *N*-donor ligands [130]. Significant decrease of Cd uptake by selenite and selenate application was reported by Shanker et al. [329], selenite being more effective and protective effect of Se(IV) against Cd-induced DNA damage and chromosomal aberrations was estimated, as well [28].

In experiment with *Brassica juncea* L., cv. Vitasso we exposed 3 weeks old plants to 12, 24, and 60 μmol dm^{-3} $CdSeO_4$, $CdSeO_3$, $Cd(NCSe)_2(nia)_2$, and $Cd(NCS)_2(nia)_2$ for 7 days [319]. The inhibition of *B. juncea* plant growth by the studied compounds was reflected in reduced length and dry mass of plant organs and it increased with increasing compound concentration, dry mass of plant organs being affected to greater extent. The toxicity of $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$ was comparable and $CdSeO_4$ was found to be less toxic. Addition of 60 μmol dm^{-3} $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$ led to considerable decrease of leaf water content, while lower loss was observed also at treatment with $CdSeO_4$ and $Cd(NCS)_2(nia)_2$. All tested compounds applied in tested concentration range caused strong reduction of leaf Chl content. Se oxidation state strongly affected Cd and Se concentration in plant organs. BAFs for roots as well as shoots related to Cd and Se decreased with increasing compound concentration. The ranges of BAF values for shoots concerning Cd(Se) were for individual tested compounds as follows: 170.5–93.7 (351.2–204.1) for $CdSeO_4$, 84.1–28.0 (45.8–13.4) for $CdSeO_3$, 132.3–62.6 (91.8–46.3) for $Cd(NCSe)_2(nia)_2$ and 191.8–81.8, for $Cd(NCS)_2(nia)$, while the corresponding ranges of BAF values estimated for roots were several times higher. From these data it is evident that (1) treatment with $CdSeO_4$ led to the highest BAF values for both plant organs, (2) $CdSeO_3$ treatment resulted in the lowest Cd accumulation in both roots and shoots and (3) application of $Cd(NCSe)_2(nia)_2$ caused higher Cd root and lower Cd shoot concentration, in comparison with $Cd(NCS)_2(nia)_2$ addition. Similarly, shoot Se concentrations decreased as follows $CdSeO_4 > Cd(NCSe)_2(nia)_2 > CdSeO_3$. The fraction of Cd(Se) from total amount of elements accumulated by the plant found in its shoots was >50% of Cd and >87% of Se at treatment with $CdSeO_4$, 39.6–46.6% Cd and 20.3–30.1% Se at treatment with $CdSeO_3$ and 49.0–

55% Cd after application of $Cd(NCSe)_2(nia)_2$. The results confirmed higher toxicity of Se(IV) compared to Se(–II) as well as toxicity increase after substitution of sulfur in $Cd(NCS)_2(nia)_2$ with Se(–II) and are in agreement with findings of Ximenez-Embun et al. [330], Zayed et al. [331], and De Souza et al. [315] who also used *B. juncea* as a model plant.

In all experiments with medicinal plants 6 weeks old plants were exposed to the same Cd compounds for 7 days as in above-discussed studies with crops, while *S. officinalis* was exposed also to their zinc analogues.

Application of $CdSO_4$, $CdSeO_4$, $CdSeO_3$, $Cd(NCS)_2(nia)_2$, and $Cd(NCSe)_2(nia)_2$ applied at concentrations 12, 24, and 60 μmol dm^{-3} reduced dry mass of plant organs, water content of shoots as well as leaf Chl content in *Hypericum perforatum* L. and these effects increased with increasing compound concentrations. Treatment with 60 μmol dm^{-3} of studied compounds caused leaf desiccation and leaf fall what was reflected in reduced shoot dry mass.

At this concentration the loss of leaf water content was the highest for treatment with $CdSeO_4$ and $Cd(NCSe)_2(nia)_2$ and it further decreased in the order $CdSO_4 > CdSeO_3 > Cd(NCS)_2(nia)_2$ [178]. Toxic metals such as Cd affect plasma membrane permeability what results in reduction of water content [177, 332]. The adverse effect of tested compounds on leaf Chl concentration decreased as follows: $Cd(NCSe)_2(nia)_2 > CdSeO_3 > CdSeO_4 \approx Cd(NCS)_2(nia)_2 \approx CdSO_4$. While Cd affects chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid (ALA) synthesis [333], Se can inhibit Chl synthesis not only by acting on constituent biosynthetic enzymes but also through lipoxygenase-mediated lipid peroxide levels and inhibition of antioxidant defense component [334]. Treatment with Se(VI) reduced Chl concentration in lettuce (*Lactuca sativa*) [335] and infiltration of adult coffee plants leaves with Se(IV) also resulted in decrease of chlorophylls, carotenoids, and xanthophylls [336].

While treatment with $CdSeO_4$, $CdSO_4$, and $Cd(NCS)_2(nia)_2$ resulted in linear increase of Cd concentration accumulated in roots with increasing compound concentration, at application of 60 μmol dm^{-3} of $CdSO_4$ and $Cd(NCSe)_2(nia)_2$ consecutive saturation of the roots with Cd was observed [178]. Similarly, also Se concentration in roots and shoots of *H. perforatum plants* increased linearly with the applied compound concentration, only at the treatment with the highest $Cd(NCSe)_2(nia)_2$ concentration saturation of the roots with Se was observed. With regards to application of individual studied compounds root Se content decreased in the following order: $CdSeO_3 > Cd(NCSe)_2(nia)_2 > CdSeO_4$, while for shoot Se concentration this sequence was opposite: $CdSeO_4 > Cd(NCSe)_2(nia)_2 > CdSeO_3$. Because the most effective Cd accumulation in both plant organs was observed with $CdSO_4$, it is evident that reduced Cd accumulation obtained with Se-containing compounds is due to Cd-Se interference. According to Shanker et al. [324] the less mobile anion SeO_3^{2-} after being reduced to selenide tends to form Cd–Se complex, which appears to be unavailable for the plants, while the more mobile anion SeO_4^{2-} is available for Cd–Se formation only after following a more complicated redox processes involving Se(VI) in selenate, Se(IV) in selenite and Se(0) species.

Portion of Cd allocated in *H. perforatum* shoots related to the total Cd amount accumulated by the plant was about 20% for treatment with $CdSO_4$ and $Cd(NCS)_2(nia)_2$ and about 12.8%, 10%, and 6% for treatment with $Cd(NCSe)_2(nia)_2$, $CdSeO_4$, and $CdSeO_3$ what are considerably lower values than the corresponding Cd portions estimated for *Matricaria recutita*, cv. Goral [320], *Brassica juncea* [319], and also *Pisum sativum* plants [261]. Portion of Se allocated in shoots related to the total Se amount accumulated by *H. perforatum* plants achieved approx.. 86%, 48.6%, and 45.9% after addition of $CdSeO_4$, $Cd(NCSe)_2(nia)_2$, and $CdSeO_3$, while the corresponding Se portions were 91.5%, 27.8%, and 25.8% in *M. recutita*, cv. Goral, 90.3%, 51.5%, and 26.4% in *Brassica juncea* [178] and 89%, 18%, and 18% in *Pisum sativum* plants [318].

In experiments with *M. recutita* plants focused on the effects of four Cd compounds ($CdSeO_4$, $CdSeO_4$, $Cd(NCSe)_2(nia)_2$ and $Cd(NCS)_2(nia)_2$) applied at concentrations 12, 24, and 60 μmol dm^{-3} two chamomile cultivars, cv. Goral [320] and cv. Lutea [302] were chosen and in the experiment with cv. Goral beside accumulated Cd concentrations in plant organs also those of accumulated Se were estimated.

In cv. Lutea application of $Cd(NCS)_2(nia)_2$ affected neither the length nor the dry mass of roots and shoots, while other three compounds partially reduced dry mass of plant organs already at application of 24 mol dm^{-3}, while in cv. Goral adverse effects were observed only for treatments with 60 μmol dm^{-3}. The accumulated Cd and Se content in roots and shoots of chamomile plants treated with Cd salts of Se oxoacids increased with increasing compound concentration in hydroponic solution and depended on the oxidation state of Se.

The ranges of BAF values for Cd related to roots in the concentration interval 12–60 mol dm^{-3} were after application of Cd compounds as follows: 582.3–209.6 (Goral) and 863–280 (Lutea) for $CdSeO_4$; 581.9–505.9 (Goral) and 555–731(Lutea) for $CdSeO_3$; 518.9–646.4 (Goral) and 633–759 (Lutea) for $Cd(NCSe)_2(nia)_2$; 673.8–335.5 (Goral) and 584–311 (Lutea) for $Cd(NCS)_2(nia)_2$. The corresponding ranges of BAF values for Cd related to shoots were as follows: 158.0–60.4 (Goral) and 155.0–70.7 (Lutea) for $CdSeO_4$; 100.1–29.4 (Goral) and 54.4–28.7 (Lutea) for $CdSeO_3$; 74.1–30.6 (cv. Goral) and 48.6–25.1 (Lutea) for $Cd(NCSe)_2(nia)_2$; 141.9–91.8 (cv. Goral) and 90.5–60.3 for $Cd(NCS)_2(nia)_2$.

The ranges of TF values estimated for Cd were 1.014–1.316 (Goral) and 0.60–0.93 (Lutea) for $CdSeO_4$; 0.483–0.512 (Goral) and 0.200–0.105 (Lutea) for $CdSeO_3$; 0.398–0.151 (Goral) and 0.27–0.12 (Lutea) for $Cd(NCSe)_2(nia)_2$; 0.737–0.905 (Goral) and 0.60–0.63 (Lutea) for $Cd(NCS)_2(nia)_2$.

In both cultivars the TF for Cd estimated with application of $CdSeO_4$ and $Cd(NCS)_2(nia)_2$ were more than two times higher than those found for $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$, whereby the highest fraction of Cd accumulated in shoots was observed for $CdSeO_4$, while the lowest one for $Cd(NCSe)_2(nia)_2$. From both cultivars Goral was found to be more tolerant to the cadmium exposure compared to the cultivar Lutea what is in agreement with the findings of Pavlovič et al. [231].

While after application of $CdSeO_4$ and $Cd(NCS)_2(nia)_2$ approximately 40% from the total amount of Cd accumulated by cv. Lutea plants were allocated in shoots and for treatment with $CdSeO_4$ in cv. Goral this portion exceeded 50%, due to treatment with $CdSeO_3$ and $Cd(NCSe)_2(nia)_2$ approximately 80% (or more) Cd from the total amount of metal accumulated by the plants remained in roots.

Se speciation significantly affected also bioaccumulated amount of Se in chamomile (cv. Goral) plant organs [320]. Due to treatment of chamomile plants with $CdSeO_4$ more than 90% from the total uptaken Se by plants was allocated in shoots, while this portion was about 30% for the treatment with $Cd(NCSe)_2(nia)_2$ and only approximately 17% at treatment with 12 and 24 μmol dm^{-3} $CdSeO_3$. These results confirmed higher mobility of $CdSeO_4$ within the plant compared with $CdSeO_3$ and are in agreements with previous findings (e.g., [178]).

In experiment with sage the effect of compounds of the type $MSeO_4$, $MSeO_3$, $M(NCSe)_2(nia)_2$, MSO_4, $M(NCS)_2(nia)_2$ with M = Cd or Zn on root and shoot dry mass and Cd, Zn, and Se bioaccumulation in plant organs of hydroponically cultivated *Salvia officinalis* L., cv. Primorska plants was investigated [321]. For treatments with Cd compounds concentration of 120 μmol dm^{-3} was applied, while for treatments with Zn compounds two concentrations (60 and 120 μmol dm^{-3}) were used. These concentrations were toxic for the plants what was reflected in wilting and desiccation of plant leaves. BAFs related to Zn, Cd, and Se accumulation in the roots were higher than those determined for the shoots. The highest BAF values related to metal accumulation (Cd or Zn) in the shoots were observed for $CdSO_4$, $ZnSO_4$, and $Zn(NCS)_2(nia)_2$ application. Oxidation state of Se in the studied Cd and Zn compounds affected not only the toxic effect of these compounds, but also the uptake and translocation of Cd and Zn into sage plants. More effective Cd translocation into the shoots was observed at application of $CdSO_4$ and $CdSeO_4$ compared to $CdSeO_3$, while the highest Zn mobility in *S. officinalis* plants was estimated at application of 120 μmol dm^{-3} $Zn(NCSe)_2(nia)_2$ and $Zn(NCS)_2(nia)_2$. The lowest Se translocation into the shoots exhibited both complex compounds of the type $M(NCSe)_2(nia)_2$ (M = Cd or Zn).

Portion of Cd allocated in *S. officinalis* shoots related to the total Cd amount accumulated by the plant was after application of Cd compounds ($c = 120$ μmol dm^{-3}) ≈80% for $CdSO_4$ and $CdSeO_4$, 67.6% for $CdSeO_3$ and 58–59% for $Cd(NCSe)_2(nia)_2$ and $Cd(NCS)_2(nia)_2$, respectively. The corresponding portion of accumulated Se represented ≈80% for $CdSeO_4$ and $CdSeO_3$ and ≈47% for $Cd(NCSe)_2(nia)_2$. While application of $ZnSO_4$ resulted in relatively low portion of Zn allocated in *S. officinalis* shoots related to the total Zn amount accumulated by the plant (43%), the highest values of this portion was obtained with application of $Zn(NCSe)_2(nia)_2$(73%), whereby for Se accumulation it represented only 28.5%. At assessment of toxic effects of complex compounds of the type $M(NCSe)_2(nia)_2$ (in which the oxidation state of selenium is (–II)) also the overall stability constants estimated for $Cd(NCSe)_2$ (199.53) and $Zn(NCSe)_2$ (4.37) have to be considered. It is evident that the stability constant for $Zn(NCSe)_2$ is very low and therefore it can be assumed that after rapid dissociation of the complex the released $NCSe^-$ ligands can interact with suitable target groups on biomolecules.

7.4 Trees: Characteristic Functions and Utilization

Wood of the trees, used by human societies for millennia, undoubtedly remains one of the world's most abundant raw materials for industrial products and renewable energy. In general, wood-working industry represents app. 40% portion from total technically utilizable potential of biomass. Besides this most important production function trees possess also further, non-production functions. Trees (both forest and fast growing trees, respectively) play a significant role in reducing erosion and moderating the climate. The last mentioned function of the trees is extraordinarily important from the aspect of global atmosphere warming. Additionally, 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. Moreover, enormous leaf biomass is after decomposition of the source of mineral nutrition in the soil (cycle of the mineral nutrients) and can also serve as a filter against various pollutants. Finally, trees and forests provide a habitat for many species of plants and animals.

It should be also considered that the response of forest ecosystems to increased atmospheric CO_2 is constrained by nutrient availability. It is thus crucial to account for nutrient limitation when studying the forest response to climate change. Jonard et al. [337] in their last study described the nutritional status of the main European tree species, to identify growth-limiting nutrients and to assess changes in tree nutrition during the past two decades. These authors emphasized that increased tree productivity, possibly resulting from high N deposition and from the global increase in atmospheric CO_2, has led to higher nutrient demand by trees. Based on found results it was suggested that when evaluating forest carbon storage capacity and when planning to reduce CO_2 emissions by increasing use of wood biomass for bioenergy, it is crucial that nutrient limitations for forest growth should be considered.

It should be emphasized that large resources of biomass energy are related mainly to forestry residues, forestry fuel wood, and fast growing woody plants (e.g., willow, poplar, black locust, and European alder). In north European countries willow and poplar have already great tradition for their plantation cultivation. However, new biotechnological approach showed that energetic plants including the trees (such are above-mentioned fast growing trees) have also significant application for environment friendly management, mainly in phytoremediation technology. Phytoremediation can be presented as a cleanup technology belonging to the cost-effective and environment-friendly biotechnology. Several types of phytoremediation technologies being used today is briefly outlined in the part of 7.5 of this chapter.

Trees such as poplar, willow, black locust, ash, or alder are not indeed fast growing species (for comparison see Masarovičová et al. [338]) but for their convenient biological features these woody plants can be used to clean up substrates contaminated by both inorganic and organic pollutants. These plants have perennial character, long lifespan, high transpiration rate, quick regeneration of removed above-ground parts, and easy vegetative reproduction (in detail see Stomp et al. [339]). Moreover, fast growing trees 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. According to Chapell [340] advantages of, e.g., genus *Populus* in phytoremediation are great number of species, fast growth up (3–5 m/year), high transpiration rate (100 L/day) and not being a part of food chains. The use of plants producing large biomass for metal extraction from soil was proposed as an alternative to hyperaccumulators (these plants are mostly characterized by low biomass production and high tolerance against toxic substances) because high biomass production establishes to compensate moderate heavy metal concentrations in their shoots. Poplars allow several cycles of decontamination, their leaves can be easily collected and the contaminated biomass substantially reduced by incineration (cf. [341, 342]). It should be stressed that precondition for effective utilization of woody plants in phytoremediation technologies is their sufficient toxic metal tolerance. Therefore, effects of toxic metals (especially Cd, Hg, Pb, and Cu) on structure and function of trees are still intensively studied. As cadmium belongs to the most dangerous environmental pollutants and has toxic and mutagenic effects on both the plants and animals, our attention was focused predominantly on this toxic metal (see next Sect. 7.4.1).

7.4.1 Effect of Bioelements and Toxic Metals on Woody Plants

For correct experimental design both, the most important and the most difficult is to prepare young individuals of woody plants from the cuttings to have good developed shoots as well as roots. Thus, in our earlier paper [343] 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., *S. cinerea* L. and two poplar species—*Populus euroamericana* cv. Gigant and *Populus* x *euroamericana* cv. Robusta cultivated under two different conditions. Stem cuttings ca 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 following conditions: air temperature 25 °C, relative air humidity app. 70%, 12 h photoperiod with irradiance 100 μmol m^{-2} s^{-1} PAR. Since effect of both different cultivation conditions and the cadmium were 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 *euroamericana* cv. Robusta were 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.

Later [344] it was compared some physiological, production, and structural characteristics of *Salix alba* L. and *Populus* x *euroamericana* cv. Robusta under two variants of cultivation: rooting in Knop nutrient solution prior to Cd treatment and direct cultivation in Cd. The measurement and equipment used have been described in detail by Masarovičová and Kráľová [345]. 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. Roots and shoots of *P. euroamericana* cv. Robusta rooted in Knop nutrient solution were more sensitive to toxic effect of Cd than plants cultivated directly in 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. Barceló and Poschenrieder [346] summarized the main morphological and structural effects of Cd on roots as follows: decrease of root elongation, root tip damage, collapsing of root hairs or decrease of their number, decrease of root biomass, increase or decrease of lateral root formation.

Similar results were published by Lunáčková et al. [347], but these authors additionally found that Cd impact increased root respiration rate of willow and poplar plants. Higher values of this physiological parameter was caused by the fact that toxic effect of Cd induced energy cost for increased metal ions uptake into the roots and for repairing mechanisms as a consequence of metabolism damages.

Nikolič et al. [348] confirmed symptoms of Cd toxicity in Cd-treated hybrid poplar plants (10–100 μmol dm^{-3} Cd): inhibited growth (plant height and biomass), decreased root length and chlorosis of the leaves. The decreased photosynthetic activity of treated plants may be connected with lower values of chlorophyll content. Gu et al. [349] investigated the effect of Cd^{2+} (10, 50 and 100 μmol dm^{-3}) on the growth of four poplar cultivars. Root growth was significantly inhibited at

100 μmol dm^{-3}. Cd accumulation increased significantly with increasing Cd concentration and with time in all organs of the *Populus* cultivars, whereby Cd was accumulated mainly in the roots. Jensen et al. [350] studied growth performance and heavy metal uptake by *Salix viminalis* in field and growth chamber trials and found that under field conditions toxic metal uptakes were 2–10 times higher than uptakes under growth chamber conditions. Vandecasteele et al. [351] investigated the growth and metal uptake of two willow clones (*Salix fragilis* 'Belgisch Rood' and *Salix viminalis* 'Aage') cultivated in a greenhouse pot experiment using six sediment-derived soils with increasing field Cd levels (0.9–41.4 mg kg^{-1}). Willow foliar Cd concentrations were strongly correlated with soil Cd concentrations. Both clones exhibited high accumulation levels of Cd and Zn in the shoots. Celik et al. [352] evaluated *Robinia pseudoacacia* L. leaves for biomonitoring of toxic metal contamination in Denizli city, Turkey. Concentrations of Fe, Zn, Pb, Cu, Mn, and Cd were determined in washed and unwashed leaves as well as in soils collected from a wide range of sites with different degrees of metal pollution (industry, urban roadside, suburban) and from a rural (control) site. All above-mentioned elements were found to be at high levels in samples collected at industrial sites, except for lead and copper which were found at high levels in samples collected from urban roadsides that associated with the road traffic. The strong correlation between the degree of contamination and concentrations in all plant leaves assessed display that the leaves of *R. pseudoacacia* reflect the environmental changes accurately.

At present [353] was published study concerning the effect of different Zn concentration on the ecophysiological response of four commercial *Salix* clones ("1962", "1968", "Drago", and "Levante") selected for short rotation coppice, and one natural clone ("Sacco") obtained from a contaminated area. Physiological parameters (net photosynthetic rate, Chl*a* fluorescence, Chl content, stomata conductance) differed in dependence on the Zn concentration and clone. At the low Zn concentration (300 mg kg^{-1}), the absence of any significant reductions in parameters investigated indicated an efficient plant homeostasis to maintain the metal content within phytotoxic limits. Stomatal limitation (observed at 750 and 1500 mg kg^{-1}, which was found in all clones after 3 days of the treatment) might be caused by indirect effects of Zn on guard cells. Commercial clone "Drago" was more sensitive to Zn stress (showing inhibition of growth), while "1962" clone showed a down-regulation of PS II photochemistry following the slowdown in the Calvin-Bensom cycle. However, the natural *Salix* clone "Sacco" performed better, compared to the other clones, due to activation of a photosynthetic compensatory mechanism.

In an other experiment we tested the sensitivity of *Salix* clone 102 against CH_3HgCl and $HgCl_2$ [354] because mercury compounds represent severe risks, often exert clastogenic effects in eukaryotes, especially by binding SH groups and acting as spindle inhibitors, thereby causing c-mitosis and consequently aneuploidy and/or polyploidy whereby from the aspect of genotoxicity methylmercury derivatives and other ionizable organomercury compounds were found to be more active in short-term tests than either non-ionizable mercury compounds (e.g., dimethylmercury) or inorganic mercury salts (e.g., mercuric chloride) [355]. Approximately 25 cm long stem cuttings of *Salix* clone 102 were cultivated hydroponically 20 days in growth chamber under controlled conditions (mean air temperature 25 ± 0.5 °C, relative air humidity app. 70%; 12 h day and 12 h night; photosynthetic active irradiance 80 μmol m^{-2} s^{-1}) in either 100 μmol dm^{-3} $Ca(NO_3)_2$ (control), or 100 μmol dm^{-3} $Ca(NO_3)_2$ with $HgCl_2$ or CH_3HgCl in concentrations 10.0, 1.0, and 0.1 μmol dm^{-3} (pH = 5.5) and then the length and dry mass of roots and shoots were determined. Despite the presence of $Ca(NO_3)_2$ applied to secure better rooting which is known to reduce toxic effects, in the presence of Hg compounds the cumulative root length as well as root dry mass were more strongly affected than the cumulative shoot length and shoot dry mass. At the concentration 10.0 and 1.0 μmol dm^{-3} the toxic effect caused by CH_3HgCl was pronouncedly higher than that of $HgCl_2$ applied in the same concentration: in the presence of organomercurial compound the growth of roots was practically completely inhibited. For example, related to the control, the inhibition of individual characteristics due to treatment with 1 μmol dm^{-3} CH_3HgCl and $HgCl_2$ were as follows: 97.5% and 29.4% (cumulative root length), 62.5% and 21.7% (cumulative shoot length), 96.4% and 62.2% and 36% (shoot dry mass). On the other hand, inhibition of root dry mass by 1 μmol dm^{-3} CH_3HgCl was 96.4% but no inhibition was observed due to treatment with 1 μmol dm^{-3} $HgCl_2$. This is in agreement with previously described results that the toxic effects of organomercurials are 1–2 orders higher than those of inorganic Hg [356, 357]. It was reported that phenylmercuric acetate inhibits both Hill activity and photophosphorylation [358] and Girault et al. [359] found that $CH_3Hg(II)$ interactions with membrane phospholipids are electrostatic in nature and the phosphate moiety is proposed as a potential binding site. The IC_{50} values related to PET inhibition in spinach chloroplasts by some organomercurials were estimated as follows: 468 μmol dm^{-3} for phenylmercuric borate, 657 μmol dm^{-3} for phenylmercuric acetate, 942 μmol dm^{-3} for phenylmercuric citrate and 627 μmol dm^{-3} for methylmercuric chloride and using EPR spectroscopy as probable sites of action of organomercury compounds in photosynthetic apparatus ferredoxin on the acceptor side of PS I and the quinone electron acceptors Q_A or Q_B on the reducing side of PS II were suggested [360]. According to Matorin et al. [361] increased toxic effect of methylmercury on *Chlorella vulgaris* resulted from the decreased capacity of PS II for reparation and damage of *Chlamydomonas reinhardtii* algal cells on the donor side of PS II and impairment of the electron transfer from Q_A to Q_B after $MeHg^+$ treatment was reported by Kukarskikh et al. [362], while decreased photochemical activity of the PS II reaction centers of diatom *Thalassiosira weissflogii* after application of $MeHg^+$ was observed by Antal et al. [363]. It could be noted that considering the adverse effects of organomercury compounds on the environment phytodetoxification of hazardous organomercurials by genetically engineered plants was proposed (e.g., [364–366]).

7.4.2 Woody Trees as Medicinal Plants

Species of the genus *Karwinskia* from family *Rhamnaceae* (common names tullidora or coyotillo) are medicinal woody plants (shrubs and trees) growing in the subtropical and tropical areas of Mexico [367]. All parts of the plant produce secondary metabolites (toxins) characterized as anthracenones [368]. One of them (T-514) was isolated and later named peroxisomicine A_1 (PXM) with antitumor effect on mammalian tumor cells [369]. Some isolated hydroxyanthracenones belonging to the genus *Karwinskia* were found to possess also antimicrobial activity, particularly against *Streptococcus pyogenes*, *Candida albicans*, *C. boidinii*, *C. glabrata* and *Cryptococcus neoformans* with minimal inhibitory concentrations ranging between 16 and 2 μg/mL [370]. Recently, Rojas-Flores et al. [371] isolated from the dried fruits of *Karwinskia parvifolia* five new "dimeric" napthopyranones, karwinaphthopyranones, possessing a methoxy group at C-5′, some of which possessed antiproliferative activities in representative human cancer cell lines, with half-maximal growth inhibitory concentrations in the micromolar range. The capability of scavenging •OH radicals by phenolic metabolites of *Karwinskia humboldtiana* leaves was investigated, as well and it was found that for the antioxidant effects are responsible metabolite such as (+)-epicatechin and flavonol derivatives quercetin, quercetin 3-*O*-glucoside (isoquercitrin), quercetin-3-*O*-galactoside (hyperosid), quercetin-3-*O*-arabinoside, quercetin 3-*O*-rutinoside (rutin), kaempferol 3-*O*-arabinoside, and kaempferol 3-*O*-rutinoside [372].

Saavedra et al. [373] found in yeast that PXM shows specific activity on peroxisomes. However, concentration of this substance in individual parts of the plant is very variable and depends on environmental conditions, especially on drought, difference in the temperature and air humidity between the day and night or summer and winter [374] and time of plant collection, too [375]. Since no information was available as to how environmental variations affect CO_2 exchange (photosynthesis and respiration) as well as production of organic substances (important for synthesis of secondary metabolites, including PXM) we estimated these parameters in the leaves of *Karwinskia parvifolia* Zucc. grown under two temperature regimes: day/night temperature of 35/20 °C (summer temperature regime, SR) and 20/5 °C (winter temperature regime, WR). These temperature regimes were similar to average air temperature in SR and WR in natural areas of the studied species—Nuevo León in Mexico. The other growth conditions were identical for both the SR and WR: photoperiod 16 h, irradiance 200 $\mu mol\ m^{-2}\ s^{-1}$, day/night relative air humidity 85/50% (in detail see [376]). We estimated net photosynthetic rate (P_N) and chemical composition (starch, reducing sugars, nitrogen, carbon, and hydrogen) of the *Karwinskia parvifolia* leaves. On the basis of the CO_2 curves of photosynthesis, the values of P_N increased in response to increasing CO_2 concentration and irradiance. Chemical composition of the leaves was different: the level of starch and the content of reducing sugars were higher in the plants cultivated under summer temperature regime (35/20 °C), however content of the N and C was higher in the plants cultivated under winter temperature regime (20/5 °C). Our study also provided the values of the other characteristics: dark respiration rate, Chl concentration, stomata characteristics, and specific leaf area. It was confirmed that higher temperature in SR significantly influences stomata apparatus properties such as stomata density, stomata length, and width. However, there were no statistically significant differences in dark respiration rate, Chl content per leaf area, and in specific leaf area between plants cultivated in summer temperature regime and winter temperature regime [376].

According to Masarovičová and Lux [377], quantitative data on physiological characteristics (mainly CO_2 exchange) and production parameters (growth and biomass formation) of the plants (e.g., *Karwinskia* species) can help to optimize conditions for both their cultivation and the production of secondary metabolites (including anthracenones) and also confirm the relationship between photosynthesis (primary metabolism) and the synthesis of PXM (secondary metabolites). Since nitrogen is often a major limiting factor for photosynthetic processes, Masarovičová et al. [378] investigated photosynthetic characteristics, biomass partitioning and PXM production of *Karwinskia parvifolia* Zucc. as well as *Karwinskia humboldtiana* Rose grown under controlled conditions at two different N supply. Two-year-old plants in pot were watered by a nutrient supply system twice daily with 455 mL of modified nutrient solution containing high nitrate supply (HN) 2.0 mmol dm^{-3} KNO_3 and 1.5 mmol dm^{-3} $Ca(NO_3)_2$ or low nitrate supply (LN) 198.8 μmol dm^{-3} KNO_3 and 150.8 μmol dm^{-3} $Ca(NO_3)_2$, and for both nutrient solution, 270 μmol dm^{-3} $MgSO_4$, 190 μmol dm^{-3} KH_2PO_4, 41 μmol dm^{-3} Fe-EDTA, 20 μmol dm^{-3} H_3BO_3, 2 μmol dm^{-3} $MnSO_4$, 0.9 μmol dm^{-3} $ZnSO_4$, 0.3 μmol dm^{-3} Na_2MoO_4 and 0.2 μmol dm^{-3} $CuSO_4$. Pots were placed for 2 months (15 May–15 July) in a growth cabinet (1600 SP, Weiss Bioclim, The Netherlands) with the following conditions: 14 h day length, irradiance at mean plant height 400 μmol m^{-2} s^{-1}, 25 ± 0.5 °C day and night temperature, $80 \pm 5\%$ relative air humidity day and night. The above-mentioned authors found significant differences in growth, CO_2 exchange (photosynthesis and respiration), Chl and nitrogen concentration between plants grown at HN and LN. At HN, the plants of both species grew faster than those cultivated at LN. Rates of photosynthesis, leaf respiration and root respiration, quantum yield, the concentration of chlorophylls, specific leaf area, leaf mass ratio and PXM concentration were higher in plants grown at HN. *K. parvifolia* responded more strongly to the nitrogen treatment than *K. humboldtiana*, in terms of growth, as well as with respect to photosynthesis and PXM concentration. Based on found results it could be concluded that it seems useful to modify biomass production through nutrients (especially nitrogen) and in this manner also to influence production of pharmaceutically effective substances (mainly PXM) that occur in *Karwinskia* species. In *K. parvifolia* the effect of nitrogen on fruit yield was also studied. It was found that nitrogen-treated plants possessed higher dry mass of the fruit than control plants [379].

Zelko and Lux [380] investigated the effect of $Cd(NO_3)_2$ on growth, structure, and development of roots of *Karwinskia humboldtiana*. Cadmium significantly reduced the growth of primary and lateral roots of *K. humboldtiana* plants cultivated in hydroponics even at the lowest concentration of $Cd(NO_3)_2$ (1 μmol dm^{-3}). Sections of root tips revealed differences between control and cadmium-treated roots, especially in dimensions and vacuolization of meristematic and cortical cells and in sloughing off the root cap border cells. The authors found that development of root endodermis was also affected by Cd and formation of the apoplastic barrier—Casparian bands—in endodermis started closer to the root apex in Cd-treated plants in comparison with control plants. Moreover, the appearance of Casparian band can precede xylem elements formation and the second stage of endodermal development—deposition of suberin lamellae was accelerated after Cd treatment.

In this context we should stress that secondary metabolites play important role in defensive mechanism of plants (especially medicinal plants) against toxic metals, because some of them can bind metal ions. As mentioned above, this additive defensive mechanism was also confirmed by Kráľová and Masarovičová [241] for cadmium and *Hypericum perforatum* L. and secondary metabolites hypericin, pseudohypericin (naphthodianthrone derivates) and quercetin that are produced by this medicinal plant. Defensive mechanisms of medicinal plants are thus connected with strategies of these plants to tolerate negative effects of toxic metals or bioelements in their non-physiological concentrations.

7.5 Phytoremediation, Phytofortification, and Nanoagrochemicals

7.5.1 Phytoremediation

One reason for interest in plant metal interaction has been the recent attention on the use of plants either to remediate toxic metal-contaminated soils or increasing the bioavailable concentrations of essential nutrients in edible portions of food crops through agronomic intervention or genetic selection. In addition, since plants are known to interact with different metals, they have been used for the “green biosynthesis” of metal nanoparticles. Such bioinspired methods are dependable, environmentally friendly and benign. In general, phytoremediation, phytofortification, and metal nanoparticles biosynthesis are thus natural green biotechnologies with using crops, medicinal plants, as well as trees.

Phytoremediation is environment-friendly and cost-effective green technology for the removing of toxic substances from the environment using convenient plant species. In general, several types of phytoremediation technologies are available for clean-up of soils and water contaminated by organic or inorganic pollutants. The most important of them are: phytoextraction (reduction of soil metal concentration by cultivating plants with a high capacity for metal accumulation in the shoots), rhizofiltration (adsorption or precipitation of metals in the roots or absorption by the roots of metal-tolerant aquatic plants), phytostabilization (immobilization of metals in soils), rhizodegradation (decomposition of organic pollutants by rhizosphere microorganisms), hydraulic control (absorption of large amounts of water by fast growing plants and thus prevent expansion of contaminants into adjacent uncontaminated areas), and phytoresaturation (re-vegetation of barren area by fast grown plants that cover soils and thus prevent the spreading of pollutants into environment) (e.g., [381, 382]).

The most frequently used phytoremediation technology is phytoextraction involving the cultivation of metal-tolerant plants that concentrate soil contaminants in their shoots. At the end of the growth period, plant biomass is harvested, dried or incinerated, and the contaminant-enriched material is deposited in a special dump, added into a smelter or the metals can be extracted from the ash [383].

Besides metal hyperaccumulators metal-tolerant species (e.g., *Hordeum vulgare, Triticum aestivum, B. napus, B. juncea, Helianthus annuus*) can accumulate high concentration of some toxic metals in the shoots. Moreover, the fast growing (high biomass producing) plant species, such as *Salix* spp. and *Populus* spp., can also be used. These trees have lower shoot metal-bioaccumulating capacity, but their efficient clean-up of contaminated substrates is connected with their high biomass production. Within the *Brassica* genus, there also exist some other species which show the tendency to accumulate high metal concentrations, and which can be characterized as metal accumulators. Some of these species grow fast and produce a high biomass. Besides already mentioned rapeseed (*B. napus*) and Indian mustard (*B. juncea*) it is also field mustard (*B. rapa*) [384].

7.5.2 Phytofortification

Phytofortification is the fortification (enrichment) of plants with essential nutrients, vitamins, and metabolites during their growth and development to be more available for human or animal consumption. As many of the metals that can be hyperaccumulated are also essential nutrients, it is easy to see that food fortification and phytoremediation are two sides of the same coin [385]. Since plants are at the beginning of food chain, improving the nutrients uptake from soil and enhancing their movement and bioavailability in the edible parts of crops or in the feed will provide benefits for humans as well as animals. Phytofortification provides a feasible means of reaching malnourished populations mainly in relatively remote rural areas, where there is limited access to commercially marketed fortified foods. There are two main challenges ahead: (1) to develop crops that have an increased content of essential elements in the edible parts of plant but that at the same time exclude toxic elements that exhibit similar chemical properties; (2) to avoid sequestration of bioelements in the inedible parts of plants (e.g., in the roots). A breeding approach to produce nutritionally improved food crops relies on genetic diversity in natural populations that can be crossbred to introduce traits/genes from one variety or line into a new genetic background [386]. Thus phytofortification could be divided into agronomic and genetic phytofortification. The first approach uses soil and spray fertilizers enriched by individual essential elements (e.g., Fe, Zn, and Se). Agronomic phytofortification has been successfully adopted in Finland as a cost-effective method for enrichment of crops by Se. It should be stressed that increasing Se content in wheat is a food systems strategy that could increase the Se intake of whole populations. Genetic phytofortification presents the possibility to enrich crops by selecting or breeding crop varieties, which enhance bioelement accumulation (in detail see [387]). According to Genc et al. [388] strategy utilizing plant breeding for higher nutrient concentration together with agronomic biofortification (e.g., applying selenate to cereal crops by spraying or adding to fertilizer) is likely to be the most effective way to improve the nutrition of populations. Because selenium as an essential micronutrient for humans and animals is deficient in at least a milliard people worldwide, Se-accumulating plants are a source of genetic material that can be used to alter selenium metabolism and tolerance to help develop food crops that have enhanced levels of anticarcinogenic Se compounds [259]. Application of selenate on soil could be used by food companies as a cost-effective method to produce high-Se wheat products that contain most Se in the desirable selenomethionine form. Increasing Se content in wheat is a food systems strategy that could increase the Se intake of whole human population [389].

Based on found results it should be emphasized the interest in the potential exploiting of hyperaccumulators of bioelements as a rich genetic resource to develop engineered plants with enhanced nutritional value for improving public health [386]. Importance of phytofortification for the humans makes this an exciting line of future research in the field of hyperaccumulation of essential elements.

7.5.3 Nanoagrochemicals

In recent years many efforts were done to minimize negative effect of fertilizers (especially synthetic fertilizers) on soil, water, and air by design of new improved fertilizers. Nanotechnology opened novel applications in different fields of both biotechnology and agriculture. Studies showed that the use of nanofertilizers causes an increase in nutrients use efficiency, reduces soil toxicity, minimizes the potential negative effects associated with over dosage and reduces the frequency of the application. Nanotechnology thus has a high potential for achieving sustainable agriculture, mainly in developing countries [390].

Studies of nanomaterials in plant systems have demonstrated that dependent on dose, nanotechnology can be leveraged in developing novel fertilizers to enhance agricultural productivity. For instance, in wheat, ZnO nanoparticles caused the stimulation of lateral roots and changed the root architecture, which could contribute in the overall uptake of nutrients. In bean, a low dose (100 mg kg^{-1}) of ZnO nanoparticles stimulated shoot growth, similar to findings in chickpea and green pea. The same ascertainment for further plant species and other metal nanoparticles were also published by Masarovičová et al. [391]. However, these authors described also negative effects of metal nanoparticles on plants depending on their concentration as well as on experimental conditions. Dimkpa [392] reminded that the delivery of mineral nutrients in nanoform is predicated on a variety of beneficial features, including timing of nutrients release, sustained release of nutrients, synchronization or targeted environmental response, and directed nutrient delivery. Mineral nutrients can be also encapsulated in nanopolymers that also could either be directly absorbed by the plant, releasing the cognate nutrient in plants, or be engineered to timely dissolve in the rhizosphere, releasing the encapsulated nutrients according to plant's need.

Originally nanoagrochemicals (nanofertilizers, nanopesticides, plant growth-stimulating nanosystems) were designed to increase solubility, enhance bioavailability, targeted delivery of substances in both, soil and plants, controlled release and/or protection against degradation resulting in the reduced amount of applied active ingredients. These substances reduce the amount of applied active ingredients by means of their enhanced bioavailability and protection against degradation and thus decrease the dose-dependent toxicity for non-target organisms (in detail see [393, 394]). These authors emphasized the further rapidly growing application of nanotechnology such as detection of pathogens and contaminants by using nanosensors and indicators, food packaging, food security, encapsulation of nutrients, and development of new functional products. It was found that nanoscale food packing materials may extend food life, may improve food safety, may alert consumers that food is contaminated or spoiled and even may release preservatives to extend the life of the food in the package. However, they also stressed that increased attention must be devoted to the impact of risk factors associated with usage of nano-size materials on the environment and possible adverse effects on non-target organisms, especially humans. Similarly, Dimkpa [392] stressed that despite potential benefits, the application of nanotechnology in plant fertilization could come with risks for environment: non-target plants, plant-beneficial soil microbes and other life forms could be affected if nanomaterials are misused.

7.6 Concluding Remarks

Global climatological changes, including "greenhouse effect", induced water deficiency in the environment and thus "blue revolution" has been started after well-known "green revolution" which appeared in the beginning of 60 years of the last century. In comparison with "green revolution", when excessive application of different fertilizers and pesticides was preferred, "blue revolution" will change the approach to water conservation and management. Mainstream plant production thus will be in the sense "blue revolution—more crops for every drop". Water, as a critical issue, needs to move to the center stage of policy-making in the whole society, as this is a time bomb ready to go off any time. Recently appeared a new approach in transforming life sciences to technologies through the "converging Technologies" that represents combination of nano-, bio-, information- and cognition technologies knows as "NBIC technologies". These "converging technologies" allow for totally new combinations of biological and non-biological materials that will open new possibilities to interfere with living organisms. However, intensive improvement of biological sciences accompanied with many novel technologies promotes new substantial issues concerning ethics. For that reason, both scientists and politicians will have to accept fundamental bioethical principles to ensure the sustainable development of human society as well as essential protection of the environment and nature.

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Part III
Phytoremediation of Aquatic Ecosystems

Chapter 8
Phytoremediation Using Aquatic Macrophytes

Amtul Bari Tabinda Akhtar, Abdullah Yasar, Rabia Ali, and Rabia Irfan

Abstract *Phytoremediation* is a plant-based technology that is also called green technology. After the discovery of hyperaccumulating plants, this technology gained increasing attention. These hyperaccumulating plants are having the ability to uptake, store, transport, and focus on large quantity of specific poisonous elements in their body parts such as aboveground parts and harvestable parts. Phytoremediation has a number of processes that are *phytoextraction*, *rhizofiltration, phytovolatilization,* etc. Both type of plants (terrestrial and aquatic) have been tested, and these are having characteristics to treat polluted soils and waters. A number of aquatic macrophytes have been found that are used for the removal of toxic contaminants such as arsenic, zinc, cadmium, copper, lead, chromium, and mercury. Some of these aquatic macrophytes are water hyacinth, water spinach, water ferns, hydrilla, and watercress. Metal uptake ability and mechanisms of many other macrophytes have been studied or investigated. Many of these studies proved that aquatic macrophytes have potential for phytoremediation. Phytoremediation is cost-effective, environment-friendly, and has gained rising appreciation. More than 400 plant species have been known that are having the ability to remediate soil and water. This chapter provides a look into new developments in *research and practical applications* of phytoremediation by using aquatic macrophytes.

Keywords Phytoremediation • Aquatic macrophytes • Hyperaccumulators • Heavy metals • Phytoextraction • Phytostabilization • Rhizofiltration • Phytovolatilization and phytotransformation

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8.1 Phytoremediation

In the past 30 years, the quantity and density of toxic waste effluents have increased. This is due to the rapid growth [1], uncontrolled disposal of waste, accidental spillage, sludge application to soils, heavy metals, and higher complexity of chemical industries. All these factors contribute towards contamination of ecosystem [2]. Freshwater resources are changed by the human activities. Due to high deterioration of water quality, this resource is deteriorating in many areas of the world [1]. Ultrafiltration, chemical precipitation, chemical oxidation, chemical reduction, reverse osmosis, electrochemical treatment, coagulation, and flocculation are traditional technologies used for the treatment of pollutants, especially heavy metals [3, 4]. All these technologies have some limitations and benefits [5].

The word phytoremediation is made of two words. The first word is Greek that is phyto which means plant, and the second word is Latin that is remedian. It means to eliminate an evil and restoring balance, in other words remediation [6]. This term has been used since 1991. There are a number of technologies in phytoremediation in which plants and some soil microbes are used. These plants and microbes are used to decrease the quantity, movement, and toxicity of pollutants in different mediums such as soil, groundwater, and other polluted media [7, 8]. In other words, phytoremediation uses the plants and their natural, biological, physical [9], and chemical activities and processes to eliminate, immobilize, and detoxify environmental pollutants in a growth medium which could either be soil, sediments, and water [10]. Phytoremediation is used to treat a variety of contaminants or pollutants in small level field and in laboratory [6, 11]. These contaminants or pollutants are heavy metals, radionuclides, petroleum hydrocarbons, polychlorinated biphenyls, chlorinated solvents, polyaromatic hydrocarbons, organophosphate insecticides, explosives, and surfactants [12, 13] in air, water, and soils with the help of plants (natural and genetically engineered) [6]. Phytoremediation is a novel, efficient, in situ applicable, nonpolluting, and solar-driven remediation strategy that is effective in its cost and is environment or eco-friendly [9, 10, 14, 15].

8.2 Mechanism of Phytoremediation (Fig. 8.1)

Plants are having power to uptake, destroy, alter, and restore the pollutants. Plants are having the ability to remediate contaminated sites [16]. Plants remediate the contaminants and contaminated sites through several methods [6]. Generally, these plants hold the pollutants without upsetting topsoil, as a result conserving its value and richness. By adding organic matter in the soil, these plants improve soil fertility.

Some of the plants break the dangerous contaminants from the ground and meanwhile their roots take water and nutrients from the polluted soil, remains, and underground water. By adopting natural processes such as by storing the contaminants in

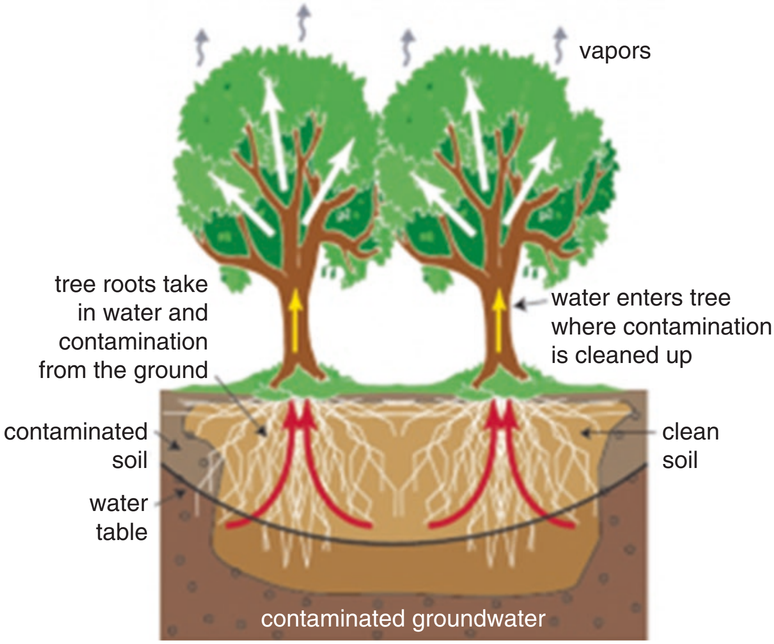

Fig. 8.1 Mechanism of phytoremediation, Source: USEPA 2012 [7]

the roots, stems, and leaves, plants can purify the pollutants. As far as their roots can get in touch converting harmful chemicals into vapors, which are released into the air. Plant breaks sorbed pollutants to less harmful chemicals into their root zone. Green plants have a huge capacity to take contaminants from the surroundings and achieve their detoxification by different processes. If the contaminated plants are left on spot for deterioration, the pollutants will be back to the soil. For total elimination of pollutants from an area, the plants must be cut and disposed of somewhere else in a less polluting way. The time period, required number, and type of species depend on the site characteristics and mostly the contaminant type. The most significant factors that have to be taken into reflection to a site where phytoremediation is used are: kind of contaminants, plant species, levels of contamination [16].

8.3 Plants Used in Phytoremediation

Some of the important plant families which hyperaccumulate the pollutants are *Brassicaceae, Euphorbiaceae, Lamiaceae, Scrophulariaceae,* etc. Indian mustard (*Brassicaceae juncea*) is a plant having large biomass. The rapid growth of plants is having the ability to accumulate nickle, lead, and cadmium in its shoots [7]. Some of the plants such as corn, sorghum, and sunflower are said to be good because their growth rate is fast and produce large biomass [17, 18]. Alfalfa (*Medicago sativa*) is an impending source of biological materials for the exclusion and revival of heavy metal ions [19]. Poplar trees, Forage kochia, Kentucky bluegrass, *Scirpus* species, Coontail, American pondweed, and Arrowhead are some others. Some of the aquatic plants are water hyacinth and duckweed [20]. Currently, a fern *Pteris vitatta* is reported to store arsenic [21].

8.4 Types of Phytoremediation

Phytoextraction (or phytoaccumulation), phytostabilization, rhizofiltration, phytovolatilization, and phytotransformation are the main phytoremediation techniques [22].

8.4.1 Phytoextraction

All over the world, phytoextraction has been gaining popularity for the last 20 years. Phytoaccumulation, phytosequestration, and phytoabsorption are some of the other names of phytoextraction. Phytoextraction involves plant roots that absorb pollutants from soil or water and accumulate them in aboveground biomass such as plant shoots [23]. *Brassica juncea* and *Thlaspi caerulescens* are commonly used plants for phytoextraction [24].

8.4.1.1 Mechanism (Fig. 8.2)

The plants take up pollutants with the help of roots and accumulate them in the root structures or then carry these into other or upper parts of the plants. A plant may carry on this process until it is removed. After removal, small amount of the pollutants stays behind in the soil, so the growth and removal cycle must regularly be repeated through a number of crops to get a considerable cleaning. After this procedure, the purified soil can sustain other plants. The essential time for removal is dependent on the type and amount of metal pollution, the length of the growing period, and the effectiveness of metal exclusion by plants [24].

8.4.1.2 Advantages and Disadvantages

This technology is appropriate for treating large areas of land, and the contamination level of these areas is low to moderate. Plant growth is not maintained in highly contaminated soils. Soil metals should also be bioavailable and subjected to assimilate by plant roots. The two properties such as high accumulation of metals and high production of biomass result in maximum removal of metals [24].

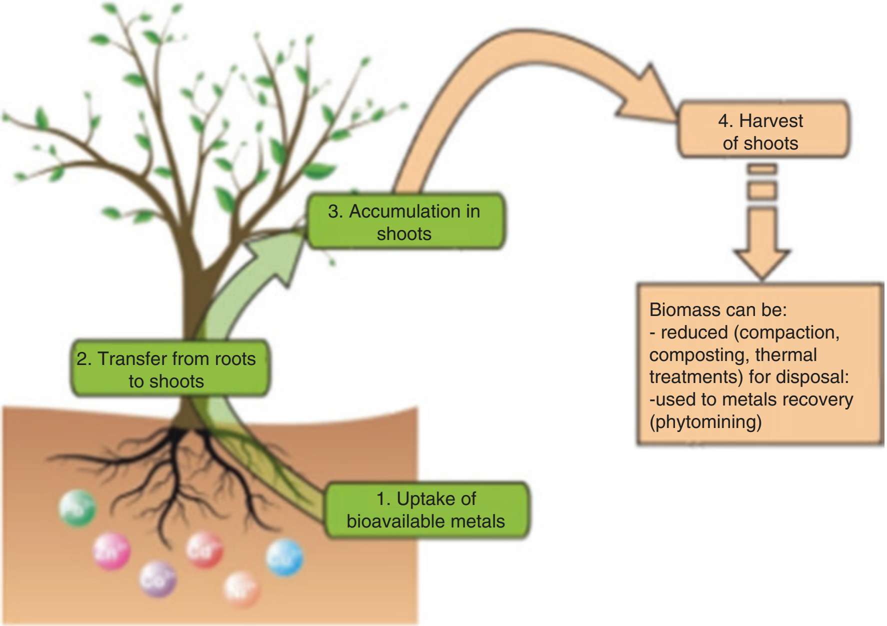

Fig. 8.2 Mechanism of phytoextraction, Source: Paulo et al. [25]

8.4.2 *Phytostabilization*

Phytostabilization also called phytoimmobilization or phytorestoration is the use of specific plants for stabilization of pollutants in polluted soils [26]. It is a remediation method that is based on plants. It stabilizes contaminants and prevents contact through wind and water erosion. It provides hydraulic control, which decreases the upright movement of pollutants. It decreases the mobility of pollutants physically or chemically by root absorption [27, 28]. This method is used to slow down the movement and availability of contaminants in the environment resultantly preventing relocation and entry of these contaminants into groundwater and food chain [29]. The primary focus of phytostabilization is storage of pollutants in soil. In this technique, pollutants are not stored in plant tissues and become less available biologically so as a result there will be less exposure to livestock, wildlife, and humans. *Agrostis tenuis* and *Festuca rubra* are commercially accessible for the treatment of lead-, zinc-, and copper-contaminated soils.

8.4.2.1 Mechanism

This process decreases the movement of the pollutants, their relocation to the underground water. This method can also be used to restore plant life at sites where natural plantation fails to stay alive due to high concentration of metals in superficial soils or physical instability to surface materials. For restoring the vegetation of polluted sites, metal-tolerant species are used. Due to which there will be less chances of transfer of pollutants in soil and water through wind erosion and uncovered soil surface. This technique is used for the treatment of lead, zinc, arsenic, cadmium, copper, and chromium [30].

8.4.2.2 Advantages and Disadvantages

Phytostabilization has many advantages over other remediation methods because it is cheap, environment-friendly, easy to apply or use, and add visual value [28]. It is most successful at those places which are having smooth soils with organic substances but is appropriate for treatment of large variety of places where large areas of surface pollution exist. Phytostabilization is not possible at highly polluted places because plant growth and life is not possible there [27].

8.4.3 Rhizofiltration

Rhizofiltration is the method or technique in which plant root stake in toxic metals from effluents accumulate and precipitate them. Water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna minor*), and pennywort (*Hydrocotyle umbellata*) are the plants used for rhizofiltration [31].

8.4.3.1 Mechanism (Fig. 8.3)

Plant roots excrete specific chemicals in the root environment that creates biogeochemical conditions that resultantly precipitate the contaminants onto the roots or in the water body. When the plant roots become flooded with the pollutants, then only the roots or whole plants are cut off for dumping [31].

8.4.3.2 Advantages and Disadvantages

Rhizofiltration is a cheaper technique that is applied for the treatment of considerable amount of metals (chromium, lead, and zinc) in surface water and groundwater. But the purpose of this technology is more difficult to achieve and inclined to failure than other methods of related cost. It requires a trained and skilled manpower for making and preservation of hydroponically grown systems. The services and specialized equipment requirement can increase operating costs [33].

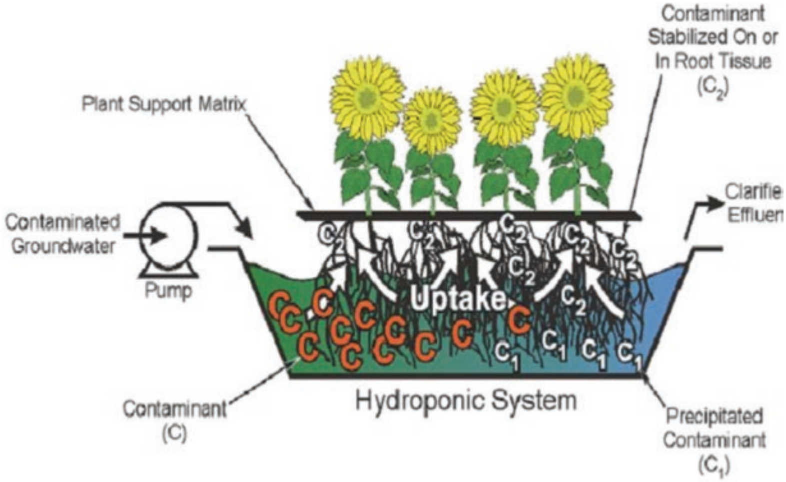

Fig. 8.3 Mechanism of rhizofiltration, Source: IGECE [32]

8.4.4 Phytovolatilization

Phytovolatilization is the method in which plants take pollutants from its surroundings and then transpire the contaminants. The transpiration is the process in which water moves in a plant from bottom root part to the upper part and then evaporated in the leaf pores. Cultivated tobacco (*Nicotiana tabacum*), Swamp lily (*Crinum americanum*), Spring wheat (*Triticum aestivum*), Mouse-ear cress (*Arabidopsis thaliana*), Water hyssop (*Bacopa monnieri*), and White clover (*Trifolium repens*) are commonly used plants for phytovolatilization [34–36].

8.4.4.1 Mechanism (Fig. 8.4)

Phytovolatilization is the process in which a pollutant is taken up by a plant and undergoes transpiration process. Meanwhile, a pollutant or a modified form of a pollutant is released by the plant into the atmosphere. Another process called phytodegradation is a linked phytoremediation process that can occur along with phytovolatilization.

8.4.4.2 Advantages and Disadvantages

Sites that use this technique of phytovolatilization may not need much supervision after the plantation of these plants. This remediation method has the additional advantages, for example, these sites are less disturbed, very less chances of erosion, and plants used in this process need not to be disposed of. Phytovolatilization would not be

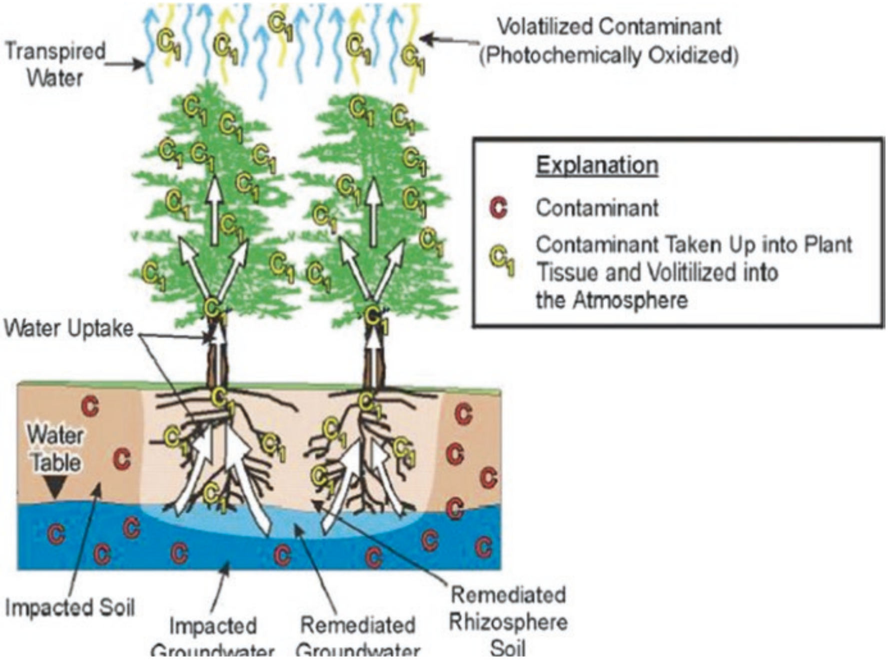

Fig. 8.4 Mechanism of phytovolatilization, Source: IGECE [32]

suitable for places which are near the highly populated sites or at some other places with distinctive weather patterns that endorse the quick settlement of unstable compounds [37]. Opposite to other treatment techniques, if pollutants have been removed through phytovolatilization, there will be less control over their relocation to other places. The use of this process is restricted because the pollutants completely remove but only changes its place such as transferring from one segment of the environment (soil) to another segment (atmosphere) and afterwards redeposited at some other place. Phytovolatilization is very contentious among all techniques of phytoremediation [38].

8.4.5 Phytotransformation

Phytotransformation is the uptake of contaminants from soil and water that are either organic or nutrient contaminants [39].

8.4.5.1 Advantages and Disadvantages

Phytotransformation is applicable on petrochemical sites and other storage areas, e.g., agricultural-based chemicals, ammunition wastes, chlorinated solvents, fuel leakages, landfill leachates [39].

8.5 Classification of Aquatic Macrophytes

Aquatic macrophytes are also called aquatic plants or water plants. These are usually presented in seven plant divisions or classes which are Cyanobacteria, Bryophyta, Pteridophyta, Chlorophyta, Rhodophyta, Xanthophyta, and Spermatophyta**.**

Depending upon the growth pattern, the aquatic plants are categorized into four major groups such as:

- Group I is also called emergent macrophytes. These are the plants which are having roots in soil and the plant growth is rising to considerable heights above the water. Examples of these plants are *Phragmites australis*, *Typha latifolia,* etc.
- Group II commonly called floating macrophytes. The plants of this group are mostly located on waterlogged sediments and are found at water depths of about 0.5–3.0 m and it includes angiosperm plants. Examples of these are *Potamogeton pectinatus,* etc.
- Group III consists of submerged macrophytes or plants. These are grown mostly below the water surface. It includes mosses, angiosperms, charophytes, and pteridophytes.
- Group IV includes free-floating plants. These are nonrooted to rock layer plants. This group is highly diversified in its habitats and characteristics.

Aquatic macrophytes or aquatic plants in comparison with terrestrial plants are more appropriate for the treatment of wastewater. Aquatic macrophytes are having many distinct characteristics such as growth rate, large production of plant body, pollutants uptake ability, and better distillation effects due to direct contact with polluted water. These macrophytes perform important functions at structural and functional levels of aquatic ecosystems. Some of the structural level functions are changes in water movement, shelter to fish and other invertebrates of aquatic habitat, and a good food source. At functional level, these macrophytes alter the quality of water by balancing oxygen, nutrient cycle, and heavy metals accumulation [40]. Aquatic macrophytes have the ability to accumulate heavy metals. This characteristic makes them attractive for research particularly for the treatment of industrial and household waste water [9, 41, 42]. The potential of aquatic plants to phytoremediation is mainly dependent on: the acceptance of plant species and difference in uptake or storage potential for the same heavy metal. In phytoremediation, some environmental factors should be maintained like chemical species, initial concentration of the metal, interface of different heavy metals, temperature, pH, redox potential, and salinity. Another phytotechnology is using the floating macrophytes for treating water in which different types of duck weed and water hyacinths have been used. Root zone plants may also be used for the treatment of small volumes of sewage water.

8.5.1 Examples of Aquatic Macrophytes for the Removal of Contaminants

8.5.1.1 *Eichhornia crassipis* (Water Hycianth) (Fig. 8.5)

Water hyacinth is a native tropical and subtropical aquatic plant. Among seven species of water hyacinth, *Eichhornia crassipis* is mostly common, grows fast and highly tolerant to pollution [43], and is used in treatment of wastewater due to its high absorption capacity of heavy metals [44]. Arsenic removal capacity of *Eichhornia crassipes* was larger than the other aquatic macrophytes due to its high production of biomass and suitable climatic conditions [41]. Plant shows high growth rate and huge vegetative reproduction [45], and it is the most troublesome weed, found in a large amount throughout the year and is very efficient in absorption of lead, zinc, manganese, cadmium, copper, and nickel by root or shoot system [26].

Water hyacinth can be the best option for the elimination of heavy metals [46]. *Eichhornia crassipes* has been used in treatment of wastewater and for improving the quality of water. It does so by reducing the levels of organic and inorganic nutrients [26]. Irfan [47] performed 1-month treatment of water at four different concentrations of chromium (Cr) and copper (Cu) by using *Eichhornia crassipes.* This plant successfully removed these heavy metals without any sign of being affected by it. *Eichhornia crassipes* removed 80.94% chromium (Cr) and 95.5% copper (Cu) during 1-month experiment.

8.5.1.2 *Azolla caroliniana* (Mosquito Fern)

Azolla is highly efficient to accumulate toxic heavy metals and can remove pollutants from wastewater [42].

Fig. 8.5 *Eichhornia crassipis*

Fig. 8.6 *Brassica juncea*

8.5.1.3 *Brassica juncea* (Mustard Green) (Fig. 8.6)

Brassica juncea, Helianthus annuus, and *Zea mays* were mostly studied in phytoremediation from 1995 until 2009. Many researches showed that *Brassica juncea* is very efficient for soil remediation and accumulates cadmium. It shows high removal efficiency of zinc because of more biomass production.

Removal efficiency of zinc, copper, and lead was compared among three species of Brassica that is *Brassica oleracea*, *Brassica carinata, Brassica juneca,* and *Brassica oleracea* showed high removal of zinc and copper in its shoots than the others zinc and lead accumulation was reported almost constant in all three species.

8.5.1.4 *Pistia stratiotes* (Water Lettuce)

Pistia stratiotes is an aquatic macrophyte that rapidly grows with large biomass. It shows high removal efficiency for removal of heavy metals due to extensive root system. Dead *Pistia stratiotes* is found very efficient and low cost alternative for the removal of diluted heavy metals like lead and cadmium for the treatment of industrial effluents [26, 48]. *Pistia stratoites* removed 77.3% chromium (Cr) and 91.29% copper (Cu) at four different concentrations of these heavy metals during 1-month treatment of water [47].

8.5.1.5 *Lemnoideae* (Duckweeds) (Fig. 8.7)

Duckweed is a free-floating aquatic plant. It grows fast in many aquatic conditions. Optimum temperature for the growth of the plant ranges from 5 to 35 °C with a wide range of pH from 3.5 to 10.5 [47].

Fig. 8.7 *Lemnoideae*

Duckweeds are mostly found in ponds and wetlands. The plant (*Lemna* species) shows high capacity for the exclusion of toxic metals from water. *Lemnoideae minor* grows well from 6 to 9 pH and accumulates up to 90% of soluble lead from water. Increased concentration of nitrate and ammonia inhibits the growth rate of *Lemnoideae minor* [49].

8.5.1.6 *Hydrilla verticillata* (Hydrilla) (Fig. 8.8)

*Hydrilla verticillata (*hydrilla*)* is an aquatic weed that forms thick layer in the whole water body. Whole plant can help in removal of contaminants. Denny and Wilkins [50] reported that shoots are more efficient in heavy metals uptake instead of roots. Hydrilla showed 98% uptake of lead when exposed to concentrated lead solution for 1 week [51].

8.5.1.7 *Spirodela intermedia* (Duckweed)

Spirodela intermedia is a floating aquatic macrophyte, shows high growth rate even under varied climatic conditions, and can accumulate cadmium, chromium, and lead from water column [52]. Plants can diminish algal production by extending itself all over the water surface and restrict the light penetration and ultimately photosynthesis [53].

Fig. 8.8 *Hydrilla verticillata*

8.5.1.8 *Schoenoplectus californicus* (Giant Bulrush)

Schoenoplectus californicus (giant bulrush) shows wide distribution geographically. It is a vascular plant that grows along the American continent below water level and takes up nutrients from sediments through its roots. It is highly tolerant to high metal concentration in streams and lakes [54].

8.5.1.9 *Ricciocarpus natans* (Fig. 8.9)

Ricciocarpus natans is a free-floating aquatic plant that can accumulate elements directly from water [55]. *Ricciocarpus natans* lack flower, stems, roots, and vascular tissues, known as liverwort.

In a study, three aquatic macrophytes *Eichhornia crassipes, Pistia stratiotes,* and *Spirodela polyrrhiza* were compared to check their removal efficiency for heavy metals. It was found that *Eichhornia crassipes* removes more metals than *Pistia stratiotes* and *Spirodela polyrrhiza* [56]. External supplementation of ethylene diamine tetra acetic acid (EDTA) was studied on *Spirodela polyrhiza* plant showed high uptake of heavy metals such as arsenic (V) and arsenic (III).

8.5.1.10 *Vallisneria spiralis* (Fig. 8.10)

An experiment was performed for 21 days on *Vallisneria spiralis* to check its capability for removal of copper (Cu) and cadmium (Cd) with different concentrations in a prepared pot containing sediment high accumulation was observed in roots and shoots by decreasing chlorophyll.

Fig. 8.9 *Ricciocarpus natans*

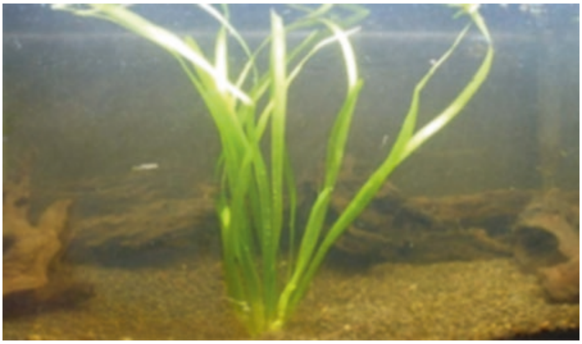

Fig. 8.10 *Vallisneria spiralis*

A positive correlation was found between the level of metals in soil and plants and/or between metals in water and plants. *Salvinia natans* showed high efficiency to accumulate different concentrations of Iron (Fe), Copper (Cu), and Zinc (Zn) at different time periods [57].

8.6 Benefits of Phytoremediation

As a natural process, phytoremediation offers many benefits.

- The process does not disturb the local environment and maintain the landscape.
- Most useful at shallow and low level contaminated sites.
- A wide variety of environmental contaminants can be treated.
- The idea is aesthetically good and has public acceptability. It is suitable for those areas where other techniques are not applicable. It is cost-effective than the other remediation techniques.

- Phytoremediation has less maintenance and installation costs in comparison to other techniques.
- The plantation on contaminated soils can prevent metal leaching and erosion. Fast growth and large biomass producing plants can also be used for energy production [58].
- Phytoremediation can help in reuse and recovery of valuable metals.
- This naturally occurring process is least harmful for the environment and surrounding people.

8.7 Drawbacks or Limitations and Challenges of Phytoremediation

Even though phytoremediation is an environment-friendly process, it does have negative aspects as well.

- It is comparatively a long-term remediation process.
- It can cause toxic effects to the food chain by transferring contaminants from water or ground to foraging animals.
- The process has a less deep remediation zone ranging from 12 in. to 15 ft.
- High metal contamination can be harmful to the plants but some species are highly efficient for the removal of toxicity.
- By the accumulation of toxic metals and contaminants, plants become harmful to livestock and general public so there should be restricted access to the site.
- It is not suitable for highly contaminated areas because plants can accumulate low to moderate level of contaminants from water and soil.

The development of phytoremediation as an eco-friendly process involves many challenges in the future, e.g., development of local capacity and to establish effective regulatory policies. There is a lack of experience using phytoremediation less available data, performance standards, and cost-benefit analysis.

8.8 The Future of Phytoremediation

Research is continuing in order to locate gene coding of plants which are having the ability to hyperaccumulate some specific heavy metals in plants. With the help of this different characteristics can be combined into a single plant species [58]. In spite of a number of challenges, phytoremediation seems to be a green remediation technology with a high potential. Unlike other physical and chemical methods for removal of heavy metals and other contaminants, phytoremediation is low cost, eco-friendly technology and does not destroy native soil microflora and fauna. Screening of local plants for phytoremediation and evaluation of the effects of different parameters during phytoremediation is in progress and for this purpose many interdisciplinary studies and researches are in process. Advance studies are identifying a number of different proteins that are involved in transportation of pollutants across membranes and vacuolar sequestration of these pollutants or heavy metals to deeply understand the mechanism of phytoremediation. For phytoremediation and phytomining of heavy metals, phytoextraction is expected to be a business technology.

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Chapter 9

Remediation of Pharmaceutical and Personal Care Products (PPCPs) in Constructed Wetlands: Applicability and New Perspectives

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Abstract Nowadays, wastewater treatment plants (WWTPs) considered not very effective in removing all types of organic compounds, including pharmaceuticals and personal care products (PPCPs). The effluent discharged containing PPCPs shows negative impact on fresh/marine waters, even at vestigial concentrations. The integration of constructed wetlands (CWs) as a biological treatment technology in WWTPs may be an option to effective removal of PPCPs, which is crucial for water bodies' protection. On the other hand, if they arrive to water bodies it is important to understand the self-restoration capacity of the system. This chapter makes an overview (based on literature and experimental data) about the effectiveness of CWs as a polishing step in WWTPs and the potential to remove contaminants if they arrive to salt marsh areas. In both cases, there is a same principle behind. CWs defined as artificially engineered ecosystems designed and constructed to control biological processes as in natural wetlands, but in a controlled natural environment.

A case study highlights the remediation potential to remove target PPCPs in both environments. Simulated CWs (spiked wastewater) planted with *Spartina maritima* and light expanded clay aggregates (LECA) as substrate. Simulated salt marsh areas (spiked elutriate soaked in sediment) were planted with the same plant but with sediment as substrate. The presence of a physical support and/or *S. maritima* decreased contaminant levels either in WWTPs or in estuarine simulated environment. Plant uptake, adsorption to plant roots/sediments and bio/rhizoremediation are strong hypothesis to explain the decrease of contaminants either in CWs or in salt marsh environment. The chapter also discusses the concept of energy production in CWs as a way to increase the competitive advantages of CWs over other treatment systems, by coupling an efficient removal together with a profitable technology, which may decrease WWTP energetic costs.

Keywords WWTPs • PPCPs • CWs • *Spartina maritima* • LECA

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9.1 Introduction

Water pollution is a relevant problem as it compromises the quality of a resource that is essential to life. In 2008, the production of hazardous chemicals (i.e., toxic chemicals defined by Eurostat) was ca.200 million tons [1]. In 2011, the European Environment Agency reported that hazardous substances, like pharmaceutical and personal care products (PPCPs), have a detrimental effect in EU fresh and marine waters [1]. PPCPs constitute a wide group of compounds largely consumed in modern societies aiming to improve the quality of daily life [2]. After utilization, e.g., pharmaceutical compounds are not completely metabolized in the body of humans and animals and as a result, metabolites, conjugates, and their native forms are excreted into the sewage system [3]. In addition, the unused and expired PPCPs are usually disposed with normal household waste or discarded into sink or toilets [4].

Wastewater treatment plants (WWTPs) receive wastewaters that contain a lot of different trace polluting compounds but are not specifically designed to eliminate all of these compounds [4–7]. Consequently, after WWTP treatment, various kinds of PPCPs and their metabolites have been detected into surface water, ground water, and even drinking water [8–12]. Upon entering the aquatic environment, and even at trace levels, PPCPs and their metabolites became a potential risk to the health of aquatic life and human beings. The available information on the ecotoxicology of these compounds is scarce, and the potential risks to the water environment are still under debate [2, 13–15]. However, it is clear that human pharmaceuticals cause e.g., antibiotic resistance in microorganisms and will negatively impact aquatic communities through feminization of male fish and affect kidneys, gills and liver in fish [13, 16].

In WWTPs, different types of treatment technologies are applied aiming to enhance organic contaminants, i.e., PPCPs removal. In fact, advanced oxidation processes, activated carbon adsorption, membrane separation, and membrane bioreactor are available to restore and maintain the chemical, physical, and biological conditions of wastewaters [17]. However, advanced treatment processes involve high capital and operational costs and selecting low-cost alternative treatments for the removal of emerging contaminants seems to be a very promising option [3, 6, 13]. Therefore, the quest for green, cost-effective, and energy sustainable technologies is a subject of debate today.

Constructed wetlands (CWs) represent an option that fits these purposes as they represent a green treatment technology, cost-effective, with low operation and maintenance requirements [18]. CWs are part of the tertiary treatment in WWTPs and may be assumed as a polishing step before the discharge for the aquatic bodies. CWs are defined as artificially engineered ecosystems designed and constructed to control biological processes as in natural wetlands, but in a controlled natural environment. CWs has been widely used to treat various kinds of wastewaters [19], such as domestic [20], agricultural [21], and industrial wastewater [22] but also storm water and acid mine drainage [23]. However, removal rate in CWs (affecting the residence time) and the effect/area of influence from the plant have been reported as limitations to this technology [6].

This chapter is an overview about the existent practices concerning PPCPs removal using CWs. The capability of a CW or a simulated salt marsh area (both planted with *Spartina maritima*) to promote the removal of two PPCPs with different physico-chemical properties, either in the presence or absence of a support matrix will also be discussed. At the end of the chapter, insights about the integration of energy production in CWs will discussed. The main aim of this concept is to increase the competitive advantages of CWs over other treatment systems, by coupling an efficient removal together with a profitable technology, which may decrease WWTP energetic costs.

9.2 Phytoremediation

9.2.1 General Aspects

Phytoremediation is an environmentally friendly technology that uses plants for the degradation, removal, and detoxification of contaminants from soils, sediments, or waters [24]. Different mechanisms can be used to immobilize, sequester, degrade, or metabolize in place (either inside or outside the plant) depending on the type of contaminant, the site conditions, the level of cleanup required, and the type of plant [25]. The phytoremediation of organic contaminants, such as PPCPs, is complex and carried out through different approaches. The contaminant absorbed by the plant and then metabolized into nontoxic metabolites (phytodegradation). The capacity to enter into the plant depends on the lipophilicity of the pollutant. It is accepted that a Log K_{ow} between 0.5 and 3 is adequate for this purpose [26]. However, contaminants can remain outside the plant. In rhizosphere, organic contaminants may be biodegraded by microorganisms that spur from root exudates (e.g., carboxylic acids, amino acids) in a synergistic action between plant and microorganisms [27]. The evolution of phytoremediation-related literature and from this, the relation with organic contaminants assessed to understand the present research tendency regarding this topic. Figure 9.1 shows the number of publications containing for the word "Phytoremediation" and then "Phytoremediation AND organic contaminants." The data was obtained from the Scopus database with the search field text = (Phytoremediation AND Organic contaminants) from 2000 to 2014. The results were refined based on: type of Literature = (Article OR Review) and subject area = (Life Sciences). The results show that the phytoremediation is intensively studied but literature regarding phytoremediation of organic contaminants represents a small percentage (between 32% in 2000 and 16% in 2014). The interest regarding phytoremediation of organic contaminants is less than the researches in phytoremediation of other organic compounds. Nevertheless, there is a growing

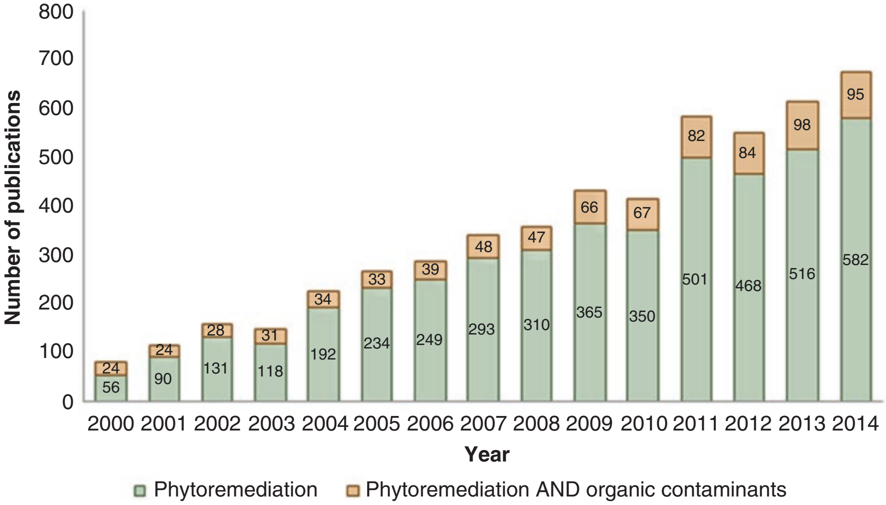

Fig. 9.1 Number of articles or reviews published on the phytoremediation area from 2000 to 2014 (Source: online version of Scopus database accessed in 26.11.2015; search field: Phytoremediation AND Organic contaminants)

tendency regarding the studies with phytoremediation and organic contaminants (including PPCPs) (passing from 24 studies in 2000 to a maximum of 98 in 2013).

9.3 Constructed Wetlands

9.3.1 General Aspects

CWs have been widely employed since its first full-scale application in the late 1960s. During the last five decades, CWs have evolved from empirical research into success, increasingly more popular applications, e.g., habitat restoration for native and migratory wildlife, anthropogenic discharge for wastewater, storm water runoff, sewage treatment, land reclamation following mining or refineries [28]. The CWs, also known as engineered wetlands, are designed to mimic the process involved in natural wetland systems but within a more controlled environment [18]. Physico-chemical properties of wetlands provide many positive attributes for contaminants remediation [29]. In sequence, CWs have also demonstrated to be a sustainable and operational technology to include in conventional WWTPs aiming for an efficient decrease of total suspended solids, biochemical oxygen demand (BOD), or elimination/decrease of various pollutants including nitrogen, phosphorus and heavy metals [30]. In recent years, the applicability of CWs for the remediation of PPCPs has been increasingly explored and proved to be successful for a variety of compounds with a simultaneous improvement of water quality [31–36].

CWs can be classified according to their hydrology (free water surface, subsurface flow, and hybrid), flow path (horizontal or vertical), and types of macrophyte (free-floating, emergent, and submerged) [6, 37]. According to the review of application of CWs for wastewater treatment in developing countries performed by Zhang et al. (2014) [38] horizontal subsurface flow (HSSF) CWs have been the most frequently employed aquatic plant-based systems to remove pharmaceutical compounds although vertical subsurface flow (VSSF) CWs and hybrid CWs have also shown good removal efficiencies for pharmaceuticals. The treatment performance in CWs is critically dependent on the optimal operating parameters and includes water depth, hydraulic load, hydraulic retention time, and feeding mode related to the sustainable operation for wastewater treatments [18]. The contaminants removal in wastewater involves a set of abiotic and biotic processes influenced by plants, substrate, and associated microbial assemblages, which assist in integral contaminant removal, while the more homogeneous conditions in WWTPs (without these dynamic interactions) induce fewer degradation pathways [30]. The physico-chemical processes contributing to contaminants degradation in CWs have not been thoroughly described [39] and it is imperative to understand the transformation processes that driven PPCPs removal, aiming to optimize CWs design for an effective contaminants removal.

CWs have advantages over the natural wetlands but also have some limiting factors. Land requirement is a limiting factor for their broader application, especially in regions where land resources are scarce and population density is high. In addition, the biological components can be sensitive to toxic chemicals (e.g., ammonia and pesticides) and peaks of contaminants in water flow may temporarily reduce treatment effectiveness. Another point is the possible re-entry of contaminants after the death of plants, which may result in a poor removal performance of CWs. To prevent this, it is necessary to develop an appropriate plant harvest strategy, with a focus on the reclamation and recycling of plant resources in CWs.

9.3.2 Salt Marsh Plants

The role of plants in CWs has been frequently discussed and several studies state their crucial role, being considered the essential component of the design of CW treatments [38]. The roots maintain the hydraulic properties of the substrate, and the shoots protect the surface from erosion while shading prevents algae growth. Besides, plants play another important role in stimulating the development and activities of microbial populations, which are supported by the rhizodeposition products (i.e., exudates) promoting the occurrence of various biological processes in the rhizosphere (e.g., transformation and mineralization of nutrients and organic pollutants) [40]. Not all plants are suitable for waste treatment since plants must be able to tolerate the combination of continuous flooding and exposure to waste streams containing relatively high and often variable concentrations of

Table 9.1 Salt marsh species reported for PPCPs removal from aquatic medium

Plants	PPCPs	References
Typha spp.	Carbamazepine, clofibric acid, and ibuprofen	[36]
Typha angustifolia	Triclosan	[44]
	Ibuprofen, diclofenac, caffeine, and methyl dihydrojasmonate	[39]
Scirpus spp.	Carbamazepine, ibuprofen, naproxen, tramadol	[8]
Scirpus validus	Caffeine	[45]
	Carbamazepine	[46]
Phragmites australis	Enrofloxacin, ceftiofur, and tetracycline	[47]
	Ibuprofen, naproxen, diclofenac, tonalide, and bisphenol A	[48]
	Ibuprofen, diclofenac, caffeine, and methyl dihydrojasmonate	[39]
Typha and *Phragmites*	Clofibric acid, carbamazepine, caffeine, methyl dihydrojasmonate, galaxolide, tonalide, ibuprofen, naproxen, ketoprofen, and diclofenac	[33]

contaminants [41]. Therefore, the study of plant species is crucial to obtain better treatment efficiency in CWs.

Salt marsh plant species are morphologically adapted to cope with environmental stress, such as, high concentrations of salt and/or insufficient water conditions. In wetlands, these types of plants have been reported to be one of the main factors influencing water quality by their capability of utilizing nitrogen, phosphorous, and other nutrients [18]. Salt marsh plants also have shown potential to remediate inorganic [42] and organic [43] contaminants. Table 9.1 summarizes studies using salt marsh plants for PPCPs removal in aquatic media simulation. The most popular salt marsh plants are *Phragmites australis*, *Typha* spp., including *Typha angustifolia*.

9.3.3 Substrates

Substrate or support matrix is considered as an important component of CWs that provides a suitable growth medium for plant and microorganisms together with a successful movement of wastewater [49]. The frequently used substrates include natural (sand, gravel, clay), artificial (light weight aggregates, activated carbon), and industrial (slag) materials [18]. Substrates can remove contaminants from wastewater by exchange, adsorption, precipitation, and complexation [36]. For this reason, the chosen materials are extremely important when designing CWs as, e.g., a material with high sorption capacity will improve contaminants removal [50]. Calheiros et al. [49] studied the treatment of tannery wastewater by *Typha latifolia* in CWs established with three different substrates. The tested substrates proved to be adequate for *T. latifolia* development with higher organic removal for the two expanded clay aggregates when compared to the fine gravel. Dordio et al. [51] showed in laboratorial batch experiments that light expanded clay aggregate (LECA) is considered a good sorbent for acidic (e.g., clofibric acid and ibuprofen) and neutral pharmaceutical compounds (carbamazepine) with removal efficiencies between 75% and 97%. Recently, biosorbents such as rice husk, pine bark, and granulated cork have also been considered as interesting alternatives to the common substrate materials in CWs due to their low cost, economical value of reuse, and easy disposal by incineration certain [6].

9.4 Case Study

The aiming of this study was to understand, in the tested conditions, the remediation potential of the different components of the system (plant, substrate) after a (simulated) PPCPs contamination before (CWs) and after effluent discharge (salt marsh area). In addition, the capacity of planted CWs and LECA as a support medium to remove contaminants were also evaluated. The first study tested the potential of CWs for PPCPs removal and the second simulates the self-restoration capacity of the salt marsh area affected by PPCPs load. In both cases, *S. maritima* was the chosen plant species. This plant species is frequently found in Portuguese estuaries and may potentially be used in CWs. Two PPCPs with different physico-chemical properties were chosen: caffeine (CAF) and oxybenzone (HMB). CAF has a Log K_{ow} of −0.77, pKa of 10.4, and solubility of $2.16*10^4$ mg L^{-1} at 25 °C. CAF is one of the most consumed stimulant of central nervous system worldwide [45]. HMB is a UV filter increasingly used in personal care products, in particular as light-filters to protect the human skin from harmful exposure to UV irradiation [52]. HMB has a Log K_{ow} of 3.8, pKa of 7.6, and solubility of 69 mg L^{-1}.

9.4.1 Methodology

The work was divided into two different parts: [53, 54]. For sake of clarity, a comparative assessment between both is carried out. Experimental design of the work is shown in Fig. 9.2.

CWs were prepared with LECA as substrate and with continuous entry of contaminants, simulating real operating parameters (residence time; Assay 1). Simulation of the salt marsh environment was carried out with sediment soaked in the respective elutriate, allowing simulation of nutrients and contaminants exchange among plants, solution, and sediment, as occurs in the natural environment (Assay 2). The effluent was collected after a secondary treatment stage in a WWTP from *Águas de Lisboa e Vale do Tejo* located in Quinta do Conde, Sesimbra, Portugal

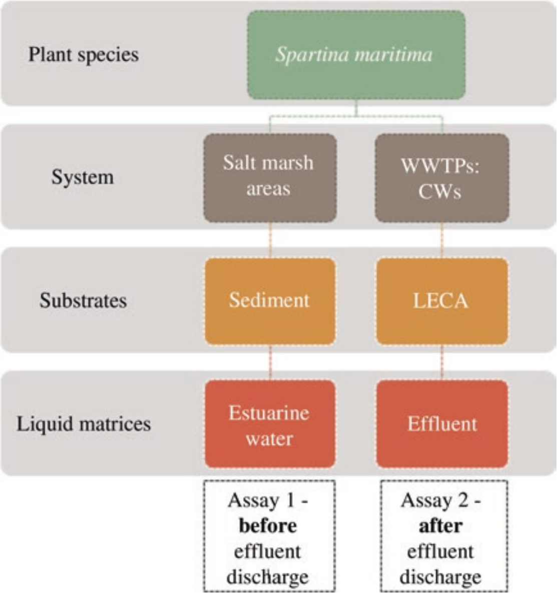

Fig. 9.2 Experimental design of the work

(38°34’13”N, 9°2’7” W). The plants, water, and colonized sediment were collected at low tide from a salt marsh, located in the Tagus River Estuary, Portugal (38º36’59.39”N; 9º02’33.41”W).

All the microcosms where wrapped in aluminum foil to protect of the sunlight and simulate real light penetration conditions. Groups of *S. maritima* were homogeneously distributed (9.0 ± 1.0 g) by different treatments and exposed to the medium (wastewater and elutriate). Plant roots were disinfected before the experiments to stop bacterial activity. The experiments to simulate CWs were carried out for 7 days, but there was three spiking periods (at days 0, 3, and 6) making the concentrations range from 0.5 mg L^{-1} to 1.5 mg L^{-1}. The experiments to simulate the salt marsh area were carried out for 10 days, and the system was spiked with 1 mg L^{-1} of each contaminant. The purpose of different spiking periods is to simulate a successive load of contaminants in CWs and a lower contaminant load in estuarine systems. Three types of controls carried out in parallel (spiked matrix with isolated presence of substrate or plant and non-spiked matrix with the presence of plant to evaluate plant vitality). Photosynthetic pigments used to evaluate plant vitality when exposed to contamination. High performance liquid chromatography (HPLC) used to quantify the levels of different contaminants in the studied matrices.

Table 9.2 Potential of remediation of plant and substrate

	CW			Salt marsh area		
	Plant		Substrate	Plant		Substrate
Contaminant	*S. maritima* vs. control[a]	Planted vs. unplanted	Unplanted LECA bed vs. control[a]	*S. maritima* vs. control[b]	Planted vs. unplanted	Unplanted vs. control[b]
CAF	(=) 0%	(+) 20%	(−) 40%	(=) 0%	(+) 19%	(+) 17%
HMB	(+) 10%	(=) 0%	(+) 10%	(+) 60%	(+) 38%	(+) 60%

Note: (+), (−) or (=) means the potential of the plant or substrate comparing (vs.) with controls
[a]Control only with wastewater
[b]Control only with elutriate

9.4.2 Results and Discussion

The presence of contaminants may influence the functions of plants and associated efficiency for contaminants removal. The evaluation of chlorophylls (*a* and *b*) and carotenoids (μg g^{-1}) of *S. maritima* exposed to PPCPs showed that this plant tolerates up to 1.5 mg L^{-1} of CAF and HMB. Table 9.2 shows the remediation potential of the system components (plant and substrates: LECA and sediment) in each simulation environment (CWs and salt marsh areas) compared with respective controls. In the CW, the presence of *S. maritima* only increased HMB remediation by 10% but did not have any effect on CAF. Also, in the simulated salt marsh area *S. maritima* had no effect on CAF remediation but promoted a decrease of 60% in HMB. *S. maritima* promoted CAF remediation in about 20% with the presence of LECA (CWs) or sediment (salt marsh area). HMB presented a different remediation behavior. The remediation was neglectable in CWs (plant, wastewater, and LECA) and was almost 40% in simulated salt marsh area (plant, elutriate and sediment). Regarding the susbtrates, the presence of sediment enhanced the remediation of HMB by 60% and of CAF by 17% in salt marsh simulation. In wastewater, LECA presented 10% of HMB remediation, but negatively affected CAF remediation.

The uptake by plants is more probable for compounds with Log K_{ow} values of 0.5–3 [26]. Recent studies show that compounds with other Log K_{ow} values may also enter the plant. Wu et al. (2013) [55] detected PPCPs with a detection frequency of 64%, and concentrations range of 0.01–3.87 ng g^{-1} (dry weight) in vegetables. Triclocarban, triclosan, and fluoxetine (Log $K_{ow} > 3$) accumulated in roots at levels higher than the other PPCPs, while translocation to leaves/stems was for compounds with Log $K_{ow} < 3$, e.g., carbamazepine. Also, (ab)/adsorption to plant roots and (bio)/rhizoremediation in liquid phase or substrate may be strong hypothesis to the enhanced remediation in the tested conditions. The higher removal of HMB, compared to CAF, explained by their octanol water partition coefficient (Log $K_{ow} > 3$) and solubility, which promotes their retention by adsorption of the solid matrices (bioconcentration in the roots or in the sediment through adsorption processes, which is higher for hydrophobic contaminants). CAF has a very high solubility and tends to remain in the liquid phase. Therefore, the presence of microorganisms (either in simulated salt marsh area or CW/liquid or solid phase) appears to favor biodegradation. The studied compounds are reported as biodegradable, being indicated as readily biodegradable, mainly HMB [56, 57].

9.5 CWs Coupling Plant Microbial Fuel Cells

The combined/integrated treatment systems present a novel pathway to improving CWs functions. The improvement of wastewater quality with simultaneous energy recovery has garnered much attention in recent years [58]. Plant microbial fuel cell (Plant-MFC) is an emerging technology, which consists in the conversion of solar energy to bioelectricity. It was patented in 2007, and the proof-of-principle was published in 2008 (e.g., [59]) and developed in an EU project 2009–2012 resulting from a spin-off company Plant-e. Plant-MFC may represent an add-in value to CWs. 50% of photosynthetic organic matter goes to soil where naturally occurring bacteria oxidize it and transfer energy rich electrons to the anode of the fuel cell. The energy can be used as electrical energy [60]. In addition, plants transfer oxygen to the rhizosphere through the root system and enhance the aerobic degradation of unutilized organic matter, nitrification and mineralization of aromatic amines [61]. Figure 9.3a presents a model of the plant-MFC. The maximum and long-term (2 weeks) power output of best performing Plant-MFC reached 0.44 and 0.222 W m^{-2} [60], a value comparable with conventional biomass–electricity chains, with potential to cover energy consumption. The technology has been scaled up to 25 m^2 in a "green electricity roof" and has a potential to be applied in wetlands [62]. In the case of CWs the "traditional" approaches, the anodic chamber is in the bottom region of the system (Fig. 9.3b).

In this region, microbes oxidize the organic matter and promote denitrification thus generating electrons (e^-), protons (H^+), and carbon dioxide. Electrical current is generated when the electrons migrate to the cathode. The voltage difference between the anode and cathode, together with the electron flow in the outer circuit, generate electrical power [63]. The electrons from the anode also react with oxygen (or other electron acceptors) at the cathode to produce water and other reduced compound. Different electrode materials can be used for the process (e.g., stainless steel mess, platinum, carbon paper, and granular active carbon). Carbon and graphite are commonly used as anode and cathode electrode materials because they offer high electrical conductivity and non-oxidative nature thus offering a good medium for the attachment and growth of microbial communities [64]. It is important to note that various operation parameters and designs have been developed lately by coupling MFC into other wastewater treatment process in an attempt to maximize the aerobic and anaerobic conditions. Different configurations can be found in the following references: [58, 65–67]. The study of the Plant-MFC concept extensively explored while the integration of CW and MFC is still in the beginning. Combining CW and MFC seems a promising green technology to be incorporated in WWTPs

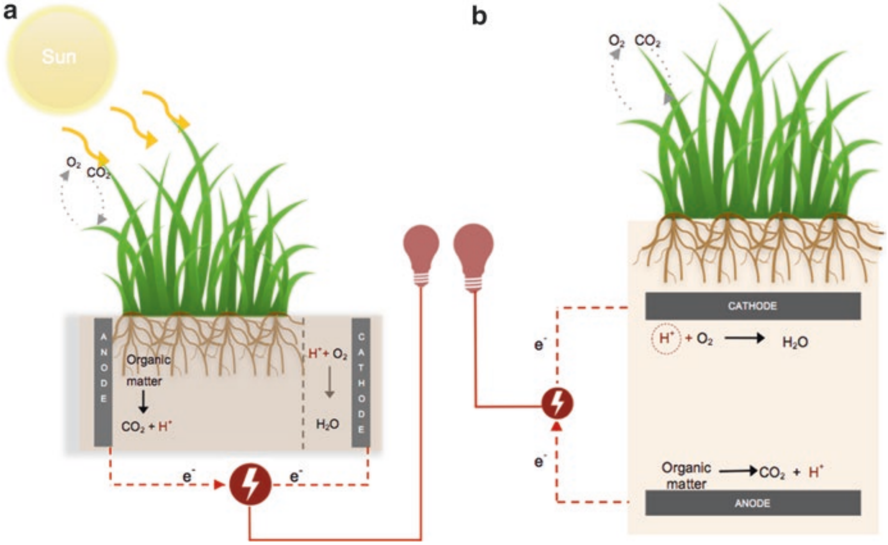

Fig. 9.3 Schematic diagram of (**a**) model of a plant microbial fuel cell producing electricity and driving a light source (adapted from [59]); (**b**) model of constructed wetland including the concept of microbial fuel cell (adapted from [67])

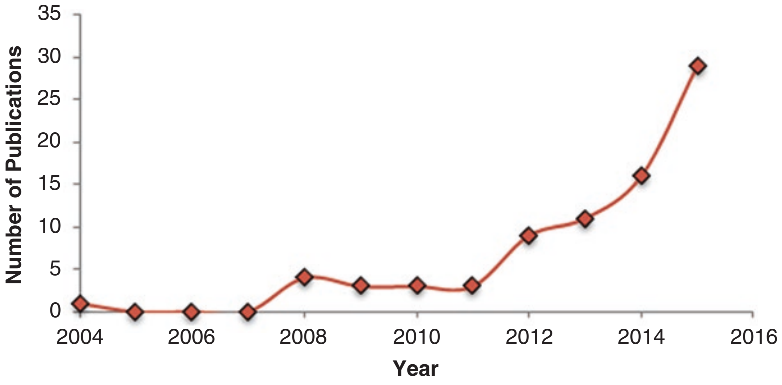

Fig. 9.4 Publications from 2004 to 2015 (Source: online version of Scopus database accessed in 26.11.2015; Search terms: Constructed wetlands AND Microbial fuel cells; Search field: Article Title, Abstract, Keywords; Document type: Article or Review)

allowing a cost-effective process to produce electricity. Several works using this technology to remove organic contaminants from wastewater with simultaneous energy production have been reported. Some examples are given below.

Figure 9.4 shows the increased number of publications with the integration of CWs and MFC, retrieved from Scopus (26 November 2015). In the online version of Scopus database, the search terms text = (Constructed wetlands AND Microbial fuel cells) with a search field: Article Title, Abstract, Keywords in a period between 2004 and 2015, were used. The results were further refined based on: type of Literature = (Article OR Review) and subject area = (Life Sciences). The study carried out by Villasenor et al. [68] operated into a HFCW-MFC using a bentonite layer to separate the lower anaerobic anode compartment and the upper aerobic cathode compartment. It was reported 95% of COD removal (mean influent concentration of 560 mg L^{-1}) and a power density of 20.76 mW m^{-2}in the CW. These authors reported that several factors influenced the electricity generation, such as the aerobic environment in the upper wetland zone, which in part, depends on the aeration potential of the plants. In general, the aeration potential of macrophytes is rather low compared with the conventional aeration systems in wastewater treatment plants.

The authors Zhao et al. [58] studied CW-MFC to treat swine wastewater operated in batch mode, in continuous, without and with air diffusion heads to aerate the cathode region. 71.5% of COD was removed (with initial concentration of 3190–7080 mg L^{-1}) and a peak power density of 12.83 mW $m^{-2}$was produced. The aeration in the cathode region significantly enhanced the performance of the CW-MFC, with the continuous mode demonstrating an average of 76.5% COD removal (average influent COD concentration of 1058.45 ± 420.89 mg L^{-1}) and a peak power density of 9.4 mW m^{-2}. Doherty et al. [66] studied the ability of the alum-sludge-based CW-MFC to remove organics from wastewater while producing electricity with different flow directions on the CW-MFC performance. They concluded that the flow direction influenced the efficiency of the system. The authors say that the simultaneous upflow–downflow CW-MFC combats the two major bottlenecks of CW-MFC power output: reducing the separation between the electrodes and maintaining anoxic conditions at the anode and aerobic conditions at the cathode.

Fang et al. [69] applied a vertical CW-MFC system to treat azo dye wastewater (aromatic compounds) and simultaneously produced electricity. The system achieved 91% of decolorization rate and a voltage output of about 610 mV. The results obtained by these authors showed that plants grown in cathode region had potential to enhance the voltage output and slightly promoted dye decolorization efficiency. Villaseñor et al. [68] reported the influence of plants in voltage, stated that photosynthetic activity affected the redox conditions in the cathode compartment, as the deposition of organic matter and O_2 in the rhizosphere increased. During the night, the voltage dropped to approximately 200 mV in the horizontal flow CW-MFC, planted with *Phragmites australis*, and gradually increased to maximum values during daylight. Liu et al. [61] have also shown the importance of plants in power density and nutrient removal of CW-MFC. The authors incorporated the root exudates of *Ipomoea aquatica* as part of fuel into the anode section of the CW-MFC and produced a power density 142% higher than that of 5.13 mW obtained from the unplanted systems. They also promoted the reduction of internal resistance. The planted CW-MFC removed 95% of COD whereas 92% of removal achieved in the unplanted CW-MFC. The average nitrogen removal efficiencies were 54% and 91% in the unplanted and planted systems, respectively. The concept of CWs coupled to MFC systems was tested with *Typha latifolia* [67]. Electricity was generated with maximum power density of 6.12 mW m^{-2} and contaminant removal was enhanced during wastewater treatment. The removal efficiencies of COD, NO^{-}_{3}, NH^{+}_{4} were of 100%, 40%, and 91%, respectively. Despite the several studies, the combination of CW-PMFC is an emerging technology and more research is required to increase the power output (as nowadays it is too low to be directly utilized) [70].

9.6 Conclusions

Population growth implies higher and faster generation of WWTP waste streams as well as higher consumption of PPCPs. These compounds are not efficiently removed in WWTP treatment methodologies and the effluent discharge into water bodies may lead to environmental and human risks. There is a need to find sustainable solutions to prevent this situation in future (by acting in WWTPs) or to remediation areas that have been contaminated throughout the times (salt marsh areas). In both environments, it is important to study the importance of "key-components" in the system, i.e., matrix, plant species and substrates. The remediation capacity of the system results from a dynamic interaction between matrix-plant-substrate components and physico-chemical properties of the PPCPs, which will promote their dispersion/dilution in liquid fraction, adsorption to solid fraction, or bio−/rhizoremediation. The concept of CWs as a green technology to remediate organic contaminants matches the purpose of Plant-MFC with the associated benefit of electricity.

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Chapter 10
Floating Wetlands for the Improvement of Water Quality and Provision of Ecosystem Services in Urban Eutrophic Lakes

Eugenia J. Olguín and Gloria Sánchez-Galván

Abstract The occurrence of eutrophic urban water bodies is widely spread globally especially in countries where sanitary infrastructure is deficient in medium and small cities. Floating Wetlands also known as Treatment Floating Wetlands or Floating Islands are a suitable option for the treatment or improvement of the water quality in urban water bodies since they show several advantages over other systems, especially that they can operate in situ and no additional surface of land is required. They have been applied for the treatment of various types of water/wastewater ranging from low nutrient to high nutrient content. Their efficiency at removal of nutrients and other type of pollutants depends on several factors being the most important ones the initial concentration of pollutants, the environmental conditions, and the characteristic of the utilized plants. Emphasis is given in the need of research at large-scale applications in situ and also in the study of the potential of FW for the provision of ecosystem services. There are very few studies oriented towards this latter issue, which is currently a very important one for understanding their benefits to the urban human communities.

Keywords Eutrophication • Eutrophic lakes • Water pollution • Phytoremediation • Emergent plants • Artificial wetlands • Ecosystems services

10.1 Introduction

It has been widely recognized that the excessive anthropogenic nutrient loading (especially nitrogen and phosphorus) promotes eutrophication. This is one of the most serious environmental problems affecting the water quality in fresh water bodies. Alterations in aquatic communities, fish mortality, reduced oxygen levels, excessive growth of algae (harmful algal blooms), and the increase of suspended solid materials are the main effects of eutrophication [1]. At high densities, harmful

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algal blooms produce odor problems and kill aquatic biota due to the release of toxins, low dissolved oxygen, and high ammonia concentrations, which are associated with their proliferation and senescence [2]. The discharge of untreated or partially treated domestic wastewater to the aquatic environment threatens public health and aquatic ecosystems. In Indonesia, the national average of access to wastewater facilities was 56% in 2010, from which only the 1% is related to sewage plants treatment systems since septic tanks are the most common wastewater infrastructure [3]. In Mexico, approximately 91% of inhabitants have access to improved sanitation; however, only 50% of all collected sewage is treated in wastewater treatment facilities [4], especially in big and most important cities, leaving behind the small and scattered communities which are mainly in rural areas.

In China, the treatment rate of urban domestic wastewater was 72.9% in 2010 [5] although in most small towns, untreated domestic wastewaters are discharged into water bodies [6].

The water pollution in urban lakes and rivers, due to the excessive input of nutrients from untreated domestic wastewaters, has been extensively reported in developing countries. Sánchez et al. [7] found that the urban lagoon called "La Pólvora," in the Grijalva River basin in Mexico, had a hyper-eutrophication level affecting the biodiversity of crustaceans and mussels. Olguín et al. [8] analyzed the water quality of the Sub-basin of Sordo River that passes through Xalapa, México.

Authors found that the concentrations of diverse parameters (nitrates, phosphates, fecal coliforms, BOD, dissolved oxygen, pH, dissolved suspended total solids), in different monitoring points, were above the permissible levels for the development of aquatic life (US-EPA). Another urban lake called "Lago de Guadalupe" located in Mexico City has been reported as a hyper-eutrophic lake with an anoxic environment [9]. Recently, Olguín et al. [10] found in the lake system "Los Lagos del Dique" in Xalapa, Mexico, a high content of nutrients, organic matter, and pathogens that exceeds the values established in the Norm of EPA for aquatic life protection and the presence of an excessive growth of algae, especially during the spring. In this case, nonpoint source pollution from storm water runoff from the surrounding streets is one of the major sources, especially during the rainy season. In China, 130 major lakes were analyzed and the results showed a high level of eutrophication in 43.5% of them while 45% had an intermediate status. All lakes are located around cities and receive large amounts of municipal sewage without an appropriate treatment [11].

10.2 Need of Environmentally Friendly Technologies

Effective solutions for the remediation of eutrophic urban lakes, the control of harmful algal blooms, and the prevention of eutrophication are a matter of urgent concern in those countries in which this problem is still prevalent in small and medium size cities. The conventional wastewater treatment processes are not suitable for eutrophic lakes since in the latter case, the nutrient concentration is lower and the water volume is huge, and land is not available for establishing treatment plants. Thus, the implementation of environmentally friendly technologies with high efficiency and low cost is required.

Phytoremediation offers various alternatives to treat polluted water, being the use of aquatic floating plants in phytofiltration lagoons one of the most widely applied for treating poultry effluents [12], municipal [13], and aquaculture wastewaters [14]. On the other hand, the use of emergent plants in constructed wetlands has also being applied for the treatment of domestic wastewater [15, 16] and agroindustrial wastewater [17–19].

More recently, another type of environmentally friendly technology using emergent plants adapted to the water environment has been usefully applied for the treatment in situ of polluted water. Such type of technology has received various names such as Floating Treatment Wetlands, FTW [20, 22], Floating Islands [21], and Floating Wetlands [10]. For practical reasons, in the foregoing text, this type of technology will be referred as Floating Wetlands (FW).

In general terms, the FW present several advantages over the other type of artificial wetlands: (a) they are applied in situ, avoiding the need of large land areas for treatment; (b) they can be built at a low cost using plastic containers supported by empty bottles for providing buoyancy [22] or floating matrices of various types [20, 23, 24]; (c) they provide a support for the upper parts of the plants (leaves and stems) and allow the roots to be hanging in the column water enhancing the plant uptake and the support for rhizospheric microorganisms and their biofilms with degrading capabilities; (d) they are able to remove nutrients [25, 26]; (e) in some cases, they can provide ecosystem services such as an increase in the dissolved oxygen of the rhizospheric zone and removal of pathogens [22, 27].

The efficiency of FW at the removal of organic matter and nutrients such as N and P varies widely according to the initial concentration of pollutants in the water, the type of plants utilized, and the environmental conditions. The initial concentration of N and P in storm water and reservoirs is much lower than in eutrophic lakes and rivers. There have been several reports dealing with this type of polluted water,

Table 10.1 Efficiency of FW at removal of organic matter and nutrients in stormwater reservoirs

Plant(s) used	% Removal averages	Environmental conditions	Highlights	References
Canna indica, Thalia dealbata and *Lythrum salicaria*	COD: 71.2% TN: 70.0% TP: 82.4%	Urban stormwater runoff sewage, greenhouse	*T. dealbata* outperformed *C. indica* and *L. salicaria* in nutrients removal	Ge et al. (2016) [46]
Scirpus californicus	TP: 50% P-PO$_4$: 45% TN: 25%	Controlled conditions with stormwater (mesocosms)	The application of floating islands as a stormwater technology can remove nutrients through plant uptake and biological activity	Chang et al. (2012) [25]
	N-NO$_3$: 60%	HRT = 30 days		
	N-NH$_4$: 55%			
Chrysopogon zizanoides (*Vetiver* grass), *Typha angustifolia* and *Polygonum barbatum*	TP: 19.1–46.0% TN: 7.8–67.5%	Water from a reservoir, in situ	Estimates of nutrient uptake rates showed that *Typha* achieved the highest uptake rates, compared to *Vetiver* and *Polygonum*	Chua et al. (2012) [40]

Table 10.2 Efficiency of FW at removal of organic matter and nutrients in effluents from wastewater treatment plants and agroindustrial activities

Plant(s) used	% Removal averages	Environmental conditions	Highlights	References
18 local species from Tampa, Florida, USA	Oxidized nitrogen $N–NO_2 + N–NO_3$: <72% TN mass removal efficiency: 61%	Wastewater effluent from treatment plant	An incremental TN removal rate for the Floating Wetland Islands was calculated to be 4.2 kg N/m^2 FWI per year	Vázquez-Burney et al. [41]
Calamagrostis epigejos, Phragmites australis, T. latifolia, Juncus maritimus, P. palustris and *E. atherica*	TN uptake: 10–15 g/m^2 TP uptake: 1–3 g/m^2	Manure and biomass digestate liquid fraction	*P. palustris* and *E. atherica* present the highest potential to be used to treat digestate liquid fraction in floating wetlands	Pavan et al. [42]
Ipomoea aquatica Forsskal	TN: 11.2% $N–NH_4$: 60.0% $N–NO_2$: 60.2% TP: 27.3%	Aquaculture wastewater, in situ	The removal mechanism of nitrogen compounds was due mostly by microorganisms, whereas the removal mechanism of phosphorus was mainly by plant absorption	Zhang et al. [43]

and the removal efficiency has been observed to vary widely and it has been reported that plant uptake plays a very important roll (Table 10.1).

On the other hand, FW have also been tested for the treatment of effluents from wastewater treatment plants and from agroindustrial activities (Table 10.2), observing also a wide variation in performance depending on the type of plant utilized and other operation parameters.

10.3 Applications of FW for the Improvement of Water Quality in Eutrophic Lakes

FWs have shown to be very efficient and convenient for the removal of nutrients in eutrophic water bodies (Table 10.3). Such efficiency varies widely with the season, the initial concentration of nutrients, and the plants utilized. There is no single optimal combination of plants; it depends on the geographical location and the local availability of each particular species. There are only very scarce studies in which the removal of pathogens (such as fecal coliforms, FC) has been quantified. In the case of the Mexican urban lakes [22], such efficiency was found to be related to the hottest season (summer).

Among the various designs that have been developed for using Floating Wetlands for treating polluted water from rivers, there is a novel design, which is called hybrid floating treatment bed (HFTB). It is a combination of a floating treatment bed (FTB) with structures to promote the attachment of periphyton to improve the nutrient removal capacity of the whole system. Periphyton is a complex assemblage of aquatic organisms such as microalgae, bacteria, protozoa, and other organisms with attaching capacity to surfaces. The combination of both, the floating plants, and their rhizosphere and the periphyton maintained the TN and TP of the river at less than 2.0 and 0.02 mg/L, respectively [28].

FWs have also demonstrated to remove toxic compounds generated by the algal blooms present in eutrophic water bodies. Bao [29] assessed the removal efficiency of microcystin-LR (MC-LR is the main variant of microcystin) in FWs planted with *Oenanthe javanica* during the treatment of water collected from a eutrophic river. The removal ranges were 38.3–48.4, 42.5–56.5, 37.5–46.9, and 30.2–42.8% for flow rates of 0.5, 2, 4, and 10 cm/s, respectively. However, the changes in flow rates did not affect the absorption of MC-LR by the plant, which would suggest the presence of an additional mechanism of removal such as bacteria degradation. MC-LR plant uptake was higher in roots than in stems and leaves at the inlet (average: 1.75 vs. 0.9 μg/kg fw) and outlet (average 1.2 vs. 0.6 μg/kg fw) of the FW in all different flow rates tested in this study. The exposition of *O. javanica* to MC from a polluted river did not inhibit its growth.

Few are the reports about the use of FWs, at large scale, to improve water quality in eutrophic water bodies. Wang et al. [30] assessed the growth of *Pontederia cordata* L. and *Schoenoplectus tabernaemontani* planted in FW established in a eutrophic urban lagoon. The macrophytes adapted successfully to the stress due to the low dissolved oxygen (1.2 mg DO/L) during the summer and the low nutrient concentra-

Table 10.3 Efficiency of FW at removal of organic matter and nutrients in polluted rivers, lakes, and ponds

Plant(s) used	% Removal averages	Environmental conditions	Highlights	References
Phragmites australis and *Canna indica*	N–NH_4: 72.0%	Polluted river water, experiments at pilot plant level	The performance of the FW was critically influenced by the maturity of the roots, during sudden increments of the hydraulic and pollutants loads	Saeed et al. [43]
	N-NO_3: −35.0%			
	TP: 57.1%			
	BOD_5: 8.2%			
	COD: 7.7%			
	TS: 23.9%			
Typha orientalis Presl, Eleocharis dulcis, and Juncus effuses	TN: 66.7%	Urban lake, in situ	The experimental units can quickly enhance water quality; it is worth promoting its application for water landscapes and environmental conservation	Lu et al. [24]
	TP: 74.4%			
	N–NH_4: 99.9%			
	N–NO_3: 93.7%			
	N–NO_2: 98.9%			
	P–PO_4: 63.5%			
	COD: 91.7%			
Pontederia cordata L., Schoenoplectus tabernaemontani	P removal:	Urban pond, in situ	*P. cordata* produced more biomass and demonstrated higher P removal performance than *S. tabernaemontani*	Wang et al. [30]
	7.58 mg P/plant of *P. cordata*			
	1.62 mg P/plant of *S. tabernaemontani*			
Pontederia sagittata and *Cyperus papyrus*	WQI (scale from 1 to 100) was upgraded from "*polluted*" (46) to "*moderately polluted*" (53) during the summer	Eutrophic urban lake, in situ	The FWs increased the DO concentration (18–25%) and decreased the fecal coliforms (85%). The plants' growth and the high productivity observed (4.87 kg/m^2 month) indicated the nutrient uptake from the water column	Olguín et al. [22]

Pontederia sagittata and *Cyperus papyrus*	COD: 71.7%	Eutrophic urban lake, in situ	The productivity of a mixture of both plants after 3 months was higher in autumn season than in winter season (4.14 vs. 2.33 kg dw/m^{2}month)	Olguín et al. [10]
	N–NH$_4$: 27.3%			
	N–NO$_3$: 61.42%			
	TDS: 39.8%			
Canna indica, Accords calamus, Cyperus alternifolius, and *Vetiveria zizanioides*	COD: 15.3–38.4%	Eutrophic river water, in situ	*Canna indica* exhibited better growth, higher DO levels, and a higher percentage of nutrient removal attributable to plant uptake	Bu and Xu [27]
	TN: 25.4–48.4%			
	TP: 16.1–42.1%			
	Chl-a: 29.9–88.1%			
Phragmites australis, Carex elata, Juncus effusus, Typha latifolia, Sparganium erectum, and *Dactylis glomerata*	COD: 29–66%	River water, in situ	The floating systems were easily installed and required few maintenance operations	De Stefani et al. [26]
	BOD:41–52%			
	TP: 15–65%			
	TN: 13–29%			
	N–NO$_3$: 12–14%			
Phragmites karka	TS: 55–60%	River water, in situ	The system is recommended as an eco-friendly river water treatment for small shallow, slow flowing (or slightly stagnant) water bodies	Billore et al. [45]
	N–NH$_4$: 45–55%			
	N–NO$_3$: 33–45%			
	TKN: 45–50%			
	BOD: 40–50%			

Fig. 10.1 Two large Floating Wetlands have been established as an efficient aquatic phytobarrier or phytofilter at the entrance of Lake 1 in the Lakes System known as "El Dique" en Xalapa, Veracruz, México [22]

tions during the winter (0.15 mg P/L and 1.15 mg TN/L). *P. cordata* was more efficient to remove P than *S. tabernaemontani* (164.4 vs. 35.1 mg P/m^2). The changes in the biomass productivity and the P concentration pointed out that the P was accumulated in stems during the summer and mobilized, during the autumn, to the inferior parts of the plants (rhizomes and roots). In Mexico, two linear FWs (17.5 and 33 m^2) have been established by Olguín et al. [22] in a eutrophic urban lake in the city of Xalapa, Mexico (Fig. 10. 1).

FWs were built with low-cost materials and planted with a combination of *Pontederia sagittata* and *Cyperus papyrus* (Fig. 10. 2). A water quality index (WQI) was calculated with the data of selected parameters, according to Olguín et al. [8]. The results showed that FWs contributed to improve the WQI, especially during the August and October's monitoring period (summer and autumn). The WQI improved from polluted (27–50) to moderately polluted (51–70), especially during summer.

10.4 Ecosystem Services Provision

An ecosystem service (ES) is a direct or indirect contribution of ecosystems to the human well-being, which generates a link between the biophysical aspects of the ecosystems and the human well-being. They have been classified as provisioning,

Fig. 10.2 The Floating Wetlands established in Xalapa, Veracruz, México, have a combination of *Cyperus papyrus* and *Pontederia sagittata* and provide various ecosystem services, including habitat for birds [22]

regulating, supporting, and cultural, according to the type of service they provide [31]. Wetlands provide all kinds of ES such as provisioning (i.e., food, fuel, wood, and freshwater), regulating (i.e., flood control, climate, water quality, water supply), supporting (nutrient recycling, soil formation, primary production, biodiversity) and cultural (i.e., recreational, aesthetic, educational) [32]. The ES assessment of wetlands can be carried out in two steps: (1) physical dimension measurement including plant harvest, removal rates of N/P, carbon fixation, and oxygen release and (2) monetary evaluation in which the SE value, in the market, is estimated [33]. Research on ES provided by wetlands has been mainly focused on large wetlands and few are the reports about small wetlands (<1 ha). However, often small wetlands have a high performance in the improvement of water quality (in terms of area), compared to large wetlands [34].

The ecosystem services (ESs) concept has been extended to the design and management of landscapes. Constructed wetlands (CW) for the improvement of the quality of wastewater, before being discharged to water bodies, is an example. CW can directly or indirectly support human well-being by providing several ES such as a clean water supply, habitats, food, aesthetic, education, and recreational benefits [35].

FW can provide other ESs different to water quality improvement. They can directly or indirectly support the human well-being providing ESs such as regulation including flood reduction and atmospheric CO_2 capture through vegetation, improvement of the air quality through filtration and/or particle absorption, NOx, and other contaminants. ESs of provision include food and the harvested biomass can be used as raw material for composting, ornamental purposes, etc. FW can also contribute to biological and genetic diversity providing habitat for plants, microorganisms, invertebrates, and vertebrates. Cultural ES include esthetic, educational, and recreational benefits [36, 37].

In the specific case of floating wetlands established in eutrophic lakes, they have demonstrated that provide at least three ES such as water quality improvement, nutrient recycling, and oxygen release. Dunne et al. [38] assessed the P removal by a FW at large scale to treat water from a eutrophic lake. The cost of the FW performance was also evaluated. FW removed 2.6 metric tons P/year mainly in particulate form. The highest removal was observed in the cold season but the performance costs were constant in all seasons. The authors concluded that an operational regime with low flow rates, in hot season, can increase the economic viability of the process.

Bu and Xu [27] compared the performance of four different emergent plants (*Canna indica, Accords calamus, Cyperus alternifolius,* and *Vetiveria zizanioides*) in FW treating the water from a eutrophic river. They found that *Canna* was the plant that exhibited better DO gradient distribution and higher DO levels compared to the other three plants. Furthermore, as a result of creating a higher number of aerobic microsites around their roots, this plant was also the one that showed a higher percentage of nutrient removal. The harvested biomass from FWs can be used as raw material to generate biogas through anaerobic digestion, which is an ES that can be quantified. Biogas has been produced from *Acorus calamus, Typha orientalis, Pontederia cordata, Canna indica, Colocasia tonoimo, Thalia dealbata,* and *Hydrocotyle vulgaris* [39].

10.5 Final Remarks

Floating Wetlands have been applied to very different types of water/wastewater ranging from storm water (low nutrient load), eutrophic lakes (medium to high nutrient content) to effluents from agroindustrial activities (high nutrient load). Their efficiency depends on several factors, the following being the most important:

- Quality of the water (initial concentration of pollutants)
- Emergent plant utilized and its rhizospheric microorganisms
- Irradiance and temperature
- Additional factors such as matrix and combination of plants

Currently, research could focus in experimental work carried out at pilot plant level in situ, especially in the case of applications for the improvement of water quality in eutrophic lakes. Likewise, there is the need to study more in depth the provision of ecosystem services provided by Floating Wetlands.

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Chapter 11

Green Aquaculture: Designing and Developing Aquaculture Systems Integrated with Phytoremediation Treatment Options

Guy R. Lanza, Keith M. Wilda, Sushera Bunluesin, and Thanawan Panich-Pat

Abstract An increase in aquaculture for global food production has been one response to the sharp reductions of the stocks of aquatic species used as a source for traditional fishing methods. Phytoremediation offers an environmentally compatible approach that can be quickly integrated into existing aquaculture systems to provide management of contaminants. The scenarios of Integrated Aquaculture–Phytoremediation systems (IAPS) provided in this chapter are not intended to be all inclusive but rather serve as selected examples of potential applications. Appropriate IAPS will be highly site specific and will depend on local conditions including geomorphology, water sources, levels of ambient soil and water contamination, the aquatic species under aquaculture, and the type of culture system used. The IAPS design must provide a good balance that insures both the removal of excess nutrients and other contaminants and an adequate supply of nutrients to support the growth of

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"*Can you help us get clean water*?" (Woman in TayPhong village, Vietnam following the loss of all of the fish in the village aquaculture pens in 2006 following a toxic spill in the Song Lan River.)

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the aquaculture products. IAPS can greatly enhance the global production of plant and animal food particularly in developing countries with warmer climates and highly diverse plant communities. IAPS that effectively removes snail-vectored parasites (e.g., fish-borne zoonotic trematodes) are especially desirable because snails are often cultured for food in aquaculture systems along with fish. The inclusion of carnivorous plants (e.g., *Utricularia* sp.) in IAPS may offer one solution. *Utricularia* sp. inhabiting wet soils and water are known to actively trap and consume aquatic animals, and it may be possible to use carnivorous plants to remove immature snails, snail eggs, miricidia, and cercariae as a treatment option in IAPS.

Keywords Phytoremediation • Aquaculture • Food security • Soil and water contaminants • Global food production • Snail-vectored diseases • S.E. Asia Aquaculture

11.1 Introduction

Increased demand on traditional global fisheries coupled with ocean contamination, altered patterns of aquatic species distribution due to climate change, El Nino effects, and altered predator prey relationships has raised international concern about sharp reductions in the stocks of available fish and other aquatic food species. One response has been the increased development of aquaculture systems to produce animals and plants for food. A major challenge confronting the growth of aquaculture activities is the increase in biological and chemical contamination associated with aquaculture to produce food.

The primary aim of this chapter is to encourage the development and use of phytoremediation options appropriate for treating and improving the soil, sediment, and water quality associated with aquaculture systems. Phytoremediation offers an environmentally compatible approach that can be quickly integrated into existing aquaculture systems and provide effective management of contaminants during aquaculture operations.

Phytoremediation offers an excellent array of plant–microbe choices that can be matched to a site-specific water quality problem in aquaculture. Matching the appropriate plant or plant community to chemical and biological contaminants can play a major role in conserving and protecting soil and water. Integrating phytoremediation options with various aquaculture systems can serve as a major tool to achieve cost-effective, low energy treatments that can support sustainable aquaculture production on a global scale.

The high diversity of plant species and their associated rhizoflora, and the favorable climate and long growing seasons typical of semitropical and tropical regions make phytoremediation an attractive and practical option in many developing countries. Although this chapter will focus on fish aquaculture in tropical systems, many aspects of the basic design features presented here could be applied to facilities in other climates and to the production of other organisms including crustaceans, molluscs, reptiles, and plants.

The approach and selected scenarios of integrated aquaculture–phytoremediation systems (IAPS) provided in this chapter are not intended to be all inclusive but rather serve as selected examples of potential applications. Appropriate IAPS will be highly site specific and will depend on local conditions including geomorphology, water sources, levels of ambient soil and water contamination, the aquatic species under aquaculture, and the type of culture system used. Sustainable IAPS will require interdisciplinary collaboration between local farmers, agriculture and fisheries scientists, engineers, and government officials.

11.2 The Global Aquaculture Industry

Current aquaculture production is on the increase representing the fastest growing sector in global livestock production [1]. The global aquaculture industry contributed 43% of all aquatic animal food for human consumption in 2007 (e.g., fish, crustaceans, and molluscs, not including mammals, reptiles, and aquatic plants) and is expected to grow further to meet the future demand. Freshwater ponds and tanks were the source for 60% of the world aquaculture production in 2008 (56% by value), despite only using 3% of the planet's water. The rapid growth noted in the production of carnivorous species including salmon, shrimp, and catfish has been driven by globalizing trade initiatives and the positive economics of larger scale intensive farming approaches [2].

The impact of climate change on future food supplies and global food security is uncertain and fisheries activity will undoubtedly face some effects influencing the sources of protein from fish and other aquatic species. Fish are an important source of protein for a substantial proportion of the world's population [3]. A portion of 150 g of fish can provide about 50–60% of an adult's daily protein requirements. In 2010, fish accounted for 16.7% of the global population's intake of animal protein and 6.5% of all protein consumed. Moreover, fish provided more than 2.9 billion people with almost 20% of their intake of animal protein, and 4.3 billion people with about 15% of such protein. Fish proteins can represent a crucial nutritional component in some densely populated countries where total protein intake levels may be low [4].

The current trend towards enhanced intensive systems with key monocultures remains strong and, at least for the foreseeable future, will be a significant contributor to future supplies. Dependence on external feeds (including fish), water and energy are key issues. Some new species will enter production and policies that support the reduction of resource footprints and improve integration could lead to new developments as well as reversing the decline evident in some more traditional systems [2].

11.3 Aquaculture in Southeast Asia

Aquaculture in Asia represents over 80% of total global production [4], and the total quantity of fish is projected to reach more than 95.6 million tons by 20,130 [5]. Southeast Asia has seen a significant increase in aquaculture beginning in the 1990s [6]. Aquaculture represents a major component of the food security and overall economies of several countries in South East Asia [7] including Thailand, Vietnam, and the Philippines. Thailand and Vietnam in particular are increasing their aquaculture activities in response to both an increasing global market and local demand accompanied by a leveling off of the yield from capture fisheries. The development of freshwater aquaculture in the Philippines, associated environmental impacts, and relevant environmental regulations and regulatory bodies was recently reviewed by Legaspi et al. [8]. They described the complex relationship between aquaculture and water quality and provided data from studies on Lake Mohicap to illustrate the potential role of paleolimnology as a tool to help achieve a more ecologically sustainable lake-based aquaculture in the Philippines.

Freshwater aquaculture in Thailand and Vietnam is mainly for domestic consumption and provides a good protein source for local use and also bolsters local food security. Small-scale freshwater aquaculture is currently providing the rural poor with high quality protein food for local consumption. Brackish water aquaculture can produce profitable products for both in-country use and export from both countries [9].

Freshwater aquaculture, mainly pond and rice-field culture, has been practiced in Thailand for more than 80 years. In 2003, total production from freshwater and brackish water aquaculture in Thailand was approximately 320,000 and 450,000 tons, respectively. The main freshwater species cultured were Nile tilapia (*Oreochromis niloticus*), hybrid catfish (*Clarias macrocephalus* X *C. gariepinus*), silver barb (*Barbodes gonionotus*), giant river prawn (*Macrobrachium rosenbergii*), and snakeskin gourami (*Trichogaster pectoralis*). The main brackish water-cultured species were giant tiger prawn (*Peneaus monodon*), whiteleg shrimp (*Penaeus vanamei*), green mussel (*Perna viridis*), blood cockle (*Anadara* spp.), and oyster (*Crassostrea commercialis*). At present, more than 50 freshwater aquatic species have been cultured [9].

Nhan et al. [6] provides a detailed description of the general operation and basic economics of integrated freshwater aquaculture, crop, and livestock production using Integrated Agriculture–Aquaculture (IAA) farming systems in Vietnam. Madsen et al. [10] has studied the freshwater snail populations in Asia and provides an excellent description of the snail disease vectors including those found in integrated fish-livestock ponds in common use by families in Vietnam [10–12]. The integrated systems described by Dung et al. [12] consist of a garden (Vaun), a fish pond (Ao Ca), and a cattle shed (Chuong) and are referred to as family VAC ponds (Fig. 11.1).

Recognizing the potential of aquaculture, since 1999, the Vietnamese government promoted diversification in agriculture, aiming to reduce the share of rice to the total agricultural output value while increasing the contribution of aquaculture

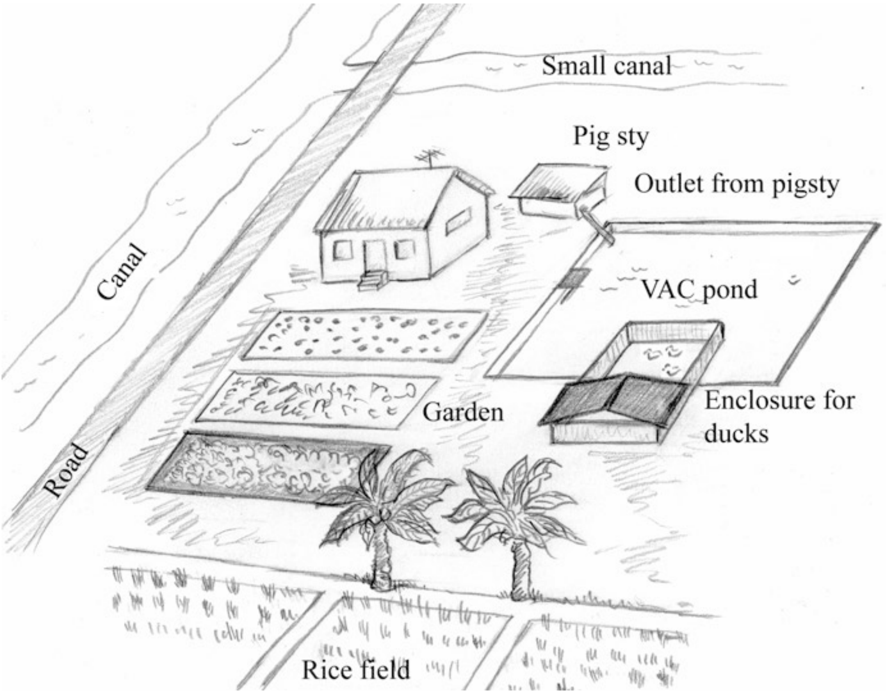


Fig. 11.1 Schematic representation of a VAC pond (Dung et al. [12], with permission of Elsevier)

to economic growth and poverty reduction [13–15]. In this context, stimulating integration between fish, shrimp/prawn, fruit, livestock, and rice production on the same farm, further referred to as integrated agriculture–aquaculture (IAA) systems, is expected to contribute to agricultural diversification and enhance its sustainability. In Vietnam, IAA-farming has been promoted through mass organizations such as the Vietnam Gardening Association and Government Agricultural Extension Agencies [6].

An important characteristic of IAA-farming is the recycling of nutrients between farm components [16, 17]. Through nutrient recycling, IAA-farming allows intensification of production and income, while reducing environmental impacts [18–20]. Intensive export-oriented *Pangasius* sp. culture in both cages and ponds is characterized by large nutrient flows supported by the use of off-farm feeds and water exchange making local nutrient recycling problematic [21–23]. Moreover, the industrial scale of the business and its sensitivity to fluctuations in global trade make it risky and the domain of the resource-rich [24]. IAA-farming in contrast appears to be a realizable approach for diversification of rice production whereby synergism between on-farm components can be realized and whole system productivity optimized rather than that of individual enterprises [18, 25].

The potential integration of farm components and attainable intensification levels of IAA-systems are in part determined by the biophysical setting and the farmer's aspirations and decisions [26, 27]. In Vietnam, the benefits of traditional VAC (garden-pond-livestock)-integrated systems [13–15] have been widely reported and recent studies have investigated IAA commercial orchard and fish production systems [6].

In the Mekong delta, freshwater IAA-farming is commonly practiced in the central region, where soil and hydrological conditions are favorable for aquaculture. Development agencies have tended to promote a rather standardized IAA-system for the region in a "conventional, linear" approach (cited in [28]). Within the central zone of the delta, however, different agro-ecologies exist and market opportunities for farming inputs and outputs differ. In particular differences between rural and peri-urban areas are likely and might be expected to have an impact on optimal forms of IAA. In Northeast Thailand, Demaine et al. [29] found that location relative to urban centers was more important than agro-ecology in determining farmer attitudes and any likelihood of intensification. Better market accessibility in peri-urban areas and access to nutrients often stimulates intensification of aquaculture compared with more rural areas [30], allowing IAA-farming to raise income and to produce cheap food for urban consumers [18].

11.4 Potential Designs of Integrated Aquaculture–Phytoremediation Systems

11.4.1 Chemical and Biological Contaminants

Sources of water used to supply aquaculture systems are often contaminated with organic and inorganic contaminants and disease causing microorganisms. For example, one major environmental challenge evident in many aquaculture systems in Southeast Asia and other areas of the world is the presence of freshwater snails that vector human and livestock diseases. Some aquaculture systems follow a polyculture approach that simultaneously produces fish, snails, and other aquatic food species for human consumption. Snails that are intermediate hosts of fish-borne zoonotic trematodes are of special concern in VAC ponds and other types of aquaculture systems. Dung et al. [12] provided an excellent description of the distribution of freshwater snails in family-based VAC ponds and associated water bodies with special reference to the intermediate hosts of fish-borne zoonotic trematodes in Nam Dinh Province, Vietnam.

In addition to the human and livestock health threats from aquatic species infected with chemical contaminants, parasites, and other pathogens, reduced marketability of fishes, snails, and other aquaculture products harboring disease organisms warrants control efforts to reduce contamination in aquaculture systems.

11.5 Aquaculture Systems in Vietnam

Figure 11.1 provides a schematic representation of a VAC pond in Vietnam [12]. High levels of nutrient pollutants (e.g., nitrogen and phosphorus) from both external and internal sources are of particular concern. Standard operations used to culture aquatic plants and animals for human and livestock consumption typically contribute additional contaminants and pathogens as waste products through the use of water contaminated with biological and chemical contaminants. These contaminants enter the aquatic food web supporting aquaculture operations and can accumulate in fish, snails, and other aquatic food sources. The lower water quality that results from the contamination contributes to both reduced overall yield of product and increases the risk of contaminated product unfit as human and livestock food sources.

Figure 11.2 presents pond nutrient flows in an integrated aquaculture, crop, and livestock system (IAA-farming) in the Mekong Delta of Vietnam [6]. Low, medium, and high pig waste nutrient input to fish farming—fruit production systems are presented as examples. The main motivations for practicing IAA-farming included increased income and food for home consumption from available farm resources while reducing environmental impacts. Fear of conflicts from the use of pesticides was given as one reason that some farmers chose to not use aquaculture.

11.6 Protocol for Integrating Phytoremediation with Aquaculture Systems

11.6.1 *Assessing Water Quality*

The first step in the development of an effective integrated aquaculture–phytoremediation system is a local water quality assessment. Major water quality problems resulting from typical freshwater pond and stream aquaculture systems are listed in Table 11.1. Increased total suspended and dissolved substances, increased biochemical oxygen demand, dissolved oxygen depletion, and increased and excessive phytoplankton which can include toxic blooms are of particular concern. Table 11.1 also lists specific contaminants in typical aquaculture systems including unionized ammonia, nitrates and nitrites, heavy metals/metalloids, (e.g., As, Al, Cd, Cu, Pb, Zn), organics (malachite green, pesticides, algicides, herbicides, petroleum hydrocarbons), and microbial pathogens and parasites (bacteria, viruses, protozoa, trematodes, cestodes, nematodes). Some of the parameters in Table 11.1 can be estimated on-site using portable field-testing kits while others will require lab testing off-site. Paleolimnology can be used to characterize the water quality history of lakes and medium to large ponds serving as aquaculture systems [8].

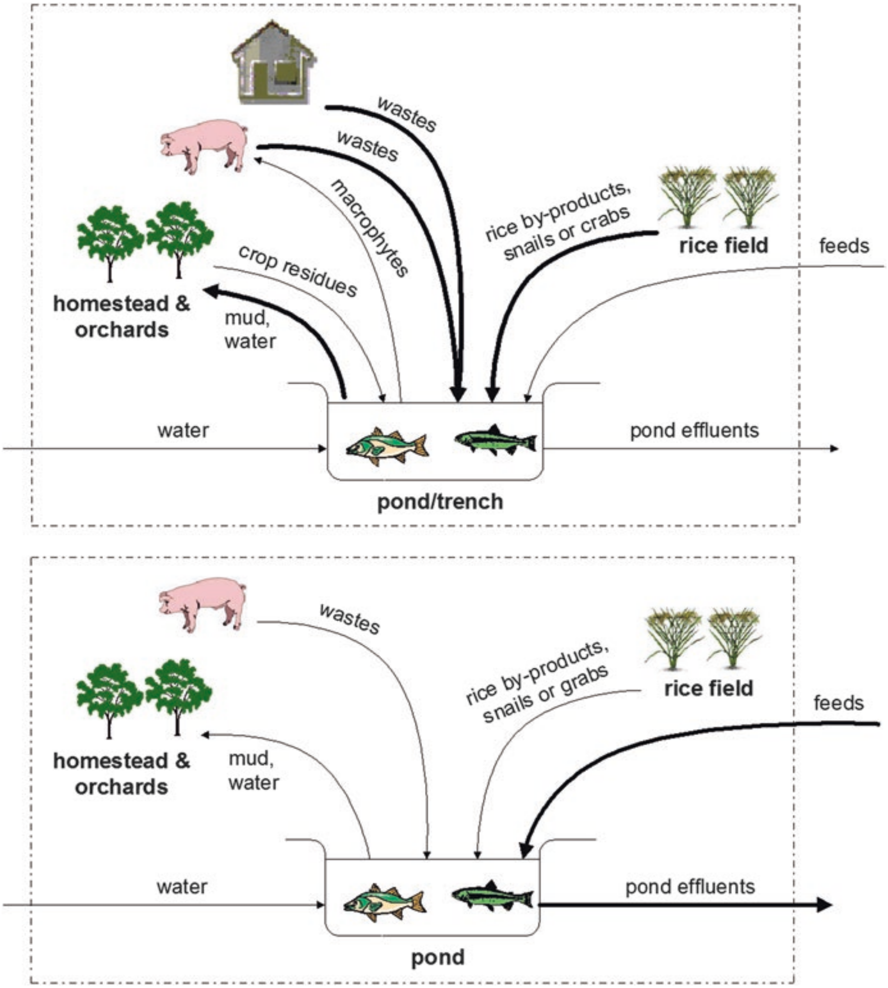


Fig. 11.2 Pond nutrient flows in an integrated aquaculture, crop, and livestock system (IAA-farming) in the Mekong Delta of Vietnam (Nhan et al. [6], with permission of Elsevier)

11.7 Selecting Plant Species

Phytoremediation options designed to control and treat the identified contaminants in aquaculture systems will also be very site specific and must be carefully planned to accommodate the individual characteristics of a particular aquatic system. Design parameters must allow for the integration of phytoremediation processes with the basic operational schemes of common aquaculture systems. Native plants with a relatively rapid growth rate and high biomass production are the most effective

Table 11.1 Water quality problems and selected contaminants in aquaculture systems

Water quality problem	Selected contaminants
Suspended and dissolved substances	Inorganic and organic materials—TSS, TDS
Nutrient loadings	Nitrates, nitrites, phosphorus, unionized ammonia
Oxygen depletion—Biochemical Oxygen Demand (BOD)	Dissolved organics, sediment oxygen demand
Increased phytoplankton and toxic blooms	Oxygen depletion, organic contaminants, microbial toxins
Increased inorganic contaminants	As, Al, Cd, Cu, Pb, Zn)
Increased organic contaminants	Malachite green, pesticides, algicides, herbicides, petroleum hydrocarbons
Microbial pathogens and parasites	Bacteria, viruses, protozoa, trematodes, cestodes, nematodes

candidates for the phytoextraction and phytostabilization of specific contaminants common in aquaculture operations. Basic knowledge about plants and water quality characteristics may be available from farmers and other local residents involved in IAA activities.

Care must be taken to avoid competition between the plant and microbe communities used to treat/remove contaminants and the processes required for cost-efficient aquaculture operations. For example, livestock and crop wastes are typically directed to aquaculture systems to fertilize the biological community that provides food for fish, snails, and other aquatic herbivores under culture (see Figs. 11.1 and 11.2). The integrated aquaculture–phytoremediation design must provide a good balance that insures both the removal of excess nutrients and an adequate supply of nutrients to support the growth of the aquaculture products. Major considerations for an integrated and sustainable phytoremediation–aquaculture system include the specific locale of the facility pond or river, climate/local weather patterns, hydrology, general land use patterns in the surrounding area, and the sources and types of major biological and chemical contaminants entering the water and sediments.

Phytoremediation research in Southeast Asia has expanded considerably during the past 15 years beginning with the initial research completed at Mahidol University and later at Burapha University in Bangkok and Bangsaen Thailand. There is a good database of plant species available in the published literature describing plants used for various applications of phytoremediation in developing countries (see for example [31]).The database can be one good source of appropriate plant species for use in designing integrated aquaculture–phytoremediation systems. Several basic factors should be considered in the process of plant selection. For example, plants used in shoreline and inflow/outflow areas should be chosen on the basis of their growth characteristics in different soils and sediments and their compatibility with other plants in the community.

Table 11.2 Examples of phytoremediation treatment options for aquaculture systems using constructed communities of plants, algae, and bacteria

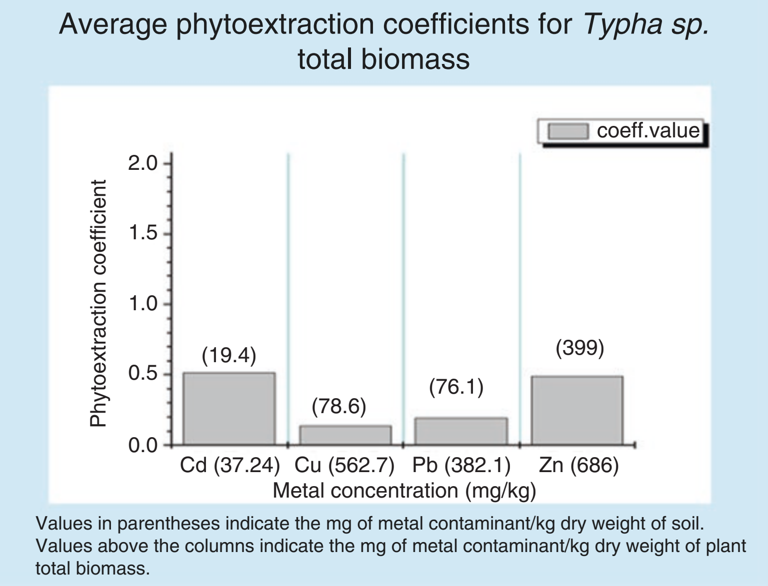
Contaminant removal system	Plants
Vegetative filter strips (VFS) [32–35]	
Pesticides	*Iris versicolor*, *Trypsacumdactyloides*, *Andropogongerardii*, *Salix nigra*
Petroleum hydrocarbons (TPH)	*Trifolium* sp., *Festua* sp., *Cynodon* sp.
Heavy metals/metalloids	*Vetiveria* sp., *Chrysopogon* sp., *Chromolaena* sp., *Typha* sp., *Leersia* sp., *Tagetes* sp., *Acidosasa* sp.
Natural and constructed wetlands [32, 36, 37]	
BOD, TSS, nutrients, heavy metals/metalloids, organics/malachite green, coliform bacteria, parasites	*Carex* sp., *Cyperus* sp., *Typha* sp., *Phragmites* sp., *Juncus* sp., *Rhizophora sp.*, *Panicum* sp., *Leersia* sp.
Limnocorrals/cages/net pens/hydroponic rafts [38–44]	
BOD, TSS, nutrients, organics/malachite green, metals/metalloid	*Lemna* sp., *Eichornia* sp., *Hydrilla* sp., *Ceratophyllum* sp., *C. indica*

11.8 Design Parameters for Integrated Aquaculture–Phytoremediation Applications

The pond and river areas available for the application of phytoremediation options to control and treat contaminants in aquaculture systems include (1) water supplying the ponds through direct inputs from inflow channels/canals and indirect inputs from non-point source runoff, (2) sediments in the ponds and rivers, (3) bank areas immediately surrounding the ponds and rivers, and (4) water exiting the pond through outflow channels/canals or downstream flow in rivers. Table 11.2 provides selected examples of potential phytoremediation treatment options for aquaculture systems experiencing common contaminants. Food security and water pollution are of increasing concern, especially in developing countries. Biomass removed from the aquaculture pond or river can be composted, used as fuel, or as food for humans and livestock if the concentration of toxic contaminants is low enough.

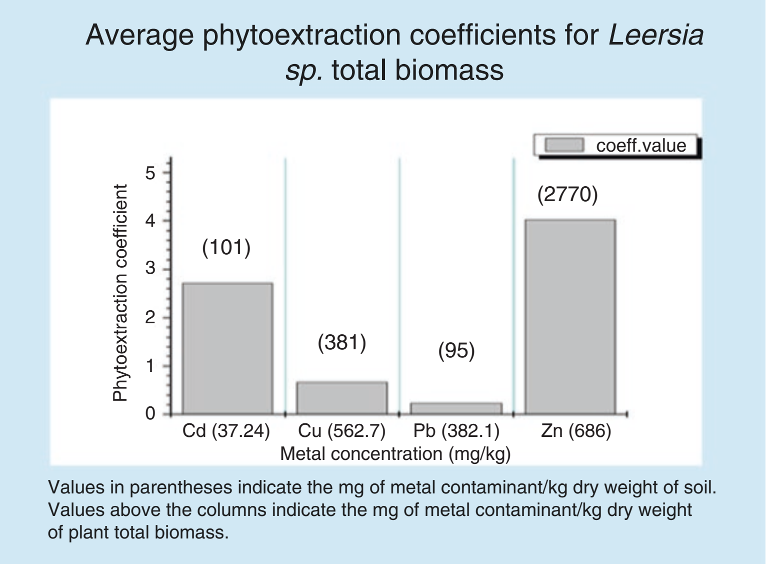
11.9 Vegetative Filter Strips and Natural and Constructed Wetlands

Vegetative filter strips (VFS) can be applied to areas immediately surrounding IAA/VAC pond shoreline areas (see Figs. 11.1 and 11.2) and to the inflow and outflow areas of the facility. The VFS plant community can be constructed using compatible native plants that are known to be effective in the treatment of specific organic and inorganic contaminant mixtures. In many cases, decorative plants including blue flag iris and marigolds (e.g., *Iris* sp., *Tagetes* sp.) with good phytoremediation potential can provide value-added benefits to farmers as products sold to floral dealers.



Values in parentheses indicate the mg of metal contaminant/kg dry weight of soil. Values above the columns indicate the mg of metal contaminant/kg dry weight of plant total biomass.

Fig. 11.3 Average phytoextraction coefficients for *Typha* sp. total biomass



Values in parentheses indicate the mg of metal contaminant/kg dry weight of soil. Values above the columns indicate the mg of metal contaminant/kg dry weight of plant total biomass.

Fig. 11.4 Average phytoextraction coefficients for *Leersia* sp. total biomass

Natural and constructed wetlands can be used to compliment VFS communities especially at the inflow and outflow areas of an aquaculture pond. Plants used in VFS and/or constructed wetlands should be matched to soil or sediment types similar to their normal habitat. For example, *Typha* sp. grows best in wet, saturated soils

Table 11.3 Inorganic contaminant uptake by *Leersia oryzoides* in different soils

Contaminant (mg/kg)	Soil type 1,2,3[a]	Roots	Shoots	SRQ**
As	1	30	10	0.30
AS	2	140	140	1.00
Cd	3	86	16	0.18
Cu	3	370	11	0.30
PB	3	91	4	0.04
Zn	3	1770	1000	0.60

All soils had percent organic content by weight between 1 and 6. Shoot/Root Quotient Data from Lanza [32], Amphia-Bonney et al. [45]

[a] Soil types 1 = Standard loam, 2 = Potting soil, 3 = Wetland sediments

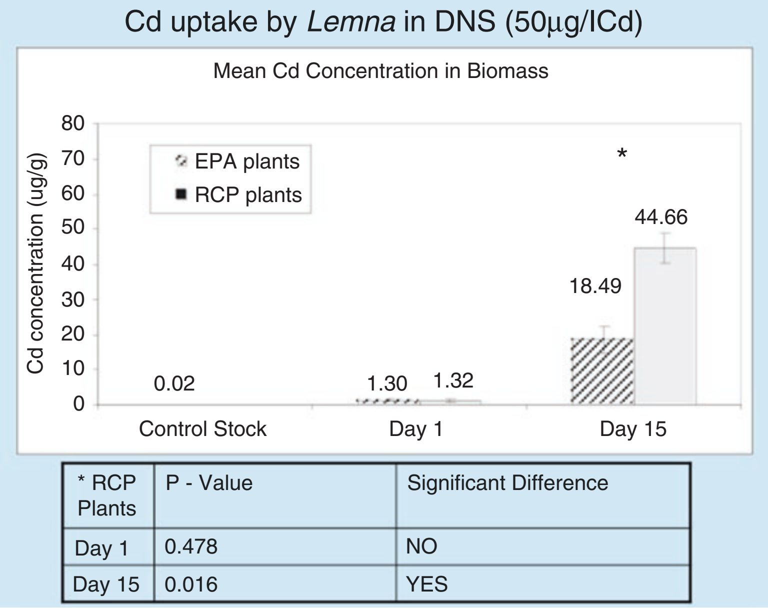
while *Leersia* sp. favors moist to dry soils. Although erratic phytoextraction patterns may occur over time, both plants can effectively remove small to moderate amounts of heavy metals/metalloids, thus preventing the contaminants from entering the aquaculture system and its food web.

Figures 11.3 and 11.4 provide examples of typical phytoextraction coefficients seen with *Typha* sp. and *Leersia* sp. from an industrial area in the USA heavily contaminated with TPH, PCB, and several heavy metals [32]. Table 11.3 displays root and shoot contaminant removal and Shoot/Root Quotients of *Leersia* sp. in test sediments and soils with varying organic content [32, 45]. Total or partial plant removal can eliminate some of the contaminants using successive plantings over time.

11.10 Limnocorrals, Cages, Net Pens, and Hydroponic Rafts

Treatment of contaminants in the pond, river basin, or canals can be accomplished with plants housed in containment structures including limnocorrals, cages, net pens, and hydroponic rafts. The site-specific characteristics of the aquaculture operation will determine which type or combination of containment structures is best suited for integration with the aquaculture process. The interaction of different contaminants (e.g., cadmium and zinc) and humic substances are important determinants of contaminant behavior and removal [38] and should be considered in designing a system. The specific absorption/adsorption characteristics of the plant are also important considerations in the planning and design of integrated aquaculture–phytoremediation systems.

In some cases, more than one type of containment structure can be used over time. Caged floating plants could be used simultaneously with hydroponic rafts. Floating Treatment Wetlands (FTW) with *C. indica* [39] could be used along with caged *Eichornia* sp. or *Lemna* sp. Since all aquatic plants absorb contaminants and then release them back to the environment when they die and decompose, the containment structure must be periodically removed, cleaned out, and repacked with



* RCP Plants	P - Value	Significant Difference
Day 1	0.478	NO
Day 15	0.016	YES

Fig. 11.5 Cadmium uptake by *Lemna* sp.

fresh plants. For example, water hyacinth (*Eichornia* sp.) and duckweed (*Lemna* sp.) listed in Table 11.2 absorb nutrients, heavy metals/metalloids, and other contaminants from water. In addition to inhibiting phytoplankton growth by competing for nutrients, the plants remove toxic contaminants such as cadmium. Studies using *Lemna* sp. collected from Rice City Pond (RCP) a cadmium-contaminated pond in the USA (see Fig. 11.5) and an USEPA reference culture of *Lemna sp.* cultivated in synthetic water (DNS) showed very good removal of cadmium after 2 weeks of culture. Using a “put and take” approach with the containment structures will prevent the return of the contaminants to the pond or river by removing biomass before death and decomposition.

11.11 Summary and Future Research Needs

Food security and water pollution are of increasing global concern, especially in developing countries. Methods to simultaneously augment food production and decrease water pollution can be valuable additions to current aquaculture operations. IAPS offers a new approach to create sustainable aquaculture systems that can provide green, low energy-low technology solutions in developing countries. If the concentration of toxic contaminants is low enough, biomass removed from aquaculture ponds or rivers can be composted, used as fuel, or as food for humans and livestock. Composting biomass can significantly reduce the volume of plant material, but contaminated biomass would have to be safely disposed of in landfills or other appropriate storage areas [46].

Typical biomass from plant material contains varying amounts of stored energy as oxygenated hydrocarbons biomass. As a result, it can serve as a reliable source of fuel if the amount produced merits collection and storage. The dry weight of *Brassica juncea* used to phytoextract lead from soil produced 6 tons biomass per hectare with 10–15,000 mg/kg lead content [47]. The use of biomass for fuel may be feasible as an augmentation to traditional solid fuels combusted under controlled conditions that do not release contaminants to the atmosphere. In the case of biomass use as food for livestock and humans, studies of nutrient removal by *Lemna* sp. from two ponds in Brazil indicated that the ponds together produced over 13 tons of biomass (68 t/ha year of dry biomass), with 35% crude protein content [36].

Using Integrated Aquaculture–Phytoremediation Systems (IAPS) can greatly enhance the global production of plant and animal food particularly in developing countries with warmer climates and highly diverse plant communities. Although IAPS will remain site specific, additional research can clarify the most efficient plant communities for many common types of aquaculture systems based on general similarities in the aquatic products grown and the waste types and loadings typically used to fertilize the food web supporting production. Information on IAPS that create a balance between the livestock fertilization supporting good aquatic product growth and excess fertilization that leads to undesirable water quality that impedes good aquatic product growth will be very useful.

The presence of various microbial pathogens and parasites present a major challenge to sustainable IAPS and aquaculture systems in general. Additional research is needed to develop IAPS that provide the effective removal of disease causing organisms common in aquatic systems used for aquaculture. One good example is provided by fish-borne zoonotic trematodes (FZT). Current research indicates that fish-borne zoonotic trematodes FZT such as *Clonorchis sinensis*, *Opistorchis viverini* (*Opisthorchiidae*), and intestinal trematodes of the family *Heterophyidae*, constitute a public health hazard in Vietnam. These parasites have been linked to consumption of raw or undercooked fish from aquaculture [11]. The FZT transmission pathways, however, are more complicated than just the presence of intermediate snail hosts in aquaculture ponds as ponds may exchange water with surrounding habitats such as rice fields and irrigation canals (see Fig. 11.1), and these surrounding habitats may be a source of snails and cercariae and contribute to FZT infection in cultured fish [11].

The fact that snails are often harvested as food from aquaculture ponds and rivers complicates the problem of FZT. Research is needed to clarify the possible inclusion of carnivorous plants in phytoremediation communities used in IAPS (see Table 11.2). Plants in the bladderwort group (e.g., *Utricularia* sp.) inhabiting wet soils and water are known to actively trap and consume aquatic animals including mosquito larvae and tadpoles [48]. It may be possible to use bladderworts to remove immature snails, snail eggs, miricidia, and cercariae as a treatment option in IAPS. IAPS may contribute to providing a holistic approach to deal with all stages of the FZT transmission cycle.

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Part IV
Special Applications of Phytoremediation

Chapter 12
Modelling Phytoremediation: Concepts, Models, and Approaches

Edita Baltrėnaitė, Pranas Baltrėnas, and Arvydas Lietuvninkas

Abstract In the phytoremediation modelling stage, which is specific due to unavoidable assumptions and limitations, the complicated nature of natural processes, and different qualifications of model developers result in the variety of phytoremediation-oriented models that differs in complication and the extent of applicability. The variety of phytoremediation models is not only naturally understandable, but also serves specificity of model application. In other words, the choice of a model and the need for detailed result depend on the prospects of the model use, e.g., for preliminary assessment of the phytoremediation effect, phytoremediation cost estimation or contaminant distribution among the plant compartments. This chapter discusses the prospects of application of the phytoremediation assessment tools, such as Phyto-DSS, BALANS, Dynamic factor method, and Hung and Mackay model used for simulating the contaminant transfer processes in the soil–plant–atmosphere system.

Keywords Phytoremediation • Modelling • Dynamic factors • BALANS • Phyto-DSS • Uptake • Chemical element

12.1 Introduction

The topic of phytoremediation, though rather new, e.g., interest in it peaked in the EU in 2002–2007, has already reached the stage of modelling of biogeochemical processes for this purpose. In the modelling stage, which is specific due to unavoidable assumptions and limitations, the complicated nature of natural processes, and different qualifications of model developers result in the variety of phytoremediation-oriented models that differs in complication and the extent of applicability. On the other hand, the variety of models is not only naturally understandable, but also serves specificity of model application. In other words, the choice of a model and the need for detailed result depend on the prospects of the model use, e.g., for preliminary assessment of the phytoremediation effect, phytoremediation cost

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estimation or contaminant distribution among the plant compartments. This chapter discusses the prospects of application of models used for modelling the metal transfer processes in the soil–plant–atmosphere system.

12.2 Different Prospects of Using Phytoremediation Models

The selection of a model for phytoremediation depends on the objectives of the operator, the tasks for applying the model, the advantages and limitations of the model, and the comprehension level of the result. Different models can be used by operators that represent particular professions, e.g., environmental researcher, environmental officer, environmental consultant, and owner of a contaminated site.

There are different prospects for use of a particular phytoremediation model. For instance, an environmental researcher might be focused on chemical element concentration in different plant compartments using a model that involves a detailed mechanism of chemical element uptake, translocation, and bioaccumulation mechanisms. Changes in the phytoremediation-related processes after soil treatment in comparison to the background situation are an important feature in understanding of process mechanisms. A food safety specialist might need more detailed simulation mechanisms to forecast contaminant concentration in plant compartments used for forage and the risk of contaminant entering the food chain. A soil researcher might be interested in the effect of soil chemical element concentration changes on the chemical element concentration in the plant [1]. An environmental officer might be looking for a model that would assist in assessment of environmental risk posed by contaminated sites before and after the use of phytotechnologies. A consultant on environmental matters might need to estimate the time required to extract chemical elements till the target concentrations in soil to evaluate the phytoremediation techniques compared to a range of other soil restoration options or to estimate the costs of phytomediation activities. An owner of a contaminated site is usually interested in decontamination costs and the efficiency of the technique based on a more spatial/regional level, and for this reason the model must be robust and estimating the macro-effects of the site. Models that integrate a soil amendment effect on the contaminant background level in soil, the effect of uptake of a contaminant by plants, and its removal by leaching in soil can assist in defining the critical loads of contaminants with soil amendments and justifying the environmental regulations.

12.3 Detailed Models vs. Robust Models

In selecting a phytoremediation model, it is important to make a clear distinction between steady-state and dynamic models. The steady-state models provide a prognosis for infinite time, while dynamic methods are more useful in predicting a time period before the modelled variable reaches the target value. Depending on the aim, one must choose a model with an appropriate level of detail in describing the processes. A disadvantage of relatively complex mechanical models is that input data for their application on a regional level is generally incomplete and values can only be roughly estimated. Even if the model structure is correct (or at least adequately represents current knowledge), the uncertainty of the output of complex models may thus still be large because of the uncertainty of input data. Simpler empirical models have the advantage of a smaller need for input data, but the theoretical basis, that is needed to establish confidence in predictions, is small, which limits the application of such models for different situations. Therefore, there is a trade-off between the model complexity (reliability) and regional application [2].

When the aim of a model is to evaluate the phytoremediation on the regional scale consisting of the receptors with different properties, it seems most rational to use a relatively simple model with an aggregated description of processes in the total considered compartment. In choosing a model, one should be aware of the consequences of simplifications, such as ignoring certain processes (complexation, chemical element cycling, etc.) and making certain assumptions (steady-state, homogenous mixing, equilibrium partitioning) [2]. In order to gain insight into the consequences of the choice of a certain model and limits, one could perform modelling with different models using various limits and compare the results. In such a way, one also gets insight into (1) the differences in vulnerability of various environmental compartments and (2) the relevance of the different processes in the systems and of the different ways of parameterizing certain processes [2].

12.4 Examples of Chemical Element Uptake Simulation Methods: Advantages and Limitations

A group of models that are described below are the examples of different modelling approaches towards chemical element uptake by plants in relation to the phytoremediation effect. The scope and a principal process of modelling, as well as advantages and limitations of the models are discussed.

12.4.1 Plant-Oriented Models

This program was named as a decision support system (DSS) and was intended for predicting the effect of phytoextraction (phytoremediation) on soil metal concentration and distribution, as well as the economic feasibility of the process in comparison to other ways of land reclamation, i.e., natural attenuation (or inaction) or the best alternative technology. The model is based on the transfer of metals through transpiration flow, and therefore water transfer through a plant and the solubility of metals in water is a basis for the algorithm for the model. Within the model, the focus is given to the soil metal concentration and the changes of it are predicted by modelling the plant water use, taking into account the metal concentration in soil solution, soil density, plant root distribution, and introduced root-absorption factor. The root-absorption factor represents the metal concentration quotient within the system "root xylem/soil solution" and lumps up the number of complex process factors that influence the uptake of metals by plant roots [3].

The model accepts a precondition that the potential uptake of chemical substance *i* through the plant of certain species *n* depends on the density of the root system. To enter the plant, the concentration (mg/kg) of chemical substance *i* at the depth *d* of soil changes, and this variation is calculated according to Eq. 12.1:

$$\Delta M_{i,z} = \frac{1}{\rho_z}\int_0^t R_z TC\varphi dt \tag{12.1}$$

where ΔM_z is variations in the concentration (mg/kg) of chemical substance *i* at the depth of soil *z*; ρ_z is bulk density (g/cm^2) of soil at depth *z*; *t* is time, days; R_z is a part of the mass of plant roots (mass of the root system at the depth of soil *z* divided by the general mass of the root system).

Among the model limitations, there are several ones including the following. Environmental conditions, such as drought, that may limit the plant growth, are not considered in the model. Root absorption factor values may vary because the metal uptake by a particular species would be different in different soil types. The model does not consider the fact that metals from the aerial parts may be further translocated within the phloem back to the below-ground compartments [4]. The model considers the uniform moisture distribution in the soil, and these conditions are not typical in a real situation. Moreover, the depth-related distribution of fine roots is not equal to the total uptake of metals by roots. This causes the remediation time to be shorter than actual. Using Phyto-DSS model, the precise initial data is required to forecast future biomass growth and metal accumulation, such as root development, metal and water uptake rates. The climate conditions must be considered very precisely because the model is sensitive to changes in water regime (rainfall, evapotranspiration). The model mainly considers metal accumulation in the above-ground plant biomass as the principal feature for phytoremediation. It also provides no possibility to simulate mixed plantations, which is usually a practical consideration in many phytoremediation projects.

Later Liang et al. [5] has modified the phytoremediation evaluation approach (Eq. 12.1) by incorporating iterative concepts (Eq. 12.2):

$$t = \frac{M_\mathrm{i} - M_\mathrm{f}}{C_{\mathrm{p}i} \cdot M_\mathrm{p}} = \frac{1000 \cdot d_\mathrm{s} \cdot A \cdot h \cdot \left(C_{\mathrm{s}i} - C_{sf}\right)}{C_{\mathrm{p}i} \cdot M_\mathrm{p}},\ i = 0,1,2,\ldots,f-1 \tag{12.2}$$

where *t* is the cycle number needed for phytoremediation (cycles), *i* is the cycle number used for phytoremediation, M_i is the initial metal concentration in soil (g/ha), M_f is the target metal concentration in soil (g/ha), M_p is the biomass production of plant species (tons/ha/cycle), d_s is the soil density (kg/m^3), *A* is the contamination area (m^2), h is the soil depth with the metal content (m), C_{si} is the metal concentration in soil after the ith harvest cycle (mg/kg), and C_{sf} is the final metal concentration in soil (mg/kg).

The transfer of contaminants in the “soil–plant–atmosphere” system was also modelled by Trapp and McFarlane [6], who used the PLANTX model for this purpose. This model considers: (a) the dynamic transfer of contaminants to plants from the soil solution and air; (b) metabolism of anthropogenic contaminants and their accumulation in the roots, stem, leaves, and fruits of the plant. The model is based on the processes of contaminants’ diffusion in the soil solution and the soil pores filled with air and roots as well as their transfer to roots by the transpiration flow, the exchange between the ambient air and leaves through their stomata due to diffusion, and metabolism and distribution of contaminants due to the growth of plants. The model can be used to predict contaminants’ concentration in plants; however, it is intended for considering organic contaminants. Boersma et al. [7] integrated the processes of contaminants’ transport and transformation into one mathematical model, CTSPAC, combining two submodels representing soil and plant, while Ouyang [8] discussed the problem of applying the model to 1,4-dioxane transfer to poplars.

Guala et al. [9] focused on the model of metal transfer from soil to plants based on the mechanism of physiological absorption of plants, which aims to assess the effect produced by it on their growth. This model is limited to the evaluation of Cd and Ni concentrations in the soil and their effect on two cereal crops (rice—*Lolium-perenne* L. and oats—*Avenasativa* L.). The model is based on the physiological mechanisms of metals’ transfer and physiological characteristics of plants described by Moreno et al. [10]. The model refers to the main relationship between the bioavailable form of metal in soil (A) and metal concentration in a tree (S):

$$A = \left(\frac{-c/\alpha + \left(f/\alpha \right) S}{-e + S} \right) \cdot S \tag{12.3}$$

where A is the available concentration of metal M^{n+} (mg/L) in the soil solution; S is the metal concentration in trees (mg/kg); α is the coefficient of absorption (L/kg/year); and c/α, f/α, and e can be fitted by experimental results in order to establish the relationship between A and S.

The model does not involve aerogenic uptake nor the flux of aerogenic contaminants and is focused on inorganic contaminants. As mentioned above, it requires experimental data to define the values of coefficients c/α, f/α, and e.

The translocation of metals from the soil to a tree embraces various chemical, physical, and biological processes (e.g., diffusion of metals in the soil and trees as well as in the area of the roots and the tree itself, adsorption and absorption in the tree and soil, the growth of the tree, and transpiration). Due to the complicated character of these processes and the action of the external factors (e.g., the climatic conditions, type of the substrate), it is hardly possible to accurately describe them by mathematical equations.

Therefore, the models which can be found in the literature describing the transfer of contaminants from soil to trees are rather simplified descriptions, usually based on a few transfer coefficients and the evaluation of the transfer of contaminants only to the whole tree organism. To create more complicated models of contaminants' translocation, huge computing resources, as well as great amounts of experimental data, allowing for validating the accuracy of the developed models, are required.

The other model, representing the uptake of chemical elements from sludge amended soil which is based on the simplified model of the contaminant's transfer from soil to plants offered by Hung and Muckay [11], is described. This model can evaluate the transfer of contaminants from soil to trees through the stem, then from the stem to leaves, from the leaves to the air, from the air to the leaves, from the leaves to the stem, and from the stem to the tree roots. In this model, uptake of contaminants from soil to plants is based on the equilibrium factors, describing the distribution of contaminants in various media (e.g., soil, water, transpiration flow), as well as on the rate of metabolism and time of diffusion. The time of the growth of the main plant organs is also evaluated.

Modelling the uptake of metals to plants is a more complicated problem than that associated with the transfer of organic contaminants (e.g., benzopyrene, naphthalene) because, unlike the organic contaminants, metals are significant for physiological processes occurring in plants, such as their growth, metabolism, and fermentation [12, 13].

Therefore, not only the factor of distribution of metals' concentrations between octanol and water (K_{ow}) is important for the accumulation of metals in plants. In this case, the coefficients of biological uptake of metals by plants and their translocation are also required for modelling.

The main Eqs. 12.4–12.6 providing for the concentrations of contaminants in leaves (C_{l}^{i}), stem (C_{st}^{i}), and roots (C_{r}^{i}), which are used in the present model, are provided below.

$$C_{\mathrm{l}}^{i} = \left(\frac{B_{er} \cdot B_{rs} \cdot B_{sl} \cdot K_{lw} \cdot C_{\mathrm{s}}}{K_{ew}} + \frac{B_{al} \cdot K_{lw} \cdot C_{\mathrm{a}}}{K_{aw}} \right) \cdot \frac{M_{\mathrm{i}}}{\rho_{\mathrm{l}}} \tag{12.4}$$

$$C_{st}^{i} = \left(\frac{B_{er} \cdot B_{rs} \cdot K_{stw} \cdot C_{\mathrm{s}}}{K_{ew}} + \frac{B_{al} \cdot B_{ls} \cdot K_{stw} \cdot C_{\mathrm{a}}}{K_{aw}} \right) \cdot \frac{M_{\mathrm{i}}}{\rho_{st}} \tag{12.5}$$

$$C_{\mathrm{r}}^{i} = \left(\frac{B_{er} \cdot K_{rw} \cdot C_{\mathrm{s}}}{K_{ew}} + \frac{B_{al} \cdot B_{ls} \cdot B_{sr} \cdot K_{rw} \cdot C_{\mathrm{a}}}{K_{aw}} \right) \cdot \frac{M_{\mathrm{i}}}{\rho_{\mathrm{r}}} \tag{12.6}$$

where B_{er} is uptake ratio of the contaminant from soil to roots; B_{rs} is uptake ratio of the contaminant from roots to stem; B_{sl} is uptake ratio of the contaminant from stem to leaves; B_{al} is uptake ratio of the contaminant from air to leaves; B_{ls} is uptake ratio of the contaminant from leaves to stem; B_{sr} is uptake ratio of the contaminant from stem to roots; $\mathrm{C_s}$ is concentration of the contaminant in the soil; $\mathrm{C_a}$ is concentration of the contaminant in the air; K_{ew} is equilibrium partition coefficient of the contaminant between soil and water; K_{lw} is equilibrium partition coefficient of the contaminant between leaves and water; K_{stw} is equilibrium partition coefficient of the contaminant between stem and water; K_{aw} is equilibrium partition coefficient of the contaminant between air and water; K_{rw} is equilibrium partition coefficient of the contaminant between roots and water; M_{i} is molecular weight of the contaminant; ρ_{l}, $\rho_{\mathrm{st,}}$ ρ_{r} are density of plant leaves, stem, and roots, respectively.

The parameters involved in the model by Hung and Mackay [11] are grouped in several groups:

- Growth and metabolism: growth rate of leaves, stem, and roots; metabolism rate of leaves, stem, and roots; duration of plant exposure to metals
- Morphological data of plant compartment: leaf surface area
- Metal characteristics: molar mass of metals, aerogenic concentration of metals, metal uptake/translocation factors within environmental and plant compartments: air/leaves, leaves/stem, leaves/stem, stem/roots, roots/stem, soil/roots; total metal concentration in soil
- Physico-chemical data of plant: transpiration rate, density of leaves, stem, and roots

The processes considered in the presented model are not associated with the specific features of contaminants. For example, the solution of a contaminant in the plant has not been modelled; therefore, this model can be adapted to modelling translocation of contaminants from the soil to a tree. In order to adapt the considered model to modelling the translocation of metals, it was extended to include [12]:

- The factor characterizing the distribution of metal concentrations between the octanol and water (K_{ow})
- The factor characterizing the distribution of metal concentrations between the soil and water (K_{d}), which depends on the soil pH and the amount of the organic material
- The coefficient of metal concentration in water (K_{T})
- The correction factors

12.4.2 Soil-Oriented Model

The model BALANS was developed in the Tomsk State University (Russia) by Prof. Arvydas Lietuvninkas [14]. The model was developed to simulate the self-purification of soil from metals that enter the soil in different ways (aerogenic metal deposition, edaphic metals, and metal entering the soil with amendment). The considered self-purification processes of the soil include: (1) metal uptake and removal with the harvest and (2) the removal of metals from the soil as the result (consequence) of their natural physical–chemical migration (Fig. 12.1) [15].

The deposition of aerogenic metals on the investigated territory is estimated based on the values of the total load of aerogenic aerosols in the form of snow

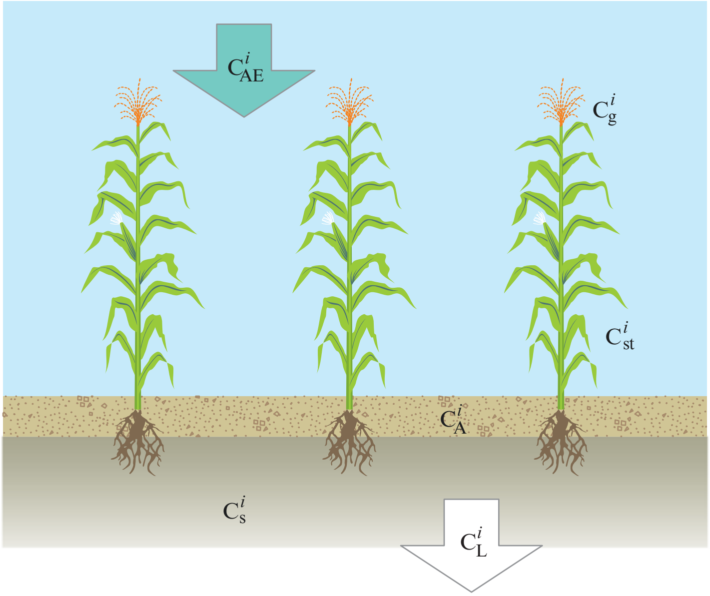

Fig. 12.1 The model BALANS is based on the systemic concept of getting of metals into the soil of the area sown with wheat together with amendments and their removal from it. C^i_{AE} is the concentration of metal *i* in mineral dust of aerogenic origin; C^i_A is the concentration of metal *i* in the amendment; C^i_L is the amount of metal *i* removed due to natural migration; C^i_s is the remaining amount of metal *i* in the amended soil; C^i_g is the concentration of metal *i* in the crop grains; C^i_{st} is the concentration of metal *i* in the crop straws

mineral dust and metal concentration in them [16]. The amount of metals bioaccumulated in the harvested crops is estimated based on biomass removed with crops and metal concentration in it. The natural removal of metals as a result of their physical–chemical leaching in the soil is estimated using empirical annual migration coefficients, corresponding to the respective geographical climatic zone and type of soil. The annual coefficient values of soil metal leaching lump up the complex soil mechanism affecting metal dissolution, sorption, complexation, migration, precipitation, occlusion, diffusion into minerals, binding by organic substances, absorption and sorption by microbiota, and volatilization. This largely facilitates simulation process being more practically attractive. The program uses annual metal leaching coefficients for Cu, Zn, Pb, Co, Ni, Cr, V, and Mo chosen by default from numerous literature sources, which correspond to the types of soil, such as *Albeluvisols* and *Podzols*, characteristic of southern taiga and mixed forest zones [17]. The model provides an option for the operator to change the coefficient values based on changed simulation conditions.

The advantages of the model include: (a) differentiation between the removal of metals by the process of natural physical–chemical migration and together with harvested crops; (b) determining the balance of metals in the “soil–plant–atmosphere” system in the long and short term, which is of particular importance in the

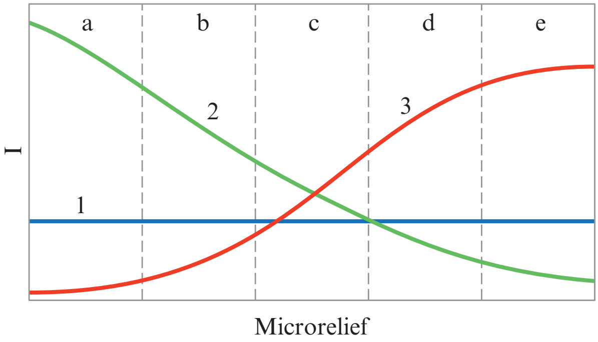

Fig. 12.2 A schematic diagram of the BALANS model describing the removal of metals from the soil (2) and their concentrations in it (3) at the constant load of mineral dust (1): I is the level of these parameters; the type of the field's microrelief: *a* is a strongly pronounced hill; *b* is a weakly pronounced hill; *c* is a flat surface; *d* is weakly pronounced lowland; *e* is strongly pronounced lowland. Note: the cases *b* and *d* are not included in the analysis of the investigation results

context of sustainable development; (c) evaluating the effect of various ways of metals' uptake by the soil (through aerogenic sources and with amendments) and their removal (due to natural migration and with the harvested crops) on the balance of metals in the soil; (d) the integration of the principles of acropetal and basipetal distribution of metals in the plant into the model of their translocation in the "soil–plant–atmosphere" system; (e) the influence of complicated processes affecting the system is expressed by the coefficients of annual contaminants' transfer, thereby creating conditions for effective practical use of the model.

The method takes into account the microrelief of the site and the load of aerogenic contaminants, on which the removal and accumulation of metals in the soil depend [14]. A schematic diagram of self-purification of the soil from metals and their accumulation in the soils, depending on the fields' microrelief, is provided in Fig. 12.2.

The aerogenic load of mineral dust is assumed to be invariable (straight line 1). On the autonomous geochemical landscape basis, the soil metal leaching is highly controlled by the type of relief. The metal leaching is much more intense in the case of autonomous geochemical landscape (a positive type of microrelief, i.e., a strongly pronounced hill) and less intense in the case of superaquatic geochemical landscape (a negative type of microrelief, i.e., strongly pronounced lowland) as expressed by curve 2 (not taking into account the extent of the process) (Fig. 12.3). Nonuniform removal of metals and the polluted soil manifests itself in their different concentration. Thus, the soil of the positive microrelief type accumulates a considerably smaller amount of these materials than the soil of the negative microrelief type (Fig. 12.3, curve 3), and the self-purification of soil is more intense in this case. In real conditions, the above difference is even more affected by the geochemical characteristics of the particular metal and conditions of the site of investigation (e.g., edaphic conditions, including the type and properties of the soil and the metal pollution load).

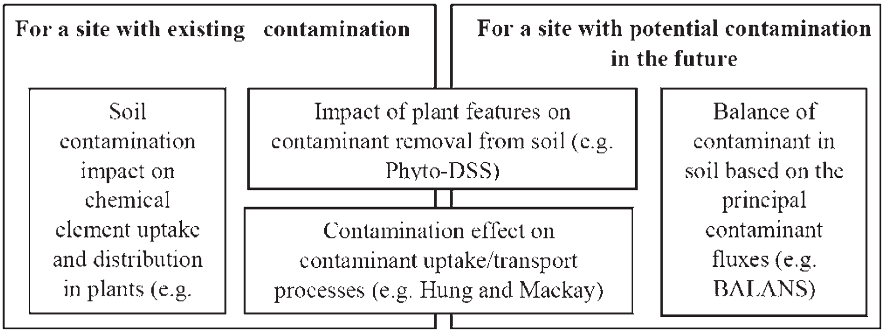

Fig. 12.3 Simulation approaches and their application for sites on two consideration levels, for a site with existing contamination, and for a site with potential contamination

12.4.3 Uptake-Process-Oriented Models

A widely known biological absorption of chemical element is based on the relationship between a plant and the soil. Consequently, it is expressed as the relationship between the concentration of chemical elements in a plant and the soil, in which it grows. This relationship is used to determine the ability of plants to accumulate chemical elements (when its value is more than one, plants are called the accumulators, meanwhile when the relationship value is equal to or is about one, they are referred to as indicators, and when this value is smaller than one, the plants are called "excluders"). The considered relationship is also used for identifying the harmful effect of metals and for determining a risk posed by them to the biota [18]. Mingorance et al. [19] used the term "enrichment factor" to compare the concentrations of metals and other chemical elements in the investigated soil or plant to those in the control objects.

However, the above-mentioned factors/coefficients, expressing the concentration of chemical elements in plants compared to that in the soil, have some drawbacks. From biogeochemical perspective, they reflect the comparison of chemical element concentration in various media (a plant and soil), but this refers only to a particular area and to particular environmental conditions characteristic of this area at a particular period of time (e.g., 10 years after the sludge was spread over the soil) [20]. *First*, from the biogeochemical point of view, the comparison of various plants based on the considered factors/coefficients could hardly be accurate because these plants could have been growing in different conditions, different types of soil, and elementary landscape, which could result in different mobility and accumulation of chemical elements in them. *Second*, it is required to compare not only the concentrations of chemical elements in plants to their concentrations in soil or the control plant, but to compare the differences in the process of chemical element uptake and its intensity with respect to the control case. The more so because in evaluating an uptake process we should compare processes rather than concentrations. *Third*, the numerical value of the relationship between the uptake of chemical elements to the investigated plant and to the control one, which could facilitate the evaluating of the variation of chemical element transfer, is lacking. However, a method based on dynamic factors can provide it. *Fourth*, it is important that the effect of natural processes, influencing the uptake of chemical elements, should be integrated into the estimate. These purposes could be achieved by introducing the higher order factors. They are calculated by comparing the value of chemical element uptake factor obtained for the investigated territory to the respective value for the control territory. The authors suggested these factors for describing five types of chemical element behavior in the soil–plant system, depending on the changes taking place in the soil (Eqs. 12.7–12.11). They are referred to as dynamic factors because of their sensitivity to the changes in variables involved in calculations [13, 21, 22].

The dynamic factor of bioaccumulation

$$BA_{dyn}^{i}=\frac{C_{\mathrm{a\_t}}^{i}\times C_{\mathrm{s\_c}}^{i}}{C_{\mathrm{s\_t}}^{i}\times C_{\mathrm{a\_c}}^{i}} \tag{12.7}$$

where $C_{\mathrm{a\_t}}^{i}$ is the concentration of chemical element *i* in the plant ashes on the treated territory, mg/kg; $C_{\mathrm{s\_t}}^{i}$ is the concentration of chemical element *i* in the treated soil, mg/kg DW; $C_{\mathrm{s\_c}}^{i}$ is the concentration of chemical element *i* in the control soil, mg/kg DW; $C_{\mathrm{a\_c}}^{i}$ is the concentration of chemical element *i* in the plant ashes on the control territory, mg/kg.

The dynamic factor of biophilicity

$$BF_{dyn}^{i}=\frac{C_{\mathrm{b\_t}}^{i}}{C_{\mathrm{b\_c}}^{i}} \tag{12.8}$$

where $C_{\mathrm{b\_t}}^{i}$ is the concentrations of chemical element *i* in the plant biomass on the treated site, mg/kg DW; $C_{\mathrm{b\_c}}^{i}$ is the concentration of chemical element *i* in the plant biomass on the control site, mg/kg DW.

The dynamic factor of translocation

$$TR_{dyn}^{i}=\frac{C_{\mathrm{v\_t}}^{i}\times C_{\mathrm{r\_c}}^{i}}{C_{\mathrm{r\_t}}^{i}\times C_{\mathrm{v\_c}}^{i}} \tag{12.9}$$

where $C_{\mathrm{v\_t}}^{i}$ is the concentration of chemical element *i* in the vegetative organs of the plant growing on the treated territory, mg/kg DW; $C_{\mathrm{r\_t}}^{i}$ is the concentration of chemical element *i* in the roots of the plant growing on the treated territory, mg/kg DW; $C_{\mathrm{r\_c}}^{i}$ is the concentration of chemical element *i* in the roots of the plant growing on the control territory, mg/kg DW; $C_{\mathrm{v\_c}}^{i}$ is the concentration of chemical element *i* in the vegetative organs of the plant growing on the control territory, mg/kg DW.

The dynamic phytoremediation factor

$$FR_{dyn}^{i}=\frac{C_{\mathrm{b\_t}}^{i}\times B_{\mathrm{t}}\times C_{\mathrm{s\_c}}^{i}\times \rho_{\mathrm{c}}}{C_{\mathrm{b\_c}}^{i}\times B_{\mathrm{c}}\times C_{\mathrm{s\_t}}^{i}\times \rho_{\mathrm{t}}} \tag{12.10}$$

where B_t and B_c denote the annual growth of a tree on the control and investigated territories, kg/ha; ρ_c and ρ_t are the soil density on the control and the investigated territory, respectively, g/cm^3.

The dynamic factor bioavailability

$$BIO^{i}_{dyn} = \frac{C^{i}_{\mathrm{bioav\_t}} \times C^{i}_{tot\_\mathrm{c}}}{C^{i}_{tot\_\mathrm{t}} \times C^{i}_{\mathrm{bioav\_c}}} \tag{12.11}$$

where $C^{i}_{\mathrm{bioav\_t}}$ is the concentration of chemical element *i* in the soil solution on the treated site, mg/kg; $C^{i}_{tot\_\mathrm{t}}$ is the total concentration of chemical element *i* in the soil on the treated site, mg/kg; $C^{i}_{\mathrm{bioav\_c}}$ is the concentration of chemical element *i* in the soil solution on the control site, mg/kg; $C^{i}_{tot\_\mathrm{c}}$ is the total concentration of chemical element *i* in the soil on the control site, mg/kg.

In addition to their biogeochemical significance, the dynamic factors have a number of advantages in practical application:

(a) They integrate the information of four various types by combining the data about the amount of chemical elements in two media (or the plant organs) and the data on the control and the polluted (treated) territory into a single value, thereby facilitating the evaluation of chemical element transfer.
(b) They are nondimensional and, therefore, easy to compare.
(c) They eliminate the risk of systematic errors in the analysis [23], thus improving the reliability of the obtained results and the quality of evaluation.

The principal input and output data used in the selected models are provided in Table 12.1.

12.5 Application Types of Phytoremediation Models

The quantitative phytoremediation methods could be classified according to their application field and characteristics. According to the element uptake evaluation level, models can be classified as those providing preliminary or screening results (e.g., method of dynamic factors) or more detailed information (e.g., Hung and Mackay

Table 12.1 Minimal input data requested by a particular model

Model	Input data	Output information
Hung and Mackay	• Molar mass of contaminant	• Concentration of contaminant in a particular plant compartment (leaves, stem, roots) after a specified period of time (years) (e.g., Baltrėnaitė and Butkus [12])
	• Contaminant concentration in soil solution	
	• Total concentration of contaminant in soil	
	• Soil density	

(continued)

Table 12.1 (continued)

Model	Input data	Output information
BALANS	• Metal concentration in soil, aerogenic dust, soil amendment (if applicable), grains, and straw (if crop is chosen as plant)	• Amount of metals leached down to soil
	• Area of a site of investigation	• Amount of metals removed from soil with crops
	• Soil density	• The half-life period of metals in soil
	• Amount of amendment (if applicable)	• See example at Baltrėnaitė et al. in review [15]
	• Annual plant yield	
	• Annual biomass increment (in case of crops, annual biomass of straw and a portion of straw mass removed from the site are entered)	
	• Type of relief	
	• Soil metal leaching coefficients (soil type is taken into account when choosing)	
Phyto-DSS	• Climate data (amount of rainfall and evapotranspiration)	• Content of metals in plant biomass after the selected time period of phytoremediation
	• Area of the site	• Increment of plant biomass after the simulated period
	• Soil type, density, and soil moisture content	• Costs of phytoremediation
	• Soil rain infiltration rate	
	• Total and bioavailable metal concentration (background and maximum contamination) in soil	
	• Metal concentration in plant biomass	
	• Root absorption factor	
	• Metal threshold concentration for plants	
Regular and dynamic factors	• Total and bioavailable soil contaminant concentration in soil of both control and treated sites	• Factors indicating the changes in contaminant bioaccumulation, biophilicity, translocation, bioavailability in soil, phytoremediation process changes (e.g., Baltrėnaitė et al. [20])
	• Total concentration of contaminant in plant (at least in roots and shoots) in both control and treated sites	
	• Soil density	
	• Annual increment biomass of plants in both control and treated sites	

model, Phyto-DSS). Such differentiation of models also defines the complexity of a model and, thus, the usability. Easy-to-use models are more practically attractive and in most cases are much welcome by the officers of environmental protection, while more complex models are better tools for researchers or others specialized in the field. In regard to the spatial/regional application of the modelled results, models can be focused on providing the results of phytoremediation effect for a single plant (e.g., Hung and Mackay model) or a simulation might be already developed to produce up-scaled results (e.g., BALANS).

Phytoremediation effect itself can be simulated of a different scope, e.g., generating information on the amount of the removed contaminants (e.g., Phyto-DSS), residual portion of contaminants in soil (e.g., BALANS) or assessment of contaminant uptake process changes (e.g., dynamic factors). The variety of model scopes defines the uptake mechanism involved in the model. For example, simulation of the transpiration flow in plant is the principal mechanism of Phyto-DSS that is more oriented to the plant organism, while the BALANS model is based on the estimation of mass balance of contaminant in soil. Sometimes, the scope of a model may refer to the contaminant uptake estimation of the present situation or defining the plausible situation in the future. In such a way, models can be used for evaluation of the contaminant uptake in the site with existing contamination or used to predict the phytoremediation effect when potential contamination occurs (Fig. 12.3).

12.6 Conclusions

A model is bad unless it is validated. Each model has a particular range of applications and is oriented to solving a special task. Models for phytoremediation process simulation vary depending on the scope, the prevailing simulation mechanism, spatial applicability of simulation results and the level of complexity. Within the plant–soil–atmosphere system, the models are classified as plant-oriented, soil-oriented or uptake-process-oriented. In regard to the mechanisms involved in simulation, the models are based on contaminant transport with a transpiration flow, mass balance of the contaminants in the soil–plant–atmosphere system.

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Chapter 13
Genetic Control of Metal Sequestration in Hyper-Accumulator Plants

Shahida Shaheen, Qaisar Mahmood, Mahnoor Asif, and Rafiq Ahmad

Abstract Heavy metal contamination is an emergent environmental dilemma all over the world, posing serious threat to environment as well as human being by disturbing the ecological balance. There are a number of physical, chemical, and biological techniques applicable worldwide for wastewater treatment, but the phytoremediation techniques are the green, sustainable, and promising solutions to problem of environmental contamination. Studies revealed that there are certain hyper-accumulator genes present in plants, which make them more metal tolerant than non-hyper-accumulator plants species where those genes are absent. In addition, hyper-accumulator plants tackle with heavy metals by activating their responsive genes for chelation, trafficking, and sequestration. Therefore, studying such hyper-accumulator genes opens a gateway for the thorough understanding of phytoremediation techniques.

Keywords Hyper-accumulator • Non-hyper-accumulator • Phytoremediation • Contamination • Tolerant

Abbreviations

Al	Aluminum
BjMT	*Brassica juncea* metallothioneins
Ca	Calcium
CaM	Calmodulin
CBL	Calcineurin B-like protein
CIPK	Calcium-interacting protein kinase
CRKs	Cysteine-rich receptor-like kinases
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
GR	Glutathione reductase

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GSH	Glutathione
H_2O_2	Hydrogen peroxide
K	Kalium (potassium)
MAPK	Mitogen-activated protein kinase
MDHAR	Monodehydroascorbate reductase
MV	Methyl viologen
MTs	Metallothioneins
OSMT	Oryza sativa metallothioneins
PCs	Phytochelatins
RLKs	Receptor-like kinases
ROS	Reactive oxygen species
SOD	Superoxide dismutase
tApx	Tobacco ascorbate peroxidase

13.1 Introduction

Wastewater released from industries makes human lives easier but brings heavy metals menace, which is disturbing the ecological balance. Heavy metals are non-biodegradable chemical species which may accumulate in different plants parts and therefore cause threats to plants and human health [1]. There are a number of techniques developed over times for remediation of heavy metals, but natural treatment systems are more effective compared to a conventional treatment system. Phytoremediation or the use of living plants to remove heavy metals from soils and water bodies and is proposed as a cost-effective and environment-friendly way to clean up the contaminants [2, 3].

In nature, plants are tolerant towards some heavy metals to some extent and assimilate these as essential nutrients. Green plants can be categorized on the basis of plant-metal interaction as hyper-accumulating and non-accumulating plants. On the basis of adaptations against heavy metals exposure, plants are divided into four main categories, metal-tolerant species, metal-resistant species, metal-tolerant non-hyper-accumulator species, metal hyper-tolerant hyper-accumulator plants species [4]. Hyper-accumulators are plant species which are able to uptake, translocate, and accumulate metals in aboveground plant tissues. A hyper-accumulator should have an intensive root uptake system and faster root-to-shoot translocation. Roots uptake metal from the soil and transport them to the stems and into the leaves. As low concentration of trace metals are present in soil so high affinity transport system is used to accumulate metal ions. A number of transporter genes are involved in this process of metal transport [5].

In most of the plants, heavy metals interaction produces oxidative stress in the chloroplast and mitochondrial membranes. This oxidative stress produce of ROS species causes disruption of intercellular and extracellular membranous organelles, ion leakage, lipid peroxidation, and DNA strand cleavage [6–8]. Most of the heavy metals are recalcitrant in nature thus causing serious damage to the environment. These are nonbiodegradable in nature but biologically can be transformed from more toxic to less toxic condition by their transformation of oxidation state and their conversion from more complex to simplest forms [9].

In nature, plants are capable of self-protection by the production of less toxic reactive compounds or by controlling metals transportation, accumulation, and metal binding with cell wall and vacuole [10, 11]. Many plants when expose to toxic concentration of metal ions try to avoid or decrease its uptake into root cells by limiting the metal ions to the apoplast, binding them to the cell wall or cellular exudates, or by reducing their long distance transportation. If this does not happened, then metals already in the cell adopted storage and detoxification strategies, along with metal transportation, chelation, trafficking, and sequestration into the vacuole. When these actions were completed, then plants trigger oxidative stress defense mechanism and synthesis of stress-related proteins and signaling molecules, such as heat-shock proteins, hormones, and reactive oxygen species [12]. This review has attempted a comprehensive description of plants mechanisms against heavy metals avoidance, transportation, accumulation, and detoxification of heavy metals contamination, and exploring the genetically based defense strategies adopted by plants against trace element excess.

13.2 Avoidance Strategy in Plants

13.2.1 Extracellular Defense Strategy of Plants Against Heavy Metals

Plants possess different intrinsic and extrinsic defense strategies for tolerance or detoxification whenever faces the stressful conditions due to the high concentrations of heavy metals. Initially, regarding metal intoxication, plants implement avoidance strategy to prevent the arrival of stress via restricting metal removal from soil or eliminating it, and control metal entry into plant roots [13]. This can be attained by some mechanisms such as restriction of metals by mycorrhizal association, metal sequestration, or complication by releasing organic compound from root [14, 15].

For heavy metals prevention or reduction of its toxicity impacts, plants develop avoidance approach against HMs entrance. Plants adapted two main pathways by taking part in enhancing its complexity in roots vicinity. For the reduction of heavy metal toxicity, plants enhance the pH of rhizosphere which released anions of phosphate. Studies revealed that South American maize variety 3 released phosphate ions without toxicity while sensitive maize variety 5 showed toxicity symptoms under Al stress [16]. Studies revealed that under Cd stress, malate is secreted from sorghum (*Sorghum bicolor* L.) roots, and citrate is secreted from maize roots [17]. Studies accomplished the fact that root exudates in plants rhizosphere decrease the level of toxicity by activating HM-binding proteins which inhibit the HM uptake [18].

Similarly, oxalate released from the root apex facilitates the prevention of Cd from entering into tomato (*Lycopersicon esculentum* L.) roots, thus in the Cd-resistant tomato cultivar (Micro-Tom) these exudates promote Cd resistance. Genetic studies revealed that under Al exposure Al-tolerant higher plants produces more malic acid than sensitive genotypic plants species [19]. Thus, it is concluded that the tolerant plant species may have adopted precipitation as an avoidance mechanism for the prohibition of the HM.

13.3 Signaling Strategy in Plant

13.3.1 Signals Transduction in Plants

In all plants, reaction towards heavy metal stress involves a complex signal transduction system that is trigger by sensing the heavy metal and is characterize by the production of stress-related proteins and signaling molecules, and finally the transcriptional activation of particular metal-responsive genes to neutralize the stress [20].

The most significant signal transduction processes consist of the Cacalmodulin system, hormones, ROS signaling, and the mitogen-activated protein kinase (MAPK) phosphorylation flow, which activates stress-related genes [21]. There are two main types of plants signaling, i.e., extracellular signaling and intracellular signaling.

13.3.2 Signaling Networks

The ROS network is highly dynamic for plants growth, development, and stress states thus producing ROS-signaling response effectively by ROS-scavenging and ROS-producing protein [22]. The production of ROS physiologically occurs as a by-product of biological reactions. During ROS production, P-450 and other cellular elements are released as a by-product [23]. Under chemical toxicity, ROS genes network is being regulated by cytochromes P-450 which slow down the ROS level in plants cells. For instance, the ROS gene network of *Arabidopsis thaliana* contains more than 150 genes for the maintenance of ROS level in plants [24]. Calcium-signaling network regulates the transmission of calcium signals through channels, pumps, and carriers that between cellular, subcellular, and extracellular parts of plants. Ca2+-binding proteins decoded and transmitted the information provided by calcium signaling for transcription by Ca2+-responsive promoter elements that ultimately regulate proteins phosphorylation [25].

13.3.3 Extracellular Signaling

When plants exposed to multiple abiotic stress stimuli, it rapidly activates signaling proteins MAPKs. An extracellular signaling ROS system has been named as “the ROS wave” that covers about 8 cm/min distance. The concept of “ROS wave” is concerned with the perception or signaling of ROS produced in the plants [26]. In many signaling network, the most important thing is the presence of transmembrane proteins that act as receptor-like kinases (RLKs) and recognize signals with their extracellular kinase containing parts then transmit them through the intracellular kinase containing parts. RLKs manage developmental and hormone responses, stomata closing and opening and stress response, and resistance against bacterial and fungal pathogens [27, 28].

About 600 members of RLK gene family has been reported in Arabidopsis. In extracellular parts of plants, the RLK groups like CYSTEINE-RICH RECEPTOR-LIKE KINASES (CRKs) have two preserved cysteine domains (C-2x-C-8x-C; DUF26 domain). Various studies revealed that on the basis of transcriptional and phenotypic analysis of CRK mutants like their extracellular domain structure, phenotype, and genotypic expression, these could be concerned in apoplastic ROS signaling [29–32].

13.3.4 Intercellular Signaling

During intercellular signaling plants, information is transmitted in the form of mobile signals, including transcription factors and membrane-associated proteins. Generally, membrane-associated proteins are significant in transcription, as small RNAs and revealed intercellular movement through mobile peptides [33]. ROS are important mediators of developmental procedures in different organisms like prokaryotes, fungi, plants, and animals through redox-sensitive transcriptional regulator genes expression. In plants, regulation of peroxidase genes is possible by novel ROS-sensitive transcription factor, UPB1 [34]. Various organelles within the cell like chloroplasts, peroxisome, and mitochondria can generate reactive oxygen species under stress situations and donate to plant stress tolerance [35]. In case of calcium intercellular signaling, calcium signatures transformed the cellular levels of calcium [36]. Cell organelles like vacuoles, endoplasmic reticulum, mitochondria, and cell wall are the store houses of Ca2+ from where these are released when it is necessary by the plant cells [37]. Similarly, cell organelles surrounded by double membrane (e.g., mitochondria, chloroplasts, and nuclei) can generate Ca^{2+} signals whenever posed to stress conditions [38].

13.3.5 Calcium Signaling in Plants

When plants come across a number of physiological stimuli or stress, like low temperature, drought, salinity stress, and pathogen or herbivorous attacks, then free calcium ions of cytoplasm enormously activated [39]. The Ca^{2+} are later on transcripted by intercellular reactions, mainly by Ca^{2+} sensor proteins which have been preserved in all eukaryotic organisms, so that by activating complex downstream signals in reaction of developmental and environmental stimuli. The physical changes of Ca^{2+} binding is measured by structural changes of sensor proteins in Ca^{2+} relying proteins [40, 41]. In response to abiotic stress, calcium signaling is produced with the regulation of cell cycle. The equilibrium of Ca^{2+} ions depends on the Ca^{2+} deficiency, Ca^{2+} transporters, efflux pumps, Ca^{2+}/H^+ antiporters, Ca^{2+} signatures, Ca^{2+} memory, Ca^{2+} sensor, and transducer proteins [42] (Fig. 13.1).

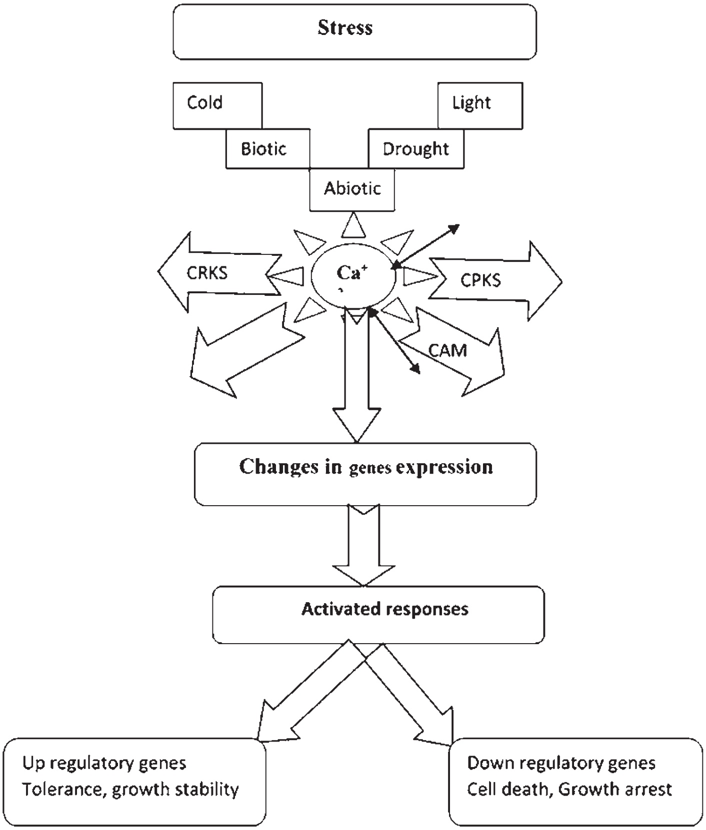

Fig. 13.1 Representation of Ca2+ signals under diverse abiotic stresses regulated by CAM, CRKS, CPKS, and CCaMK for the activation of regulatory genes

13.3.6 Genes Involved in Calcium Signaling

Calcium and protein kinases significantly take part in signaling pathways against environmental stress in plants. The first calcium-dependent, calmodulin-independent protein kinase activities were reported in pea (*Pisum sativum*) extracts 20 years ago [43]. Various studies have shown that Ca-regulated proteins and kinases [44]. Such as CaM protein [45] neurin B-like 218 (CBL) 8 proteins, CDPK genes [46], and CBL-interacting protein kinase (CIPK) [47] are associated to abiotic stress response in plants.

By the activation of protein kinesis, calcium sensors or Ca^{2+}-binding proteins detect the high concentration of Ca^{2+}. In response to the genes, expression of regulatory proteins produced by protein kinesis can enhance transcription factors by phosphorylation and by changing the metabolism that ultimately results in producing phenotypic responses for enhancing stress tolerance [37]. Because of the rise of calcium level two types of genes, specifically up regulating or down regulating are overexpressed and produces stunt growth or death of plant cells. These consist of known quick stress-responsive genes in addition to genes of nonspecific function [48]. Recent studies show that calcium signaling plays a significant role in some pathways, for example, in *Arabidopsis*, Ca^{2+}-signaling pathway can also control a K^+ channel for low-K response in the presence of a blue light receptor phototropins, which successfully promotes growth and plant development [49]. Calcium signaling is correlated with the sucrose-signaling pathway that is an essential source of fructan synthesis [50]. Under abiotic stress, calcium signaling controls the cell cycle progression.

13.3.7 ROS Signaling in Plants

Plants can sense, transduce, and translate ROS signal into suitable cellular response with the assistance of redox-sensitive proteins, calcium mobilization, protein phosphorylation, and gene expression. ROS can be sense directly by some important signaling proteins such as a tyrosine phosphatase through oxidation of conserved cysteine residues [51]. ROS can also regulate many enzymes in signaling, such as protein phosphatases, protein kinases, and transcription factors [52], and transmit to other signal molecules and the ways forming part of the signaling network that regulate the response downstream of ROS [53]. Usually, the power, lifetime, and size of the ROS-signaling pool rely on balance between oxidant production and removal by the antioxidant. By using mutants that lack in key ROS-scavenging enzymes, Miller and coworkers determined a signaling pathway that is operated in cells in response to ROS accumulation [54]. In tomato leaves, ROS produced in cell walls of vascular bundle cells, as a result of wounding and produce H_2O_2 from wound-inducible polygalacturonase which is acted as a second messenger for the activation of defense genes in mesophyll cells, but not for signaling pathway genes in vascular bundle cells [55]. Tracing or detoxification of the unnecessary ROS is achieved by well-organized antioxidative system that contains the nonenzymatic as well as enzymatic antioxidants [56].

13.3.8 Genes Involved in ROS Signaling

ROS are a sort of free radicals, reactive molecules, and ions that are obtained from O_2. It has been evaluated that about 1% of O_2 used by plants is transformed to ROS [57]. The most frequently existing ROS are O_2, $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$. As O_2 is a completely nontoxic molecule as in its ground state it has two unpaired electrons with equidistant spin which form it paramagnetic and, therefore not likely to take part in reactions with organic molecules unless it is activated [58]. Naturally, in living organisms the production of ROS is responsible for the intracellular communication system that regulated the response to environmental stresses [59]. When the plants are under salinity stress, the target of the ROS is regulated by vesicle trafficking complexes [60].

At low concentration, ROS have been concerned as second messengers in intracellular signaling cascades that mediate several plant responses in plant cells with closing of stomata [53, 61, 62], automatic cell death [63, 64], gravitropism [65], and achievement of tolerance to both biotic and abiotic stresses [54, 66] (Fig. 13.2).

13.3.9 Genetic Control of ROS Production

Studies revealed that under abiotic conditions signaling is produced due to oxidative stress, which result in activation of defense genes giving out specific adaptive responses [67]. The system involved in up regulation of mRNA due to ROS production can occur by redox-sensitive second messenger systems (e.g., MAP kinase activation) [68]. Plants genetics analysis showed that ROS signaling in Arabidopsis plants enhances the antioxidative defense by rising the antioxidative genes expression and activation of the genes of inducible stress proteins [69]. The specific effect of ROS-mediated signaling is related with the confirmation of definite genes expression. Some specific promoters and transcription factors have been recognized as a producer of oxidative stress-responsive elements [70]. Under chilling and salt stress, overexpression of a cytosolic APX-gene taken from pea (*Pisum sativum* L.) in transgenic tomato plants (*Lycopersicon esculentum* L.) improves the oxidative injury [71]. Likewise, tolerance against oxidative stress can also be improved by overexpression of the tApx genes in tobacco or in Arabidopsis [72]. Several studies revealed those in plants, under environmental stresses MDHAR show overexpression [73]. Gene expression analysis of wheat showed two varieties of tolerant wheat bHLHs (bHLH2: CA599618 and bHLH3: CJ685625) that have been affected by salinity [74]. Another gene family WRKY plays effective regulatory role in plants under biotic

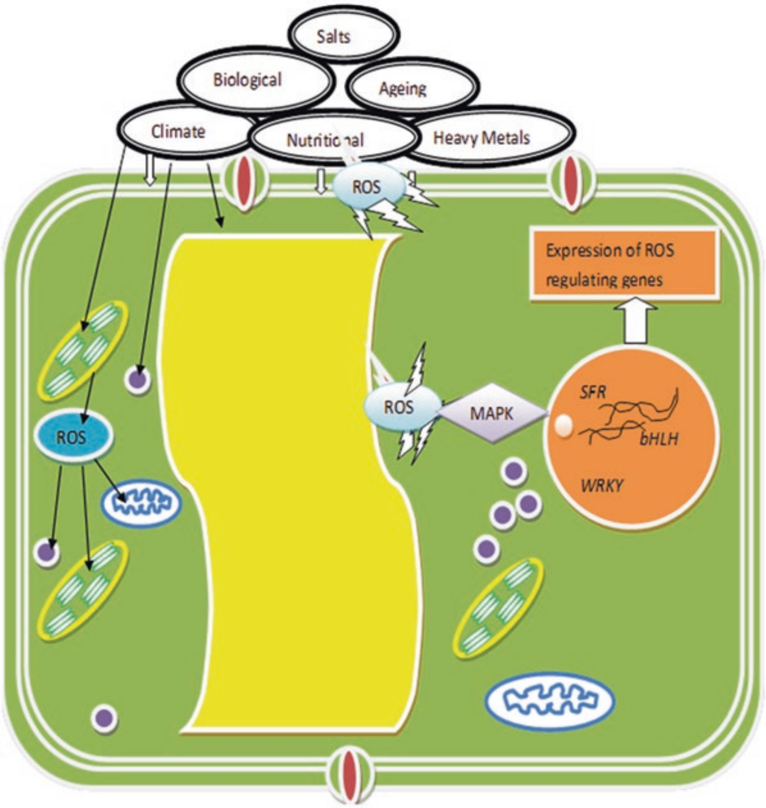

Fig. 13.2 Representation of the transduction pathways involved in ROS signaling in response to abiotic stresses. External stimuli produce ROS in chloroplast, mitochondria, and peroxisomes; these activated MAPK cascade. These mitogen-activated protein kinesis that regulate transcription factor and synthesis of genes (SFR, WRKY, bHLH, etc.) to overcome the negative effects of stress

and abiotic stress conditions like cold, drought, wounding, salinity, UV, H_2O_2, salicylic acid (SA), viral and bacterial attack [75, 76].

Overexpression of Arabidopsis MDHAR gene in tobacco enhanced salt tolerance and mitigated polyethylene glycol stress [77]. Tomato chloroplastic MDHAR overexpressed in transgenic Arabidopsis improved its tolerance to temperature and methyl viologen-related oxidative stresses [78]. Likewise, regulation of the gene-encoding cytosolic DHAR was observed in *L. japonicas*, which was proved to be more tolerant to salt stress than other legumes. This increase of DHAR was associated with its action in AsA recovery in the apoplast [79]. Transgenic potato overexpressing Arabidopsis cytosolic AtDHAR1 proved higher tolerance to herbicide, drought, and salt stresses [80].

Kwon et al. [81] verified that simultaneous expression of Cu/Zn-SOD and APX genes in tobacco chloroplasts increased tolerance to methyl viologen (MV) stress relatively to expression of either of these genes alone. Similarly, improved tolerance to multiple environmental stresses has been produced by instantaneous overexpression of the genes of SOD and APX in the chloroplasts [82–84] SOD and CAT in cytosol [85] and SOD and GR in cytosol [86]. Moreover, the instantaneous expression of numerous antioxidant enzymes, such as Cu/Zn-SOD, APX, and DHAR, in chloroplasts has shown to be more efficient than single or double expression for developing transgenic plants with improved tolerance to various environmental stresses [87]. So, in order to attain tolerance to multiple environmental stresses, increased importance is now given to produce transgenic plants overexpressing multiple antioxidants.

13.4 Detoxification Strategy in Plants

13.4.1 Detoxification Mechanism in Plants

Main detoxification mechanisms in plants are

1. Transportation to storage parts
2. Chelate formation
3. Compartmentalization in subcellular parts
4. Removal from the plant body [88].

13.4.2 Metal Transporters

Transport system of metal ions is very complex and miscellaneous. Metal transport system is involved in uptake of metal, its translocation to various plant organs and metal liberation in subcellular parts together with metal storage in vacuoles [89]. For long distance or intercellular transport in plants and subcellular compartmentalization of metals, low-molecular-weight chelators, such as glutathione, phytochelatins, histidine, or citrate, play a crucial role. By selective metal chelation and trafficking or by internal transporter selectivity, there is a requirement to make a distinction between metal cations of different elements.

A wide variety of transport proteins occurs that belongs to different families including

- The zinc-regulated transporter, iron-regulated transporter protein (ZIP) family
- The cation diffusion facilitator (CDF) family
- The P1B-type subfamily of P-type ATPases
- The natural resistance-associated macrophage protein (NRAMP) family
- The yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily
- The copper transporter (COPT) family
- The Ca2+-sensitive cross complementer 1 (CCC1) family
- The iron-regulated protein (IREG) family

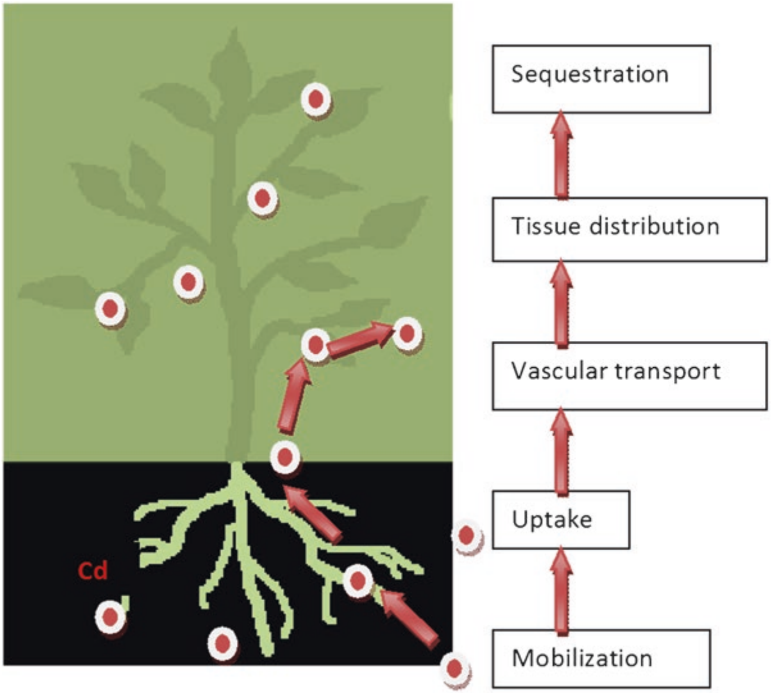

Fig. 13.3 Mechanisms involved in heavy metal transport

Other membrane protein families that have been found to involve in transition metal transport are the cation exchanger (CAX) family and three subfamilies of ATP-binding cassette (ABC) transporters, the multidrug resistance-associated proteins (MRP), the ABC transporters of the mitochondria (ATM), and the pleiotropic drug resistance (PDR) transporters [90, 91]. A metal transporter is involved in metal detoxification and metal hyper-accumulation [89].

Metal transporters are involved in:

1. Uptake from the soil to root
2. Translocation from the root to the shoot
3. Detoxification by storage in the vacuoles (Fig. 13.3)

13.4.3 Uptake from the Soil to Root

Transporter genes involved in cellular uptake of metals from soil have been identified by researchers**.** A number of ZIP transporters are found to be involved in Zn uptake across plasma membrane [89]**.** Fifteen potential ZIP genes may be identified in the *Arabidopsis thaliana* genome [92].

Transport properties of plant metal transporters that mediate metal entry into the cytoplasm, for example, the Zn transporter AtZIP1 have been analyzed upon heterologous expression in yeast by measuring metal uptake into yeast cells [93]. IRT1 is one of the most important members of ZIP family, involved in iron uptake from the soil [93]. Puig et al. [94] found that AtZIP2 and AtZIP4 are involved in cellular accumulation of zinc and copper. OsIRT1 and OsIRT2 have been proposed to contribute in Cd uptake [95]. Milner et al. [96] found that expression of *AtZIP1* is localized to the root stele and is a vacuolar transporter while AtZIP1 expression was also found in the leaf vasculature and is localized to the plasma membrane. Functional studies with *Arabidopsis AtZIP1* and *AtZIP2* T-DNA knockout lines suggest that both transporters play a role in Mn (and possibly Zn) translocation from the root to the shoot. AtZIP1 may play a role in remobilizing Mn from the vacuole to the cytoplasm in root stellar cells and may contribute to radial movement to the xylem parenchyma. AtZIP2, on the other hand, may mediate Mn (and possibly Zn) uptake into root stellar cells, and thus also may contribute to Mn/Zn movement in the stele to the xylem parenchyma, for subsequent xylem loading and transport to the shoot [96].

13.4.4 Translocation from the Root to the Shoot

In phytoextraction, transport of metals from root to shoot is of utmost important. The study of Zn and Cd hyper-accumulators provides the evidence of involving P-ATPase also called HMA (Heavy Metal transporting ATPase) as an important factor in their transport from the cystol of root cells into vascular tissues [97]. The HMAs divide into two groups: those transporting monovalent cations (Cu, Ag) and those transporting divalent cations (Pb, Cd) group [98]. Eight HMAs has been identified in Arabidopsis and *Oryza sativa* [99]. Analysis of the complete genomic sequence in Arabidopsis shows the division of these eight HMAs in two groups: HMA1–4 for the transport of Zn/Co/Cd/Pb and HMA5–8 for the transport of Cu/Ag [100], while HMA2 and HMA4, are involved, in the transport of Zn and Cd. HMA4 confers increased Cd tolerance when expressed in yeast [99].

Transport of cadmium from root to shoot is a control process and most of the Cd is stored in roots. To increase root-to-shoot translocation of Cd, transformation with the genes of high biomass responsible for high root-to-shoot translocation such as HMA4 can be done. In *A. thaliana,* HMA2 and HMA4 genes are involved in the transport of Zn and also nonessential Cd to the shoots [101, 102]. Studies show that both proteins encoding for Cd loading in xylem and are plasma membrane contained proteins [101]. The P1B-type ATPases (also known as HMAs) have a major role in translocation of metal ions against their electrochemical gradient by using ATP as energy. All living organisms including humans, yeast, and plants contain HMAs [100]. In Arabidopsis, HMA4 is found to be more expressed in vascular tissues of root, stem, and leaves. It has been characterized and its role in Cd detoxification has been confirmed in Arabidopsis [100]. HMA4 plays a role in xylem loading of Zn and Cd, and hence in the control of translocation to shoots in *Arabidopsis halleri* and *Thlaspi caerulescens*. *A. halleri* plants (from a Cd-hyper-tolerant accession) with a lowered expression of HMA4 translocated less Zn from the root to the shoot and were more sensitive to Cd and Zn treatments [103].

Work of Courbot et al. [100] leads to conclusion that elevated expression of HMA4 P_{1B}-type ATPase is an efficient mechanism for improving Cd/Zn tolerance in plants under conditions of Cd/Zn excess by maintaining low cellular Cd^{2+} and Zn^{2+} concentrations in the cytoplasm. HMA2 and HMA4 are the only P1B ATPases identified so far, which are predicted to have a long C-terminal domain. It is interesting that the CC dipeptides and the His-rich domains are found in the prolonged C termini of HMA2 and HMA4 and not in the N-terminal domain, where HMA domains are always found. The N-terminal end of HMA1 also harbors a poly-His domain [104] (Table 13.1).

13.4.5 Detoxification by Storage in the Vacuoles

In the hyper-accumulation of Zn, Ni, and Cd, an enhanced capacity of metal storage in leaf vacuoles seems to play an important role [98]. ABC transporter is involved in many physiological processes. Several members of ABC transporter are involved in vacuolar sequestration of metals. Hmt1 is found to be involved in transport of PC-Cd complexes in the vacuoles of *S. pombe* [89]. Some members of the MATE family were shown to function as cation antiporters that remove toxic compounds from the cytosol by exporting them out of the cell or sequestering them to vacuole [105].

Members of CDF family involved in the cytoplasmic efflux of metal cations from cytoplasm to organelles like Zn^{2+}, Cd^{2+}, and have been named MTP (metal tolerance protein). CDFs are highly expressed in *A. halleri* and *T. caerulescens*: MTP1, MTP, and MTP11. AtMTP1 suggested being involved in Zn tolerance and basal Zn accumulation in leaves. MTP11 and especially MTP8 are close homologues of ShMTP8 that give Mn tolerance when expressed in yeast and when ectopically overexpressed in *A. thaliana* [93] (Fig. 13.4).

Mechanism of metal transport in plant cell. Heavy metals enter into cytosol through metal transporter from cytosol into vacuole via metal transporters.

HM—High Metal
LM—Low Metal
ZIP—Zinc-regulated transporter, iron-regulated transporter protein
NRAMP—Natural resistance-associated macrophage protein
CAX—Cation exchanger
ABC—ATP-binding cassette
MT—Metallothioneins
PC—Phytochelatins

Table 13.1 Gene expression of various abiotic stress conditions in plants

Type of stress	Genes involved	Plant species	References
Heat	AtCaM3	*A. thaliana*	Xuan et al. [145]
Heat	AtCaM7	*A. thaliana*	Lu et al. [146]
Heat	OsCAM1-1	*O. sativa*	Wu et al. [147]
Salt	GmCaM4/5	*Glycine max*	Park et al. [148]
Heat	TaCaM1-2	*Triticum aestivum*	Liu et al. [149]
Salt	AtCML8	*A. thaliana*	Park et al. [150]
ABA, droughts, salt	AtCML9	*A. thaliana*	Magnan et al. [151]
Salt	AtCML18/CaM15	*A. thaliana*	Yamaguchi et al. [152]
Heat, cold, ABA	AtCML24/TCH2	*A. thaliana*	Delk et al. [153]
ABA, salt	AtCML37/38/39	*A. thaliana*	Vanderbeld and Snedden [154]
ABA, drought	AtCML42	*A. thaliana*	Vadassery et al. [155]
Cold, heat, drought, Salt.ABA	OsMSR2	*O. sativa*	Xu et al. [156]
Heat	AtPP7	*A. thaliana*	Liu et al. [157]
Heat	AtCBK3/CRK1	*A. thaliana*	Liu et al. [158]
Cold, heat, Salt.ABA, H_2O_2	AtCRCK1	*A. thaliana*	Yang et al. [159]
Cold	AtCRLK1	*A. thaliana*	Yang et al. [160, 161]
ABA, H_2O_2, ROS, dehydration	OsCCaMK/DMI3	*O. sativa*	Shi et al. [162]
ABA, ROS	ZmCCaMK	*Z. mays*	Ma et al. [163]
ABA, salt	TaCCaMK	*Pisumsativum*	Pandey et al. [164]
Salinity	DHAR	*Oryza sativa*	Chen and Gallie [165]
Drought, ozone	DHAR	*A. thaliana*	Ushimaru et al. [166]
Cu, Zn	AtZIP2, AtZIP4	*A. thaliana*	Puig et al. [94]
Cd	OsIRT1, OsIRT2	*O. sativa*	Clemens et al. [95]
Mn	ShMTP	*A. thaliana*	Delhaize et al. [167]
Cu/Ag	HMA5–8	*A. halleri*	Courbot et al. [100]
Zn/Co/Cd/Pb	HMA1–4	*A. halleri*	Courbot et al. [100]

13.4.6 Metal-Binding Genes

Plants have been authentically proved to minimize the harmful effects of metal toxicity, by pathways or methods relating to the binding of heavy metals to cell wall and its transporation [106, 107]. Generally, the synthesis of metal-binding peptides based on the production of metallothioneins and phytochelatins [108]. Usually, chelation is the most common intracellular system for the maintenance of low concentrations and detoxification of free metals in plant cytoplasm that can be achieved by thiol compounds (which contain sulfhydryl/thiol groups; such as a tripeptide glutathione, GSH, γ-Glu-Cys-Gly; phytochelatins, PCs; metallothioneins, MTs), and

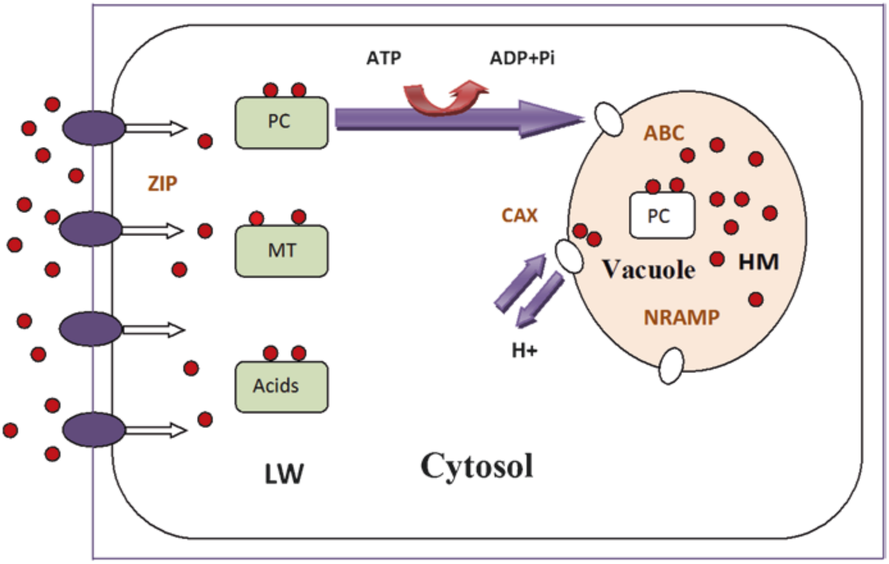

Fig. 13.4 Mechanism of metal transport in plant cell. Heavy metals enter into cytosol through metal transporter from cytosol into vacuole via metal transporters

also by non-thiol compounds (such as organic acids and amino acids) [109–118]. Studies revealed that peptides that have either histidines (GHHPHG) 2 (HP) or cysteines (GCGCPCGCG) (CP) be engineered to Lam B and expressed on the surface of *E. coli*. Surface demonstrated that CP and HP enhanced the bioaccumulation fourfold and twofold [119].

13.4.7 Phytochelatins

Phytochelatins are a family of cysteine-rich, thiol-reactive peptides that attach many toxic metals and metalloids, producing good messengers for genetically better phytoremediation system [120]. The general structure of PCs is (g-Glu-Cys) n-Gly, where n differs from 2 to 11 [121]. Practical descriptions of an unusual phytochelatin synthase, LjPCS3, of *Lotus japonicus*, have been acknowledged in an extensive variety of plant species and some microorganisms [122].

Phytochelatins plays an important role in biosynthesis and detoxification of heavy metals [122]. After production, PCs combine with heavy metal ions and make possible their transportation as complex into the vacuole, where they finally produce complexes of high molecular mass, which is the key method that utilizes to bind heavy metal ions in both plants and yeasts [122]. Genes concerned in the production of PCs are phytochelatin synthases, such as g-glutamyl cysteine trans peptidase [121].

Overexpression of phytochelatin synthase in *Arabidopsis thaliana* seedlings causes tolerance of arsenic, but hypersensitivity to cadmium and zinc [123]. The same fact was observed in other transgenic plants with diverse PCS genes show diverse phenotypes, including heavy metal tolerance (by or lacking accumulation) and hypersensitivity to heavy metal ions [124]. Overexpression of pytochelatin synthase in tobacco: distinctive effects of AtPCS1 and CePCS genes on plant response to cadmium [125]. Phytochelatin synthase of *Thlaspi caerulescens* increases tolerance and accretion of heavy metals when expressed in yeast and tobacco. PCs are a group of cysteine-rich, thiol-reactive peptides that combine many toxic metals and metalloids, thus producing best messenger for genetically improved phytoremediation pathways [120].

13.4.8 Metallothioneins (MTs)

Apart from PCs that are the product of enzymatically formulated peptides, MTs are formulated resultantly by mRNA translation [126]. While PCs in plants may primarily deal with Cd detoxification, MTs appear to elaborate the attraction with a larger series of metals such as Cu, Zn, Cd, and As [127]. MTs demonstrated unusual properties and performance that depends on their presence in a type of plants and are extremely mottled in terms of their molecular characteristics and structural qualities [128]; they probably contain a number of various activities in plants than a few other living creatures. In plants, these ligands are concerned to negate the toxicity of HMs by cellular sequestration, homeostasis of intracellular metal ions, and metal transport modifications [129–131].

Additionally, MTs play an important role in HM detoxification, actively involved in cellular-related events including ROS scavenger [132], maintaining of the redox level [133], repair of plasma membrane [134], cell proliferation, and its growth and repair of damaged DNA [135]. There are numerous endogenous and exogenous agents other than HMs that are able to bring the synthesis and expression of MTs. Of these, osmotic stress, drought, intense temperatures, nutrient deficiency, release of different hormones, natural and dark-induced tissue decay, injuries, and viral infections can be mentioned [12, 127, 136].

Ectopically expressed MTs in transgenic plants are proved to increase their tolerance towards metal intoxication. Kumar et al. [137] showed that OSMT1e-p, a type 1 MT extracted from a salt-tolerant rice genotype (*Oryza sativa* L. cv. Pokkali), participated in tolerance for copper and zinc toxicity when ectopically expressed in transgenic tobacco. They evaluated that tobacco plants in which gene have been inserted possessed to hold more quantity of Cu^{2+} and Zn^{2+} in their roots or lower leaves, considerably decreasing the HMs ions transportation and quantity in leaves and harvestable plant parts. Zhigang et al. [138] accomplished that the ectopic expression of BjMT2, a metallothionein type 2 from *Brassica juncea*, in *Arabidopsis thaliana* enhanced copper and cadmium tolerance at the seedling phase but intensely decreased root growth when there was no heavy metal treatment. This tendency may propose that ectopic expression of MTs in transgenic plants may proceed in host plant in a nonspecific method and in a different way effect the organ growth.

13.5 Conclusion and Future Prospects

The present review outlines the impact of abiotic stresses on plants. Most of the investigations done so far mainly described the genetic investigation of plants against abiotic stress; this review involved in genetically based defense and detoxification pathways mainly Ca and ROS signaling, transportation, chelation, and detoxification has been discussed in detail. Under stress conditions, plants activate specific molecules which enhance plants tolerance and the development of defense mechanisms in it. It has been observed that the activation of defense genes cascade transmit various signals in cell organelles under various biotic stress conditions [40].

As abiotic condition produces oxidation stresses that overexpressed a number of stress-induced proteins, this review could provide fundamental information about antioxidant and regulatory genes production. As ROS have regulatory function as signaling molecules, this feature may open a gateway to physiological, molecular, and evolutionary research perspectives. Due to the importance of ROS, it is central to modern plant biology to obtain a comprehensive understanding of the processes where ROS have regulatory roles. Studies revealed that ROS signaling with ozone as a tool is significant for the transmission of distinct from of ROS signals to chromatin reformation and transcriptional regulation [140].

Therefore, the elaboration of transcriptomics and proteomics analysis will be more helpful in understanding the bioinformatics and mutant studies. It has been observed that ROS signals play an important role in intercellular Ca2+ signals, Ca2+ influx which can be regulated by various PAs and Spm4+ proteins [141]. There is need to highlight the mechanism and interrelation of Ca2+-efflux systems with ROS and AtMPK6-signaling under biotic and abiotic stresses along with the overexpression of regulatory genes against stress. Xing et al. [142] observed various proteins kinesis like MKK2-MPK4/MPK6 and MEKK1 activation in salt, cold, drought, and wounding stress can phosphorylate MPK4 which is also significant in abiotic stress signaling. Studies also revealed that ABA is a key hormone in inducing abiotic stress responses in plants like barley showed pronounced effect of ROS and PAs in salt-sensitive variety then salt-tolerant plants [143]. Likewise, ABA is an important hormone under biotic and abiotic stress in plants [144]. But the links between ABA and MAPKs under biotic and abiotic stresses has not yet been properly studied at cellular and subcellular levels in plants. Similarly, little work has been done on K+/Na+ -signaling pathways under biotic and abiotic stresses like Ca^{2+} homeostasis. Therefore, plants responses towards multiple abiotic stresses would be another interesting area of future transgenic hyper-accumulator plants production.

In future, it is needed to identify molecular interaction of chelators with metal transporters. Further investigation is needed to determine more about functional significance and biological role of transporter genes, especially, overexpression in plants with greater biomass to increase their potential use in phytoremediation processes. Furthermore, these genes can provide better understanding in the analysis of gene regulation in metal-rich environment as well as metal-deficient environment. Similarly, silencing of transporter genes in edible crops may decrease metal bioaccumulation in food chain. Likewise, there is a greater area of exploration in terms of 3D structures of protein and functional analysis of the candidate genes. In future, gene cloning and plant transformation can be done to determine efficiency of metal transporter genes in transgenic plants. Thus, the application of powerful genetic and molecular techniques may surely be helpful in designing of hyper-accumulator transgenic plants for bioremediation.

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Chapter 14

Engineered Nanomaterials for Phytoremediation of Metal/Metalloid-Contaminated Soils: Implications for Plant Physiology

Domingo Martínez-Fernández, Martina Vítková, Zuzana Michálková, and Michael Komárek

Abstract Nanomaterials, including engineered nano-sized iron oxides, manganese oxides, cerium oxides, titanium oxides, or zinc oxides, provide specific affinity for metal/metalloids adsorption and their application is being rapidly extended for environmental management. Their significant surface area, high number of active surface sites, and high adsorption capacities make them very promising as cost-effective amendments for the remediation of contaminated soils. The alleviation of the toxicities of metal/metalloids by their immobilization in the soil stimulates the growth and development of plants during phytoremediation, but there is a body of evidence indicating that nanomaterials themselves can yield both beneficial and harmful effects in plant systems at the physiological, biochemical, nutritional, and genetic levels. Nanoecotoxicological studies are providing a good understanding of their interactions with plants, and an increasing number of publications have attempted to clarify and quantify their potential risks and consequences for plants. However, many results are contradictory and the safety of engineered nanomaterials still represents a barrier to their wide, innovative use in phytoremediation. Both their positive and negative effects on plants will have to be taken into account to evaluate their applicability, and the scientific community faces a challenge to understand deeply the factors which can determine their relevance in environmental science and technology.

Keywords Nanoparticles • Oxides • Stabilization • Immobilization • Nanoremediation • Toxicity

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14.1 Introduction

Soil contamination by metals (e.g., Cd, Cu, Mn, Ni, Pb, Zn) and metalloids (e.g., As, Sb) is a current global phenomenon endangering safe agricultural production and groundwater quality. Therefore, society, economics, and science are involved together in the common need for novel and environmentally friendly techniques for soil remediation. Recently, nanotechnology has offered a new generation of environmental remediation technologies that can provide cost-effective solutions to some of the most challenging environmental clean-up problems [1]. While various industrial sectors produce a large number of products containing nanomaterials, nanotechnology is also used in environmental management. Nanoparticles (NPs, materials with at least two dimensions between 1 and 100 nm) and nanomaterials (NMs, materials with at least one dimension smaller than 100 nm) [2–4] have the potential to revolutionize agricultural systems, environmental engineering, safety and security, water resources, and numerous other life sciences [5]. The smaller particle sizes, the higher specific surface area, and thus the higher number of reaction sites for metal adsorption represent the main advantages of NMs [6]. Nano-sized metal oxides, including nano-sized iron oxides, manganese oxides, aluminum oxides, zinc oxides, titanium oxides, and cerium oxides, have specific affinities for metal/metalloids adsorption and their application has been rapidly extended for environmental tasks [7–9]. Although they can exist naturally in the environment, they can also be produced/engineered intentionally [10], through methods that are becoming simpler, more effective, and cheaper. Concerning the economic aspect, it is clear that the doses of these compounds required for adsorption are lower when applied as engineered NMs because of their huge reactivity, while the contact times needed for the metal/metalloids adsorption are shorter in comparison with conventional adsorbents. In this sense, their application seems to be profitable [11].

14.2 Nanoparticles for Environmental Remediation

For sites contaminated by metal/metalloids, successful remediation is complicated by the fact that these pollutants do not degrade spontaneously, and it is not usually possible to excavate all the contaminated soil. Therefore, chemical stabilization of the metal/metalloids in these soils, through adsorption, surface precipitation, structural incorporation, or ion exchange, is a viable option for such sites as this technology immobilizes the contaminants in the soils and thus reduces their mobility, bioavailability, and bioaccessibility. In fact, this first step—the reduction of their toxicity—is crucial for the establishment of a vegetation cover on the contaminated sites during phytoremediation. The use of engineered NMs for remediation purposes can then enhance the natural attenuation processes, a key mechanism for the re-establishment of sustainable environmental systems. It has been claimed that nanotechnology has great potential as an environmentally cleaner technology, including alleviation of the toxicities of various metal/metalloids [12]. As a result, several studies have appeared in various journals dealing with the metal-NMs-mediated diminution of metal toxicity [13–15]. In general, this remediation technology involves (a) NMs transport along with the solution to the contaminated zone; (b) attachment to soils in the contaminated zone; and (c) reaction with the target contaminants to form less toxic or less mobile products [6]. The principal removal mechanisms for the most common inorganic contaminants can be divided into five categories: (1) adsorption (Cr, As, U, Pb, Ni, Se, Co, Cd, Zn, Ba); (2) reduction (Cr, As, Cu, U, Pb, Ni, Se, Co, Pd, Pt, Hg, Ag); (3) oxidation (As, U, Se, Pb); (4) precipitation (Cu, Pb, Cd, Co, Zn); and (5) co-precipitation (Cr, As, Ni, Se). The overall reaction processes are strongly influenced by a number of factors, in particular the NMs chemical properties and structure, the presence of more than one contaminant species, the pollutant characteristics, and the hydrogeochemistry of the aqueous environment (pH, redox conditions, natural dissolved species, etc.) [6, 16].

Since the amendments applied for the stabilization process need to be cost-efficient and suitable for different soil types and should not pose a risk to environmental compartments, application of engineered NMs in remediation technologies provides a very interesting alternative to soil excavation and dumping, ex situ soil washing, etc., because these are generally disruptive and costly. Nanoparticles have been studied as adsorbents of metals and their characteristics (i.e., large surface area, high number of active surface sites, low intra-particle diffusion rates, and high adsorption capacities) make them very promising for the cost-effective treatment of polluted soils [17, 18]. Then, nanoremediation, defined as the use of nanoparticles for environmental remediation, has the potential not only to reduce the overall costs of cleaning up large-scale contaminated sites, but can also reduce clean-up time, eliminate the need for treatment and disposal of the contaminated soil, and reduce the availability of some contaminants [19–21]. This is reflected in the increasing number of publications on this subject and the rising level of funding for remediation projects [6, 22–26].

To date, researchers have mainly focused their attention on the removal of metals from aqueous solutions rather than soil-bound metals, which may be absorbed by plants and subsequently spread into the human food chain. The nanoremediation of contaminated soils is a topic that has been researchedless, compared to the removal of pollutants from water or wastewater [21]. Based on this idea, soil columns can be set up for ex situ remediation, and a liquid suspension of NMs can be added to extract or to immobilize the contaminants (typically metals); the species adsorbed onto the NMs can be removed by applying mild gravitational (centrifugal) or magnetic (in the case of magnetic NMs, such as magnetite) gradients [21]. As a result of these previous leaching experiments with aqueous solutions, and thanks to the demonstrated potential of engineered NMs, they have been gradually incorporated into new in situ strategies for phytoremediation. Most of the information about their behavior in aqueous systems can be extrapolated to the soil solution, which is important since the physical/chemical properties of NMs are one of the most important factors that control their behavior in the environment although obviously it must be modified to take into account the new conditions in the soil. In this context, different hydrochemical parameters—such as pH, Eh, ionic strength, and aqueous chemistry—can change the aggregation kinetics and transformation of engineered NMs and their subsequent behavior. Similarly, natural organic matter alters their stability through electrostatic and steric interactions. The transformation process for NMs is also altered by a confluence of factors, depending on the characteristics of the NMs and of the environmental receptors.

Although the use of plants for phytoremediation and their capacity to accumulate and tolerate high concentrations of metals have been explored, and a significant amount of literature is available, the same is not true regarding NMs, and understanding the response of plants to NMs would be a key element in identifying mechanisms involved in stress tolerance and NMs toxicity [27]. According to Juganson et al. [28], 936 was the total number of publications (as sum of all the materials) found in the Thomson Reuters Web of Science™ for "environmental remediation" using NMs (for a search done on March 19th, 2015), with 303 publications for $nTiO_2$, 219 for nFeOx, 110 for nAg, 74 for nZnO, 36 for nCuO, 16 for $nCeO_2$, and the rest for other NMs like fullerenes and carbon nanotubes (See chart in Fig. 14.2).

14.2.1 Main Types of Nanomaterials for the Adsorption of Metals and Metalloids

Metal NMs display size-dependent properties, such as magnetism (magnetic NPs), fluorescence (QDs), or photocatalytic degradation (metal oxide NPs), that have biotechnological applications in sensor development, agrochemical degradation, and soil remediation [29]. Nanoparticles and nanomaterials are mainly classified according to their dimensionality, morphology, or uniformity [30], but the classification according to their chemical properties is the most accepted and useful.

14.2.1.1 Iron Nanooxides: Nanogoethite, Nanomaghemite, Nanomagnetite

Iron oxides represent natural components of soils and exist in many forms, including mainly goethite (α-FeOOH; prevailing in temperate climatic areas), hematite ($\alpha\text{-}Fe_2O_3$; prevailing in warm-dry climate zones), maghemite ($\gamma\text{-}Fe_2O_3$), and magnetite (Fe_3O_4). These iron oxides play a crucial role in soil systems due to their ability to adsorb potentially toxic elements such as metals and metalloids [31–33]. Synthetic iron-based NMs are thus interesting candidates for the removal of metals and metalloids from contaminated waters and soils, or their stabilization therein, due to their increased specific surface area and modified surface structure, which strongly affect their reactivity and chemistry [34–37]. During the remediation process, they can be applied directly as nano iron oxides or in the form of their precursors (i.e., nZVI, nano zero-valent iron) ([38] and references therein). The use of iron-based NMs for the in situ immobilization of trace elements limits the potential leachability of metals/metalloids and thus prevents their transport into deeper soil layers and groundwater [39].

Due to its abundance and the presence of surface hydroxyl groups, goethite is significantly involved in the transport and transformation of nutrients and contaminants—including inorganic/organic anions, cations, and some gases [40]. Synthetic nanogoethite (nFeOOH) has been successfully used for the removal of Cu from aqueous solutions, showing photocatalytic activity and a high adsorption capacity for Cu [41].

Maghemite is a common weathering product in soils of temperate, tropical, and subtropical climatic regions, usually formed during the oxidation of magnetite. Synthetic maghemite (nFe_2O_3) is a promising material for the removal of inorganic contaminants as it is readily available, inexpensive, and can be easily separated and recovered because it is magnetic [42]. Nanomaghemite has been deeply investigated due to its efficient removal of the most-toxic form of As (arsenite, As (III)) [43–47]. Moreover, it has been demonstrated that it is an important scavenger of Cr(VI), Pb(II), Cd(II), Cu(II), and Zn(II) from aqueous solutions and thus could be a useful sorbent for water and soil remediation [4, 42, 48, 49]. The use of nFe_2O_3 has been reported to be useful for promoting the growth of plants in a contaminated soil [50], mainly due to the immobilization of Zn from the soil pore water (available to plants) with the consequent reduction of its toxicity to the roots and aerial parts. Adsorption of Pb(II) by nFe_2O_3 occurs mainly through the formation of inner-sphere complexes, while Cd(II) is likely adsorbed as a mixture of inner- and outer-sphere complexes [42]. The effectiveness of the adsorption of these metal/metalloids is affected by the modification of the atomic structure on the particles surface with decreasing size of nanomaghemite [43]. Also, the presence of other components in the soil solution—such as citrate complexes and organic acids [51], or other nutrients [52]—influences the sorption process. For example, PO_4^{3-} has been described as a competitor for arsenite and arsenate immobilization by nanomaghemite [53] due to their similar outer electronic structures.

Magnetite is a mixed-valence magnetic iron oxide, containing Fe^{2+} and Fe^{3+}, and it can be formed in the soil through (bacteria assisted) weathering of ferrihydrite [54]. Immobilization of As in soils using nanomagnetite (nFe_3O_4) was performed by Zhang et al. [32], who reported higher stabilization efficiency of nFe_3O_4 compared to iron sulfide or nZVI. In another study, nFe_3O_4 proved to be an efficient amendment for the removal of Pb from aqueous solutions, yielding fast adsorption with a maximum capacity of 36 mg Pb g^{-1} [17]—which was much higher compared to, for example, goethite [55]. Moreover, the behavior of Pb was not affected by the presence of other ions such as Ca, Ni, Co, or Cd. Additionally, desorption and regeneration tests showed that nFe_3O_4 can be used repeatedly without loss of their adsorption capacity [17]. Shen et al. [56] investigated the influence of pH, temperature, and particle size on the adsorption of metals from aqueous solution by nFe_3O_4. Under room temperature at pH 4 with an average particle size of 8 nm, $\geq 85\%$ of Cu^{2+}, Cd^{2+}, Ni^{2+}, and Cr^{6+} were removed, yielding the maximum at $pH > 7$ for divalent metals and at pH 2 for hexavalent Cr. In contrast, coarse particles showed values of maximum adsorption capacity about seven-times lower [56].

Recently, the synthesis and utilization of iron NMs (nFeOx) with novel properties and functions have been widely studied, both for their nano size and for their magnetic characteristics [11, 36, 57–59]. Typical iron NMs syntheses involve routes including chemical precipitation [60], sol-gel, hydrothermal, dry vapor deposition, surfactant mediation, microemulsion, electro-deposition, and sonochemical methods [36]. Iron oxide composites such as clay–iron oxide magnetic composites and magnetic zeolites can be synthesized and used for the removal of metallic contaminants from water [61]. If the size of the magnetic NPs is reduced to below a few nanometers, they become superparamagnetic. Using an external magnetic field, these particles change their direction. Therefore, the remediation efficiency may be enhanced by combining metal binding and selective adsorption properties with separation of magnetic nano-sorbents from the system, since their magnetic behavior (either ferromagnetic or superparamagnetic) depends on the particle size [36]. Gómez-Pastora et al. [11] illustrated that engineered nFeOx have very high adsorption capacities for metals/metalloids in polluted waters; moreover, their magnetic properties facilitate their collection from the solution—allowing their further reuse. The recovery of magnetic NMs by the use of magnetic gradients [62, 63] represents a promising alternative for sorbent applications.

14.2.1.2 Nano Zero-Valent Iron (nZVI)

A vast number of studies have demonstrated the applicability of nano zero-valent iron (nZVI) as an amendment for remediation of metal/metalloid-polluted water systems [6, 11, 64–66] or trace elements immobilization in contaminated soil [16, 39, 67–69]. The possible mechanisms by which nZVI stabilizes metal/metalloids include adsorption and/or surface precipitation, redox reduction, and co-precipitation in the form of metal iron oxides or oxyhydroxides [6] ([66] and references therein). The particles of nZVI have a core-shell structure, which gives them characteristics typical of both iron oxides (sorption) and elemental Fe^0 (reduction) [6]. The iron core (up to 98% Fe) is covered by a shell composed of iron oxides and hydroxides (FeO, Fe_2O_3, FeOOH). Furthermore, the surface of nZVI particles has a significant influence on their stability and mobility in the environment and it prevents their rapid oxidation. Their increased specific surface area results in much higher reactivity but, on the other hand, the reaction of particles smaller than 20 nm is so fast that their reaction capacity may be depleted before they get to the contaminant. Thus, attaining the optimal balance between the reactivity and lifetime of nZVI needs to be guaranteed for in situ applications [66, 70, 71]. Detailed overviews of nZVI reactivity have been provided by O'Carroll et al. [6] and Yan et al. [72], while the reaction mechanisms have been recently reported by Filip et al. [73].

When nZVI is exposed to air or water, it is oxidized, forming a layer of iron oxides or hydroxides on the surface that is responsible for the subsequent adsorption process [6, 64, 74]. The reaction process is strongly dependent on pH. Under alkaline conditions, a negatively charged surface is favorable to metallic cations adsorption, while the high pH values limit the adsorption of metallic anions. Therefore, nZVI can interact with arsenate and chromate oxyanions at low pH, mainly through electrostatic interaction with the positively charged groups on the surface [45, 56, 75].

Different forms of nZVI are available, including powder, mineral oil suspension, or aqueous suspension. The synthesis methods include formation of the nanomaterial from atoms or molecules through physical/chemical methods (nucleation, vapor condensation, precipitation, agglomeration) and physical/chemical methods to breakdown a bulk material to the nanoscale size (thermal decomposition, thermal reduction of oxide compounds, or pulsed laser ablation) [66, 71]. The synthesis method influences the size, shape, and composition of iron NPs and thus their actual reactivity. Particles of nZVI prepared by the reduction of goethite or hematite are generally bigger (up to 100 nm) and of irregular shape, while formation using $NaBH_4$ provides smaller, regular-shaped particles up to few tens of nm in size [66]. The borohydride reduction of ferrous salts is the most common method of nZVI synthesis for laboratory-scale experiments [71, 76]. However, the industrial application is precluded since highly reactive particles with a significant tendency to agglomerate are produced by this procedure and the overall expenses are high [70, 71]. The easy and cost-effective synthesis of highly reactive NMs, such as nZVI, is a priority of the academic community and nZVI producers. In this context, a method using leaf extracts from different trees was performed to obtain "low-cost" nZVI particles [77].

In order to prevent particle aggregation and to improve nZVI reactivity, various innovative surface modifications/coatings have been developed [6, 66]. Among the traditional agents for nZVI stabilization are poly(acrylic acid), poly(methyl methacrylate), poly(ethylene glycol), polyaspartate, and others. Several stabilizers of natural origin have been tested also, including xanthan gum, guar gum, potato starch, alginate, and chitosan. Bimetallic NPs represent nZVI particles coated with noble metals [6]([78] and references therein). Although the surface properties of nZVI may change, the modifiers generally ensure the transport of stabilized NPs and the reaction with the target contaminant only in the polluted zone. The specific surface of Fe^0 can be 4–15 $m^2\ g^{-1}$, while up to >40 $m^2\ g^{-1}$ can be reached for surface-stabilized particles [6, 71]. The use of several composites such as bentonite-nZVI has been reported also [79].

Due to its abundance, easy accessibility, high reactivity, and efficiency for risk element stabilization, nZVI has become a widespread remediation amendment both on a laboratory scale and for in situ applications. However, as nZVI is a redox-active material, this being important for treatment of redox-sensitive elements (e.g., As, Cr) [14, 80], the impact on soil microbial communities needs to be investigated. According to Li et al. [81], rapid and complete oxidation of Fe^0 eliminates its effects on bacteria due to passivation within a few hours. Significant impacts on microbial diversity were reported for nZVI-treated soil contaminated with Pb, whereas there were no effects on microbial activity in Zn-contaminated soil [16].

Efficient treatments of contaminated soils with nZVI have been reported, resulting in Pb and Zn immobilization [16, 69] as well as decreased availability of As [39]. However, nZVI was a less efficient amendment for in situ soil remediation of Cr, compared to other sorbents studied by Chrysochoou et al. [82], while successful remediation of Cr from ore processing residue [83] and wastewater [14, 80] was achieved upon the application of nZVI. Further studies are needed in order to assess the long-term impacts of nZVI on the environment in terms of contaminant stabilization, while minimizing the effects on soil characteristics.

14.2.1.3 Manganese-Based Materials

Manganese oxides (including hydroxides and oxyhydroxides) together with Fe oxides occur naturally as erosion products in almost all soil types, mainly as coatings on soil particles and pores or in the form of concretions and nodules with poorly crystalline (even amorphous) structure [84]. Compared to Fe oxides, Mn oxides are generally less abundant in soils but appear more efficient in the immobilization of some metals [85, 86]. This is mainly due to their large specific surface and (usually) low value of pH at point of zero charge (pH_{pzc})—the reason for their negative surface charge in usual soil conditions [87]. Their specific structure, formed by sheets (layers) or tunnels in most cases, allows the accommodation of water molecules or various cations in interlayer or tunnel regions [84]. Manganese oxides possess strong oxidative properties and thus take part in many oxidation-reduction and cation exchange reactions. For this reason, Mn oxides are not suitable amendments for soils contaminated with Cr as they are able to readily oxidize Cr(III) to the more toxic and mobile Cr(VI) [88, 89]. On the other hand, this oxidizing nature can be beneficial in the case of contamination with As; Mn oxides have proved efficient in the oxidization of the more mobile and toxic As(III) to As(V) [90–93].

Due to their promising properties, many studies focused on the synthesis and testing of engineered Mn nanooxides, which are potential agents for environmental clean-up. Manganese oxide NPs can be prepared both by classical chemical routes [94–97] and by biotechnological means, using the activity of microorganisms like bacteria or fungi [98–100]. In fact, biogenic oxidation of Mn(II) represents also the prevailing route for the formation of Mn oxides in soil. Although thermodynamically favored, Mn(II) oxidation in the environment solely by chemical means is very slow. On the other hand, when the process is mediated microbially, the reaction rate can be increased by several orders of magnitude [101, 102]. As in the case of other nanoadsorbents, the first studies dealing with these materials focused mainly on their synthesis, characterization, or adsorption properties with respect to targeted compounds; in this case, metals/metalloids. Based on these data, possible applications—including remediation—can be proposed. In this context, application of Mn nanooxides for soil remediation appears relatively safe as nanoscale biogenic Mn oxides are natural and ubiquitous soil components. Although numerous studies have been published dealing with the adsorption performance of Mn-based NPs [100, 103–108], their application for the direct remediation of contaminated soil, together with assisted phytoremediation, is still rather scarce. Della Puppa et al. [109], together with Michálková et al. [110] and Ettler et al. [111], studied the adsorption properties and stabilizing potential of partially nanoscale amorphous Mn oxide (AMO) with regard to Cd, Cu, Pb, Zn, and As in contaminated soils. In these studies, after application to contaminated soils, AMO was able to decrease significantly the amount of targeted metal/metalloids in the soil solution, even showing a higher sorption capacity for Cd, Cu, and Pb than engineered nanomaghemite [51, 110]. On the other hand, higher dissolution of this agent in acidic conditions—connected with unwanted oxidation and dissolution of soil organic matter—was recorded. For this reason, AMO appears a suitable amendment for neutral and slightly alkaline soils.

14.2.1.4 Other NMs

In addition to Fe- and Mn-based NPs, there exists a wide variety of novel, engineered NMs potentially usable in the remediation of soil and water contaminated with metals/metalloids (see chart in Fig. 14.2, according to Juganson et al. [28]). To date, one of the most studied NMs is $nTiO_2$, also the most studied photocatalyst worldwide. Besides its applications targeting the decomposition of various organic compounds, dyes, etc., the process of photocatalytic reduction can be used to remove various toxic metal ions as well. Numerous studies have examined the potential of $nTiO_2$ for the reduction of highly mobile and toxic Cr(VI) to Cr(III) [112–114] and the immobilization of toxic As(III) species [115]. Other materials (Zn/Al-based nanocomposites) were shown to be very promising as they not only behaved as adsorbents but also had photocatalytic properties, being able to adsorb the highly toxic Cr(VI)—that was subsequently reduced photocatalytically to Cr(III) [116]. Nanoparticles of hydrous Ce oxide were reported as another material suitable for adsorption of Cr(VI) from aqueous solution [117]. Nanoparticles of nMgO, $nTiO_2$, and nZnO were found to be efficient adsorbents for Cr in soil contaminated by leather factory waste, decreasing significantly the exchangeable Cr fraction while increasing the residual fraction [118]. Carbon nanotubes represent another promising type of engineered material, being efficient in the adsorption of various divalent metals from aqueous solution [119]. In the study of Jośko et al. [120], application of multiwalled carbon nanotubes reduced the phytotoxicity of sediment contaminated with various organic and inorganic contaminants.

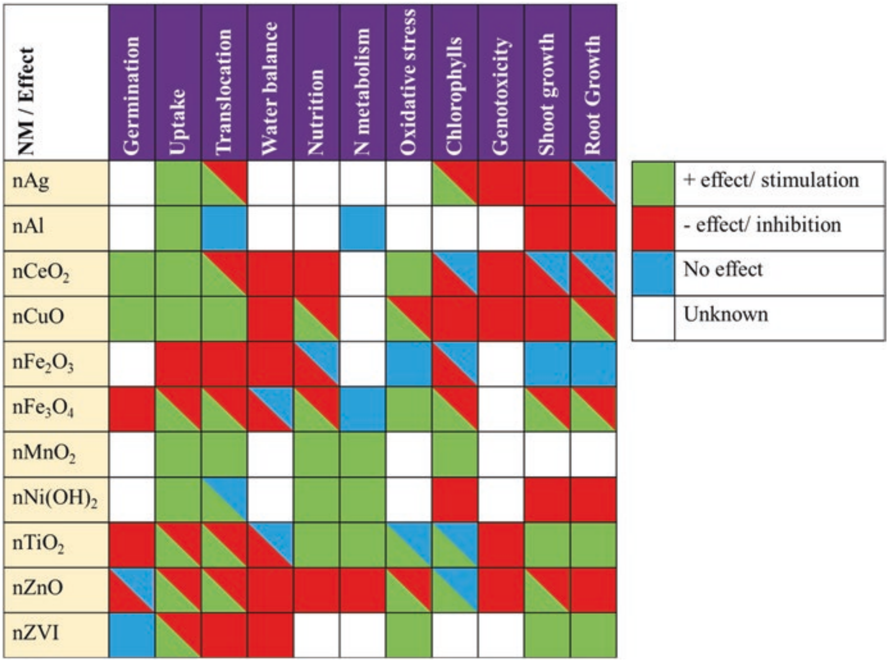
14.2.2 Nanomaterials in the Environment

The global production of engineered NMs was estimated to be 260,000–309,000 metric tons in the year 2010; of which about 8–28, 0.4–7, and 0.1–1.5% were estimated to have ended up in soils, water bodies, and the atmosphere, respectively [121]. The use of NMs in environmental remediation will inevitably lead to the release of NMs into the environment and subsequent ecosystems. Once in the environment, NMs may persist for a long time or be taken up by organisms and transferred between organisms of different trophic levels, thus acting as an ecotoxicological hazard, and undergo biodegradation or bioaccumulation in the food chain [121–123]. Plants are considered to represent both the first sink for the accumulation of NMs from the surrounding environment and the point of entry for their bioaccumulation in the food chain [124]. For this reason, emerging studies have focused on the general consequences of NMs uptake by plants, as their effects on the biomass production and plant response are very relevant to phytoremediation. Although nanotechnology has the potential to solve problems that cannot be solved by the full-scale products, one important aspect in nanoremediation, the safety of NMs, still represents a barrier to their wide innovative use and is hindering their full application; hence, intensive studies must be done before their use. As concluded recently by Schaumann et al. [125], further assessment of environmental impacts on the fate and effects of NPs is needed.

14.3 Consequences of Nanomaterials for Plants

Nanoecotoxicology is a branch within toxicology which focuses on measuring the toxicity of NMs that enter into contact with organisms like plants, bacteria, fish, and invertebrates [126]. A good understanding of the interactions of NMs with the plant system is of paramount importance for assessing their toxicity and trophic transport [127, 128]. To understand and quantify the potential risks for plants, the mobility, bioavailability, toxicity, and persistence of manufactured NMs need to be studied. An increasing number of published studies have attempted to understand the interactions between NMs and plants, and several reviews have already examined the implications of NMs in food crops [8, 129–131]. As can be seen in Fig. 14.1, which shows the general trends of the effects of NMs on plants according to the published literature to date, there is sufficient evidence that NMs can yield both beneficial and harmful effects in plant systems at the physiological, biochemical, nutritional, and genetic levels. The interactions between plants and NMs can shed light on the environmental consequences of nanotechnology, but, in contrast to the huge amount of research done on the bulk chemicals as environmental hazards, the research on NMs toxicity is markedly scarce [128] and it needs to be improved.

There are many factors which must be taken into account during nanotoxicological studies, and this makes it very complicated to understand the real consequences for plants since even small differences in the design of the experiments can produce different results. For example, for most NMs, relatively high concentrations are needed to cause observable toxicity in plants and the toxicity threshold is species dependent [132, 133]. Owing to their insolubility in water, NMs in general have a limitation for toxicity experiments [128]. Moreover, most plants showed visible signs of recuperation from NMs toxicity—indicating that the toxicity was temporary [4]. Auffan et al. [134] pointed out that chemical stability under physiological redox conditions appears to be a condition for the non-toxicity of metallic NMs. Nevertheless, metallic NMs with strong oxidative or reductive properties can be cytotoxic and genotoxic. Consequently, the nanotoxicological research on the uptake and accumulation of NMs by plants, and their subsequent response, has sometimes generated controversial data [27, 135, 136]. However, when taken together, the apparent differences in the toxicity of NMs to plants may arise from



NM / Effect	Germination	Uptake	Translocation	Water balance	Nutrition	N metabolism	Oxidative stress	Chlorophylls	Genotoxicity	Shoot growth	Root Growth
nAg	⬜	🟩	🟩🟥	⬜	⬜	⬜	⬜	🟩🟥	🟥	🟥	🟥🟦
nAl	⬜	🟩	🟦	⬜	⬜	🟦	⬜	⬜	⬜	🟥	🟥
$nCeO_2$	🟩	🟩	🟩🟥	🟥	🟥	⬜	🟩	🟥🟦	🟥	🟥🟦	🟥🟦
nCuO	🟩	🟩	🟩	🟥	🟩🟥	⬜	🟩🟥	🟥	🟥	🟥	🟩🟥
nFe_2O_3	⬜	🟥	🟥	🟥	🟥🟦	⬜	🟦	🟥🟦	⬜	🟦	🟦
nFe_3O_4	🟥	🟩🟥	🟩🟥	🟥🟦	🟩🟥	🟦	🟩	🟩🟥	⬜	🟩🟥	🟩🟥
$nMnO_2$	⬜	🟩	🟩	⬜	🟩	🟩	⬜	🟩	⬜	⬜	⬜
$nNi(OH)_2$	⬜	🟩	🟩🟦	⬜	🟩	🟩	⬜	🟥	⬜	🟥	🟥
$nTiO_2$	🟥	🟩🟥	🟩🟥	🟥🟦	🟩	🟩	🟩🟦	🟩🟦	🟥	🟩	🟩
nZnO	🟥🟦	🟩🟥	🟩🟥	🟥	🟥	🟥	🟩🟥	🟩🟦	🟥	🟩🟥	🟥
nZVI	🟦	🟩🟥	🟥	🟥	⬜	⬜	🟩	⬜	⬜	🟩	🟩

🟩	+ effect/ stimulation
🟥	- effect/ inhibition
🟦	No effect
⬜	Unknown

Fig. 14.1 Matrix of the described effects of NMs on plant physiology during environmental remediation. The matrix has been created using the general effects of each NM from the literature compiled in this book chapter. In some cases, contradictory responses have been detected (two colors in the same box), denoting that the toxicity of NMs to plants is not completely understood

their chemical (reactivity) and physical properties (size, form, aggregation) and the dose (high or low concentrations), exposure time, plants (species, age, physiological status), and experimental conditions (in hydroponics, soils, field, glasshouse, etc.) used. Many of the responses to NMs by plants have been evaluated to find out how NMs improve growth when used as soil amendments to reduce or mitigate the toxicity of contaminants; but their effects must be studied in isolation, to ascertain the effects of the NMs themselves on the plants. If the addition of NMs to a contaminated soil can potentially ameliorate metal-induced damaging effects on growth, by the reduction of metal availability and toxicity, this stimulation of growth may mask the potential negative effects caused by NMs. The effects on key physiological processes in plants of engineered NMs with potential use for phytoremediation, reported to date, are described below.

14.3.1 Germination

Seed germination tests represent one of the simple and rapid tools for assessing the phytotoxicity of NMs. The recorded effects of NMs on seed germination fall into all possible classes—being negative, nil, or positive, depending on the kind of NM, the species, and the concentration used [10, 27, 129]. For example, strong to total inhibition of germination after seed exposure ton Fe_3O_4 has been reported for lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), and spinach (*Spinacia oleracea*) [137, 138]. However, for the same NMs, Barrena et al. [139] reported low to nil toxicity against cucumber and lettuce germination. Nano-CuO did not affect germination, but inhibited growth of *Zea mays* seedlings [140] and enhanced the seed germination and shoot-to-root ratio of lettuce [141]. Exposure of pea (*Pisum sativum*) seeds to nZnO had no impact on germination [142].The effects of nZVI on germination have been reported to be concentration dependent [143, 144].

Although widely used, germination tests have to be interpreted carefully. When evaluating the influence of NMs on seed germination in relation to the phytoremediation of soil, attention should be paid to the experimental approach as different experimental designs may give different results. Classical ecotoxicological studies dealing with NMs and their effects on seed germination are usually performed with the seeds directly exposed to NMs in the suspension. But, in the context of assisted phytoremediation, this experimental approach appears to be not very suitable. In this case, NMs are applied to soil—the aim being to immobilize contaminating metals, decrease their solubility and toxicity, and promote thus the plant growth. The soil solution from contaminated soil amended with NMs thus represents a system completely different to that of a pure NMs suspension. For this reason, NMs destined for use in assisted phytoremediation should be tested not just directly in suspension; their influence on the soil solution composition and, subsequently, the influence of the soil solution obtained or the amended soil itself on seed germination should be examined too, as the results of these tests could vary significantly.

14.3.2 Uptake of NMs by the Roots

Most of the available studies on phytotoxicity of NMs have focused mainly on toxicity symptoms of plants, and relatively few have examined the mechanisms of NMs phytotoxicity, uptake, translocation, and bioaccumulation [136]. The roots are the first organ which can suffer from NMs interference in the soil, and for that reason there is an urgent need to evaluate the impacts on plant physiology of NMs, together with their potential ecotoxicity and interactions with the key processes in the rhizosphere [145–147]. Possible interactions of NMs with plant roots include adsorption onto the root surface, incorporation into the cell wall, and uptake by the cell [148, 149]. For NMs to enter the root stele, they have to either cross the cell wall and plasma membrane of an endodermal or exodermal cell or cross a root cell wall of a cell exterior to the endodermis/exodermis and move into the stele symplastically [135]. They may be transported from one cell to another through plasmodesmata. However, the exact reasons why only some plant species readily take up several NMs are still unknown and remain to be explored [131]. To cross an intact cell wall, it has been hypothesized that NMs have to move passively through a cell wall pore, suggesting that plant uptake is highly size selective. Even so, NMs could be incorporated passively into the apoplast of the endodermis; they would be then subjected to the highly size-selective permeability of the membranes before reaching the central cylinder [135]. It is generally assumed that it is difficult for NMs bigger than 20 nm to penetrate through the cell wall since the cell wall pore sizes vary from 2 to 20 nm [4, 8, 150–152]. According to Burello and Worth [153], NMs with a diameter larger than 20–30 nm act often as bulk materials; thus, the "true nanoeffects" are attributable to NMs with smaller size. Roy and Bhattacharya [4] suggested that NMs can enter plant cells by binding to carrier proteins, through aquaporins and ion channels, by creating new pores, or by binding to organic chemicals in the environmental media. Endocytosis may be also an important pathway by which NMs enter plants [154] since NMs could theoretically activate membrane receptors and induce endocytosis.

The high reactive capacity of NMs—due to their high specific surface area—can stimulate their adhesion to the epithelial root cell wall. Nanomaterials of all compositions also have the potential to aggregate, due to Van der Waals forces or other interactions [155]. As a result, NMs may aggregate along the roots, blocking their proper water uptake and disturbing thus the whole plant physiology and ultimately affecting their growth and development [3]. Aggregation of NMs appears to change the color of the roots surface by covering the epithelial cells [152, 156]. It can affect also the interactions of the plant with the external medium through mechanical disruption of membranes and cell walls, blocking the pores and diminishing the root hydraulic conductivity [156]. Despite their adherence to the surface, due to binding and electrostatic attraction by a limited number of cell surface cation exchange and binding sites on the negatively charged root surface [157], some NMs do not seem to move through the surface of the roots. In this way, inhibition of plant growth may not derive directly from chemical phytotoxicity of NMs. Instead, toxicity may result from the physical interactions between the NMs and plant cell transport pathways [129, 156]. Anyway, even though potential aggregation might dramatically increase the size of the NMs and reduce their mobility [149, 158], Whitley et al. [159] showed that NMs may remain unaggregated in soil pore water for an extended period of time, suggesting that NMs are likely to be bioavailable to plants.

Although some NMs can be found in plant cells and tissues [8], no uptake or toxicity has been reported specifically for nFeOx, which may be due to the adherence of these materials to soil particles. Zhu et al. [124] reported no measurable uptake of nFeOx by pumpkin (*Cucurbita maxima*) grown in either soil or sand, or of nFe_3O_4 by lima bean plants. Similarly, Wang et al. [160] reported no uptake of nFe_3O_4 (25 nm in diameter) by pumpkin plants. For poplar, it appeared that some of the nZVI penetrated through the membrane and was internalized in the root cells [161]. Zhou et al. [162] reported the adsorption of nCuO (55 nm in size) onto the *Triticum aestivum* root surface. As defended by Lü et al. [163], metallic NMs can affect the epithelial root cells but they seem not to have an important effect in the xylem of the plants. In spite of the adsorption of $nCeO_2$ aggregates on the root surface observed by Majumdar et al. [164], Ce accumulation increased linearly with increasing exposure concentrations, corroborating previous studies in other edible plants like tomato [165], rice [166], soybean [167], corn [168], and cucumber [169].

The concentration-dependent linear increase in Ce accumulation in roots suggests uptake through simple diffusion. According to Pradhan et al. [170], nano-sized Mn oxides (nMnOx) were readily taken up from soil by the roots of *Vigna radiata* and transported to the leaf, where nMnOx acted as a cofactor in a series of enzymatic reactions during the assimilation of nitrate into organic nitrogen compounds. In a nano-ZnO translocation study by Hernandez-Viezcas et al. [171], the use of μXRF images led the authors to conclude that Zn was obtained from nZnO by the roots, but it was not adsorbed on the root surface. This interesting research indicated an important concept because the authors were careful to separate the effects produced by nZnO from those related to and produced by Zn released from the NMs.

The type of roots and their architecture, their age, and the species are also crucial factors in the response to and uptake of NMs by roots. For example, lignin can act as a barrier to reduce the permeability of foreign materials in cells. This could be a reason why, in the study by Ma et al. [161], nZVI was able to enter the root cells of poplar plants, while the relatively high lignin content in the cell wall of *Typha latifolia* prevented nZVI from passing through it.

14.3.3 Translocation and Accumulation

The available literature indicates vaguely that NMs are found in plant cells and tissues, and even though some studies report NMs internalization in roots, no translocation to the shoots was found [4, 130]. However, NMs could potentially be taken up by plant roots and transported to shoots through vascular systems, depending upon the composition, shape, size, and plant anatomy [129]. There are many physico-chemical differences between plant species—such as variations in hydraulic conductivity, cell wall pore size, and root exudate chemistry—that could influence NMs bioaccumulation. For example, Zhu et al. [124] observed accumulation of nFe_3O_4 (min. size 20 nm) in *Vigna radiata* (mung bean) and pumpkin grown in an aqueous medium, but did not observe bioaccumulation when conducting this same experiment using *Phaseolus lunatus* (lima bean) or *Phaseolus limensis*, in either soil or sand. Corredor et al. [172] investigated xylem transport of NMs by injecting graphite-coated iron NMs into the pith cavity of the leaf petiole of pumpkin plants, and a very homogenous population of approximately 46-nm NMs was found in the xylem at a distance from the injection site, suggesting that NMs larger than 46 nm were not transported. Recently, lack of uptake and translocation for nFe_2O_3 were demonstrated by Martínez-Fernández et al. [156], without the presence of these NMs in the sap of *Helianthus annuus*, possibly as the result of aggregation of NMs on the root surface. Another study examined the importance of size on uptake by exposing wheat plants to $nTiO_2$ ranging from 14 to 655 nm [173], concluding that NMs greater than 140 nm were not taken up and NMs greater than 36 nm were not translocated into the aerial portions of the plants. The accumulation and translocation of $nCeO_2$ were dose dependent [174], but they accumulated mainly in the root tissue [166, 175]. Birbaium et al. [176] also reported no uptake after exposing 3–5-week-old corn plants to 37-nm-diameter $nCeO_2$ for 14 days. The work by Schwabe et al. [177] showed that $nCeO_2$ with a size range of 17–100 nm is at least partially available for uptake by pumpkin. Translocation of Ce has, however, been documented in previous studies of cucumber [169], corn [178], and beans [164]. Ma et al. [161] reported that nZVI was able to move into the root cells of poplar plants while such internalization was absent in the case of *Typha latifolia*, maybe because the aggregate of nZVI was too large for the xylem tissues to transport. In both cases, upward transport to the shoots was insignificant. Although Lin and Xing [179] reported accumulation of the nZnO in the protoplast of endodermal cells, they found no evidence that the particles were translocated into the shoots and leaves, possibly as the result of NMs aggregation in the exposure media. Wang et al. [180] observed xylem- and phloem-based transport and biotransformation of nCuO (20–40 nm) as well as nCuO transport from roots to shoots via the xylem and translocation back to roots via the phloem.

In contrast, recent studies show that Au, Ag, CuO, and ZnO NMs are readily taken up and translocated by plants, either as NMs or in their ionic form [181]. Nanomaterials may accumulate and/or increase the concentrations of the component metal in the fruits/grains of agricultural crops, have detrimental or beneficial effects on the agronomic traits, yield, and productivity of plants, induce modifications in the nutritional value of food crops, and transfer within trophic levels. So, it is important to establish whether a more predominating trend of NMs accumulation exists and whether the metals involved follow the same trend as the chemical form available in the soil or in the water [27].

14.3.4 Water Balance

Because of their relevance to the proper growth, nutrients uptake, stress, and biomass production of plants, more studies of NMs are needed at the root–soil interface, including measurements of plant water relations [182]. As described above, many researchers consider that the observed toxicity exerted by NMs in plants is based on physical plant–NMs interactions. The presence of NMs on the root surface could alter the surface chemistry of the root such that it affects how the roots interact with their environment [183]. For example, it is known that metals/metalloids can reduce the root hydraulic conductivity, with consequent decreases in plant water content, turgor potential, and growth [184, 185], but how metallic NMs influence the transport of water through the roots is not known. Martínez-Fernández et al. [50, 156] found a reduction of the root hydraulic conductivity in plants of *H. annuus* treated with nFe_2O_3 in hydroponic culture, but no changes in the internal water status of plants grown in a contaminated soil treated with the same NMs. The work by Trujillo-Reyes et al. [186] suggests that the reduction in dry biomass production in plants exposed to nFeOx is most likely due to the particle aggregation on the surface of the root, which affected water entrance, resulting in growth reduction. Asli and Neuman [187] also found that exposure to $nTiO_2$ (30 nm) caused a reduction of the water transport capacity in sections of primary roots, their effect being proportional to the exposure time in hydroponic culture. The $nTiO_2$ also inhibited transpiration, reduced root hydraulic conductivity in *Zea mays* root apices, caused cell wall pores to constrict, and resulted in minor inhibition of shoot and root growth. Other work suggests that NMs increase the expression of aquaporins in roots [188, 189], maybe as a response by the plant to compensate a reduction in root hydraulic conductivity. Trujillo-Reyes et al. [190] showed that, although Fe ions/NMs did not affect the water content of lettuce plants, Cu ions/NMs reduced their water content, root length, and dry biomass. Nano-CuO (size: <50 nm) was reported to reduce the transpiration volume in plants [191], also related with an up-regulation of proline-biosynthesis genes under nCuO exposure in *Arabidopsis thaliana* [192]. Nano-CuO stress induced high accumulation of proline (a water-stress indicator in plants), and the degree of accumulation was associated closely with the nCuO concentration [193, 194]. The accumulative transpiration rate in plants indicated that transpiration was highest for the controls and gradually decreased as the concentration of nZVI increased in poplar, *Typha latifolia* [161], and peaplants after nZnO exposure [142, 179].

14.3.5 Nutrients Uptake

A damaged water transport system implies a lower capacity to pass water to the shoot, affecting the transport of all the dissolved elements and causing a deficiency of them in the shoot, according to the plant requirements. Generally speaking, exposure to NMs involves changes in the nutritional status of the plants and development is negatively affected, but positive effects have been documented as well. Studies revealed that the lower uptake of nutrients is related to the fact that NMs clog the root openings and inhibit both hydraulic conductivity and nutrient uptake in roots [156, 187], although NMs with high specific surface areas may also help to sequester nutrients on their surface.

The effects of NMs on plant nutrition have been reported in few studies [195, 196]. Martínez-Fernández et al. [156] detected a significant reduction of trace elements concentrations in shoots and roots of *H. annuus* exposed to nFe_2O_3, without changes in their concentrations in the sap. However, the concentration of Mo in the roots increased with the dose of nFe_2O_3, maybe due to the close relationship between the Fe and Mo uptake systems and because the uptake of Mo can be facilitated under higher Fe concentrations in the external medium [197]. Iron oxide NMs have been reported as facilitators of iron and photosynthates transfer to the leaves of peanut [40]. In bean (*Phaseolus vulgaris*), nCuO decreased the shoot Fe, Zn, and Ca levels, but not that of Mg, while K showed little change and Na increased [195]. Aluminum, Ca, and Zn concentrations in roots and leaves were higher in plants exposed to Cu NMs, compared with the control treatment [190]. Silicon NMs used to mitigate the Cu toxicity increased the contents of Mg, Ca, K, and P in the root and shoot of pea plants [15].

There are different effects related to the nitrogen uptake and metabolism in plants during the interaction with NMs. Manganese NMs affected the assimilatory process by enhancing the net flux of nitrogen assimilation in mung bean plants [170]. Nano-sized TiO_2 can also have a positive effect on plants through promotion of the uptake of nitrate, which accelerated the transformation of inorganic nitrogen into organic nitrogen [27], due to increased nitrate reductase activity, and could also protect chloroplasts from aging in soybean and *A. thaliana* [198, 199]. Exposure to $nTiO_2$ also increased biomass, photosynthesis rate, and enzyme activity in spinach [200–202], related to enhanced N_2 fixation from nitrogen photoreduction and the stimulation of RuBisCo activity. Cai et al. [203] reported stimulation of the removal of Cu and nitrate from aqueous solution, after the application of bimetallic Fe/Ni NMs. On the other hand, the presence of nZnO in the environment is potentially hazardous to the *Rhizobium*–legume symbiosis system [142], this interaction being an important factor for plant growth and crop productivity as it provides bioavailable nitrogen to the plants. The presence of nZnO in the rhizosphere affected the early interactions between rhizobia and the host plant as well as nodule development, and subsequently delayed the onset of nitrogen fixation [142].

14.3.6 Oxidative Stress

Nanomaterials can mediate significant elevations in reactive oxygen species (ROS) generation and its subsequent consequences (such as membrane damage), as well as the modulation of antioxidant defense system components and cellular redox homeostasis in plants. Iron nanooxides can significantly increase the antioxidant enzyme activities, but their effects seem to be related more to the changes in the mineral composition in the plant than to the presence of nanoscale forms of Fe [190]. Iron NMs toxicity studies have primarily focused on Fe(II) and its oxides, and little is known about the toxicity specific to others NMs such as nZVI. However, nZVI produces Fe(II) and iron oxides through oxidation, and nZVI can produce free radicals which are highly reactive and cause oxidative stress [204]. This could be one of the mechanisms behind the toxic effects of nZVI on plants. Further studies on *A. thaliana* evidenced that nZVI triggered high plasma membrane H^+-ATPase activity, resulting in stomatal opening that was fivefold higher than in unexposed plants [205]. Nano-MnOx has been reported to increase the activity of the electron transport chain by binding with the CP43 protein chain of photosystem II [206]. The nMnOx enhanced the oxygen evolution process, being a part of the water splitting complex in the light reaction of photosynthesis, hence improving the photophosphorylation capacity [206]. Nano-CuO stress also induced modulation of antioxidant enzymes activity, and nCuO treatment caused oxidative damage to rice seedlings, as evident from high ROS-scavenging antioxidant enzymes activity and enhanced malondialdehyde levels [193], and maximally disrupted the plant-defense system by oxidative stress [207]. The accumulation of $nTiO_2$ in plants does not appear to induce oxidative stress in the leaves [173]. Biochemical assays with nZnO indicated increases in the specific activity of CAT (in the root, stem, and leaves of *Prosopis juliflora-velutina*), but no evidence of chlorosis, necrosis, stunting, or wilting, even after 30 days of treatment [171]. In ROS formation and release, the conversion of fatty acids to toxic lipid peroxides occurs, leading to the disruption of biological membranes [208] and consequently the entrance of and damage by NMs and metals, causing TBARS (thiobarbituric acid reactive species) formation—which damages the membrane permeability. This specific report showed that, by increasing the concentrations of the nZnO, higher values for the TBARS were observed. Silicon NMs protect pea seedlings against Cr(VI) phytotoxicity, by reducing Cr accumulation and oxidative stress and up-regulating the antioxidant defense system and uptake of nutrient elements [15].

14.3.7 Chlorophylls

Nano-sized materials may interact with the proteins associated with photosystems, the starch-synthesizing machinery, and/or carbohydrate translocation [209]. Since chlorophyll content is considered as an index of the total light harvesting complex and the electron transport components, present in chloroplast membranes [210], it is used as a stress indicator in plants. Studies of the bioavailability of nFe_2O_3 in *A. thaliana*, performed by Marusenko et al. [211], suggested that the Fe-NMs were not used for chlorophyll production. Iron NMs reduced the accumulation of chlorophylls in the leaves of *Lactuca sativa* [190] and *Helianthus annuus* [156], this effect being related to the reduction of the root hydraulic conductivity and the transport of dissolved nutrients from the solution, especially for Mg since this nutrient is associated with the synthesis of chlorophylls. In an experiment with $nCeO_2$, Zhang et al. [131] related a reduction in the chlorophylls content with the physical adsorption of the NMs on the root surface, and the consequent blockage of Mg uptake by the roots. Nano-CuO was reported to decrease chlorophyll content significantly in wheat [212], soybean [213], and *A. thaliana* [192], and in *Vigna radiata* in an in vitro experiment [194]. On the other hand, *A. thaliana* plants treated with bulk $ZnSO_4$ had a smaller amount of chlorophyll and were shorter compared with the plants treated with nZnO [211]. In an in vitro experiment carried out with *Petroselinum crispum* by Dehkourdi and Mosavi [214], $nTiO_2$ caused a significant increase in the chlorophyll content of seedlings. Higher chlorophyll contents were also recorded in leaves of *Brassica juncea* treated with Ag NMs [215].

14.3.8 Genotoxicity

Plants have been used as indicator organisms in studies of genotoxicology, facilitating data interpretation for a complete understanding of the effect of NMs [216–218]. Genotoxicity may be produced by direct interaction of NMs with the genetic material, by indirect damage from NM-induced ROS, or by toxic ions released from soluble NMs [174, 219, 220]. Nanoparticles that cross intracellular membranes (diameter between 8 and 10 nm) may be able to reach the nucleus, through diffusion across the nuclear membrane or transportation through the nuclear pore complexes, and interact directly with DNA [220] and influence DNA replication and transcription of DNA into RNA. However, as expected, this effect is very conditioned by their size. The NMs aggregates could also mechanically damage the chromosomes. Nano-CuO is able to enter the nucleus of plant cells and mediate direct oxidative damage to DNA [221]. Nano TiO_2 (~100 nm in size) was found to be genotoxic as well as cytotoxic in plant systems [222]. Kumari et al. [223] showed a direct relationship between the increase in the number of aberrations and the increase in the concentrations of the NMs, by analysis of changes in the chromosome morphology caused by nZnO in root cells of *Allium cepa*, and explained these results based on ROS activation. According to López-Moreno et al. [174], the toxicity may rise either due to the interaction of the DNA with the Zn ions leached out from the nZnO or its direct interaction with the nZnO. But the absence of nZnO in plant tissues, as shown by the XANES results, failed to confirm the main reason behind the genotoxic response in soybean. It is not clear from these studies whether the genotoxicity in plants is caused by the NMs themselves or their biotransformation within the plants. Ma et al. [129] pointed out that one of the most urgent needs in plant–NMs interaction studies is to determine the genetic response of the plants and the genes that are up-regulated/down-regulated in plants exposed to NMs, but this knowledge is in its infancy still. More research needs to be focused on the differences in toxicity of NMs in relation to their respective bulk counterparts, and on the effects of the ions produced inside or outside the organism exposed to the NMs.

14.3.9 Growth and Biomass Production

During recent years, the number of peer-reviewed papers related to nanoecotoxicology has increased exponentially. On the one hand, there are abundant references for positive effects of NMs on growth and biomass production: $nTiO_2$ in spinach [200–202]; $nCeO_2$ increased root and stem elongation in cucumber [167]; nCuO enhanced lateral root formation in *A. thaliana* [192]; nCuO enhanced the lignification of root cells in *Glycine max* [213]; nFeOx increased root elongation in pumpkin [160]; etc. On the other hand, negative effects also appear very often in the related literature: Al_2O_3 NMs in tobacco plants [224]; nCuO reduced root length in *Landoltia punctate* [225]; nCuO in *L. sativa* and *M. sativa* [196]; $nCeO_2$ decreased stem elongation in corn and inhibited root elongation in alfalfa and tomato [167].

Many researchers have considered the biomass production as a response to the stress, concluding that some external factors (treatments) affect it positively or negatively according to the stimulation or inhibition of the growth, development, and productivity of plants in comparison to untreated controls. However, the biomass production is only the mere consequence of the huge combination of the positive

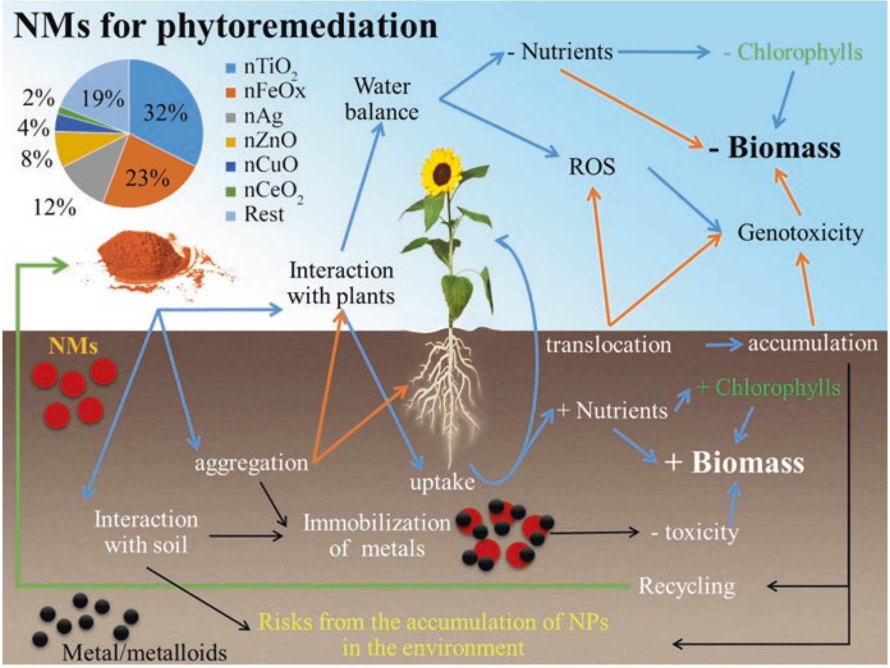


Fig. 14.2 Once in the soil, the engineered NMs can interact with the metal/metalloids in the soil solution, aggregate, or interact with the roots of the plants. According to the balance among all the positive and negative effects at different plant physiological levels, the plant will show the overall effect on biomass production; but, even when apparent changes in growth are not manifested, intrinsic and important effects can happen in the plants. In accordance with Juganson et al. [28], the chart shows the percentages of each type of NM within the total number of publications in the Thomson Reuters Web of Science™ for "environmental remediation" by NMs

and negative effects (Fig. 14.1) at different plant physiological levels (Fig. 14.2). In contaminated soils, the application of engineered NMs can decrease the contaminant availability in the soil solution and thus enhance the growth of plants. Nevertheless, as described above, there are many steps previous to the complete understanding of the real consequences for plants exposed to NMs. In fact, and generally speaking, long-term exposure to metallic NMs affects growth negatively [226–228]. Only when the individual effects of engineered NMs on plants and their applicability are properly evaluated, can concise conclusions be obtained to decide about their use during phytoremediation tasks.

Sunflower plants treated with nFe_2O_3 in a Zn-contaminated soil showed a 25% increase in shoot biomass, related to the Zn-adsorption capacity of the NM [50]. These results highlight the applicability of this NM as an amendment during phytoremediation due to its immobilization of metals in the soil, stimulating the growth of plants by making the contaminants less available. However, an additional experiment with the same species in hydroponic culture showed that treatments with the same nFe_2O_3 reduced the functionality of the roots, changed the nutrient status of the plants, and led to reductions in the shoot macronutrient concentrations and chlorophyll content [156].

Aspects like stem and root elongation, root gravitropism, architecture of the root system, and number of lateral roots can help to describe the direct effect that NMs have on the growth of plants. Trujillo-Reyes et al. [190] found that iron NMs (Fe/Fe_3O_4) reduced root size and changed root architecture, as well as affecting the root water content and the chlorophylls accumulation in the leaves of *Lactuca sativa*. Exposure of peas to nZnO had an impact on root length [142], decreasing the number of first- and second-order lateral roots. When the concentration of nZnO was increased in the medium, the shoot and root lengths declined. Extended treatments with nZnO also resulted in shorter root length than in controls without the NMs in radish, rape, ryegrass, lettuce, corn, and cucumber during seed incubation [132]. Controversial results were also found for nZnO treatments, which increased the lengths of the shoots and roots compared with the control for peanut [229] and *Brassica juncea* [230]. Ma et al. [231] observed that, although $nCeO_2$ inhibited the root elongation of lettuce, six other plant species were unaffected. Also, the dose is crucial: Ma et al. [232] noted that $nCeO_2$ at concentrations less than 250 mg L^{-1} significantly increased *A. thaliana* biomass, but, above 500 mg L^{-1}, biomass and chlorophyll production were reduced and lipid peroxidation was evident.

According to the Thomson Reuters WoS, 770 peer-reviewed papers on nanoecotoxicology that corresponded to the keywords "nano* AND ecotoxic*" were published between 2006 and March 2015 [28]. The rapidly increasing number of scientific publications on ecotoxicity of NMs over the past decade has inspired several review articles summarizing the existing data in the field. However, each review has focused on specific aspects and parameters of NMs testing; therefore, it is difficult to get an overview of all the factors (and their values) that might influence the toxicity of NMs. All these results will be an important factor to take into account with regard to the applicability of NMs for long-term use in phytoremediation tasks, but they will be especially useful when the causes become clear.

14.4 Limitations and Drawbacks of the Use of NMs

The use of NMs is not exempt from limitations. For example, the proneness to rapid passivation of some NMs [233], susceptibility to geochemical conditions [234], and possible environmental and human health threats of various NMs [235]. The high reactivity and heterogeneous size distribution of NMs may have adverse impacts on the sorption efficiency, which negatively affects the long-term performance and overall applicability. The mobility and sorption capacity of such particles are limited by three principal mechanisms: (1) nanoparticle aggregation followed by gelation, caused by poor colloidal stability, (2) nanoparticle oxidation/corrosion followed by the formation of corrosion precipitates, and (3) nanoparticle trapping from solution by interaction with other components (i.e., mineral surfaces and organic matter) or via microbial removal [71]. In order to prevent them, the particles can be coated with certain organic or inorganic materials. The development of various surface stabilizers and modifiers—such as biopolymers and alginate—has made the NMs much more versatile [11, 59, 236].

In spite of their nano size, NMs may be able to pervade very small spaces in the subsurface and remain suspended in groundwater, allowing the particles to travel farther than larger, macro-sized particles; in practice, the NMs used currently for remediation do not move very far from their injection point [237]. In fact, the introduction of NMs into soil is one of the most difficult aspects to consider in in situ soil bioremediation [21]. Since NMs have the potential to aggregate [155], either during manufacture or during wastewater treatment, this may dramatically reduce their bioavailability, mobility, and toxicity [158, 238], and consequently limit their effectiveness [233, 239]. The aggregation of some NMs supports the need for polymer or other coatings to modify their surface, in order to improve mobility [240].

The regulatory framework generally assumes that NMs possess toxicity and risk equivalent to those materials with larger particles, but the smaller size of NMs results in entirely different physico-chemical properties. Since knowledge about NMs regarding their interaction with biota and their toxicity is scarce, their full-scale application and usage for soil remediation is still problematic. For example, the report by The Scientific Committee on Emerging and Newly Identified Health Risks does not even mention or define engineered NMs.

14.5 Prospective Work Plan in Phytoremediation with NMs

As described in this book chapter, NMs appear to offer faster and cheaper remediation solutions, and their use at sites around the world is beginning to be explored. Comprehensive utilization of nanotechnology at the present time and unprecedented application of NMs in products will certainly create significant amounts of new-generation waste in the near future [30]. NMs are a reality, but future research efforts need to be directed towards finding new methods for nanoremediation, recognition of the biological effects of NMs in the environment, and creation of the bases of nanobiomonitoring. Recently, a database working group was established in the framework of the European Union Nano Safety Cluster [241]—which highlights that research efforts are necessary to promote science-based regulations for nanotechnology. Detailed research on the biogeochemical behavior of NMs in soil systems, and on the potential advantages and drawbacks of their use in chemical stabilization combined with phytoremediation, is being undertaken by the scientific community, but a much broader view is still needed about their use. Each step in this research will have the potential to provide better knowledge for the use of engineered NMs during remediation tasks and thus to provide a very significant benefit to society, by evaluating the impacts and safety of NMs application to soils contaminated with metal/metalloids.

Most experimental studies with NMs have been conducted in batch systems using model compounds and model media, instead of natural systems. Understanding the fate of NMs and their effects in natural environments also requires more realistic experimental setups [125]. Mesocosm experiments complement laboratory experiments and, as a best practice, can be combined with laboratory experiments to further develop the process of understanding the aging and functioning of NMs [125] as well as their transformations through their life cycle [242]. So, more realistic and holistic studies are needed in future investigations, including long-term experiments in more complex environmental media. Critical knowledge gaps and incomplete studies mean that the mechanisms for the removal of metals by NMs from contaminated soil proposed by different researchers are often contradictory [14]. These discrepancies in the literature can be primarily related to methodological and experimental shortcomings, such as inadequate NMs characterization, lack of consideration of NM aggregation or dissolution, lack of proper controls, or the use of environmentally irrelevant NM concentrations and/or exposure conditions. However, it is now evident that, under certain circumstances, NMs are bioavailable and toxic to several key terrestrial ecoreceptors [135].

New perspectives regarding the combined use of engineered NMs have been proposed and open a huge field for new research. For example, magnetic Fe-NMs have been demonstrated to increase the reactivity and cation exchange capacity of biochar [243, 244], increasing the uptake of nutrients by the plants since the magnetic Fe-NMs can also increase nutrient availability and decomposition of soil organic matter. Also, due to their antimicrobial properties, NMs may increase the resistance of plants to stress and produce indirect plant growth stimulation. Detailed knowledge of NMs ecotoxicity to bacteria and other soil microorganisms is also lacking. It is essential to understand the diversity of the aspects involving engineered NMs and plants if major advances in new fields are to be made.

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Chapter 15
Phytoremediation Application: Plants as Biosorbent for Metal Removal in Soil and Water

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Abstract Phytoremediation for metal-contaminated soils was started about 40 years ago, and the phytoremediation for organic pollutants is more recent. Phytoremediation has gained extensive attention and much progress in remediation of inorganic and organic contaminants and as the means for enhanced phytoremediation. Phytoremediation of various inorganic pollutants such as Cd, Cr, Pb, Cu, Zn, Co, Ni, Se, Cs, and As has been extensively studied. This is mainly based on the use of natural hyperaccumulator plants with exceptional metal-accumulating capacity, which can take up metals to concentrations at least an order of magnitude greater than the normal plants growing in the same environment. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to heavy metals.

Keywords Phytoremediation • Heavy metal contamination • Hyperaccumulator plants • Phytoextraction • Phytostabilization • Phytovolatilization • Rhizofiltration

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15.1 Introduction

Phytoremediation for metal-contaminated soils was started about 40 years ago, and the phytoremediation for organic pollutants is more recent. Phytoremediation has gained extensive attention and much progress in remediation of inorganic and organic contaminants and as the means for enhanced phytoremediation. Phytoremediation of various inorganic pollutants such as Cd, Cr, Pb, Cu, Zn, Co, Ni, Se, Cs, and As has been extensively studied. This is mainly based on the use of natural hyperaccumulator plants with exceptional metal-accumulating capacity, which can take up metals to concentrations at least an order of magnitude greater than the normal plants growing in the same environment. These plants have several beneficial characteristics such as the ability to accumulate metals in their shoots and an exceptionally high tolerance to heavy metals.

At present, there are totally more than 400 species of hyperaccumulator plants for As, Cd, Mn, Ni, Zn, etc. Phytoremediation is a general term including several processes, in function of the plant-soil-atmosphere interactions. For heavy metal-contaminated soil, four processes of phytoremediation are recognized: phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration. The first two mechanisms are the most reliable. The different forms of phytoremediation require different general plant characteristics for optimum effectiveness [1].

15.2 Definition and Concept

Phytoremediation can be defined as the process, which uses green plants for the relief, transfer, stabilization, or degradation of pollutants from soil, sediments, surface waters, and groundwater. Some plant roots can absorb and immobilize metal pollutants, while other plant species have the ability of metabolizing or accumulating organic and nutrient contaminants [2]. Multifarious relationships and interactions between plants, microbes, soils, and contaminants make these numerous phytoremediation processes possible. The term phytoremediation, from the Greek phyto, means “plant”, and the Latin suffix remedium, “able to cure” or “restore”. It can be used for a wide range of organic and inorganic contaminants [2]. Phytoremediation processes are most effective where contaminants are present at low to medium levels, as high contaminant levels can inhibit plant and microbial growth and activity [3]. Mechanisms involved in the uptake, translocation, and storage of micronutrients are the same involved to translocate and storage heavy metals [1].

Phytoremediation is considered an economical and environmentally friendly method of exploiting plants to extract contaminants from soil [4]. This process is relatively cost-effective compared with other remediation techniques. However, a thorough economic analysis for this process is unavailable. Most phytoremediation studies are directed at the biological, biochemical, and agronomic processes [5]. An economic outlook, instead of simple estimates of the cost advantages of phytoremediation over other techniques, has not been reported.

15.3 Advantages and Limitations of Phytoremediation Mechanisms

Phytoremediation, like other remediation technologies, has a range of both advantages and disadvantages. The most positive aspect of using phytoremediation is as follow: (1) more cost-effective; (2) more environmentally friendly; (3) applicable to a wide range of toxic metals, and (4) more aesthetically pleasing method. On the other hand, phytoremediation presents some limitations. It is a lengthy process, thus it may take several years or longer to clean up a site and it is only applicable to surface soils [6].

Prior to phytoremediation field trials, extensive research was performed in laboratories and greenhouses. Some of this work explored the effects of plants on removal of contaminants from spiked soil and soil excavated from contaminated sites. Many of these experiments provided valuable insights into the types and specific mechanisms of phytoremediation of organic contaminants [7]. Some organic compounds can be transported across plant membranes. Of these, the low molecular weight compounds can often be removed from the soil and released through leaves via evapotranspiration processes (phytovolatilization). Some of the non-volatile compounds can be degraded or rendered non-toxic via enzymatic modification and sequestration in plants (phytodegradation, phytoextraction). Other compounds are stable in the plants and can be removed along with the biomass for sequestration or incineration.

15.4 Basics of Phytoremediation Process

The discovery of metal-accumulating properties in certain plants leads to the development of phytoremediation technology. Research in the field of phytoremediation is aiming to develop innovative, economical, and environmentally compatible approaches to remove heavy metals from the environment. Even apart from the metal hyperaccumulating property of the plants, the presence of ground cover with plants helps to shield people from direct contact with the soil and prevents the blowing of contaminated dust around the neighbourhood [8].

15.5 Types of Phytoremediation Technologies

Depending upon the process by which plants are removing or reducing the toxic effect of contaminants from the soil, phytoremediation technology can be broadly classified as follows [9].

15.5.1 Phytoextraction

This is the process of using pollutant-accumulating plants to remove metals or organics from soil by concentrating them in harvestable plant parts.

15.5.2 Phytotransformation

This is the partial or total degradation of complex organic molecules by their incorporation into plant tissues.

15.5.3 Phytostimulation

In this process, the release of plant exudates or enzymes into the root zone stimulates the microbial and fungal degradation of organic pollutants.

15.5.4 Phytostabilization

This is a method that uses plants to reduce mobility of contaminants (both organic and metallic contaminants) by preventing erosion, leaching, or runoff and to reduce bioavailability of pollutants in the environment, thereby preventing their migration to groundwater or their entry into the food chain [10].

15.5.5 Phytovolatilization

This is the technique of using plants to volatilize pollutants or metabolites. This technology can be used for volatile organic carbons (VOCs) and for the few inorganics that can exist in volatile forms such as selenium and mercury [10].

15.5.6 Rhizo-Filtration

This is the use of plant roots to absorb or adsorb pollutants, mainly metals, but also organic pollutants, from water and aqueous waste streams.

15.5.7 Pump and Tree

This method is the use of trees to evaporate water and simultaneously to extract pollutants from the soil [11].

15.5.8 Hydraulic Control

It is the controlling of water table and soil field capacity by plant canopies [12].

15.6 Plant Selection Considerations

Plant species for phytoremediation are selected based on their root depth, the nature of the contaminants and the soil, and regional climate. The root depth directly impacts the depth of soil that can be remediated. It varies greatly among different types of plants and can also vary significantly for one species depending on local conditions such as soil structure, depth of a hard pan, soil fertility, cropping pressure, contaminant concentration, or other conditions [13].

The cleaning depths are approximately phytoremediation. It has been reported that for phytoremediation, grasses are the most commonly evaluated plants [14]. They have been more preferable in use for phytoremediation because compared to trees and shrubs, herbaceous plants, especially grasses, have characteristics of rapid growth, large amount of biomass, strong resistance, effective stabilization to soils, and ability to remediate different types of soils [2]. They are pioneers and usually are adapted to adverse conditions such as low soil nutrient content, stress environment, and shallow soils [15]. The large surface area of their fibrous roots and their intensive penetration of soil reduce leaching, runoff, and erosion via stabilization of soil and offer advantages for phytoremediation. Wild plants such as grasses can produce closures above ground quickly and reduce dispersion of the dust of tailings [16].

Shrubs and trees produce extensive canopy cover and produce deep roots to prevent erosion in the long term. In addition, shrubs or trees provide high nutrient to the grass while lowering water stress and improve soil physical properties [17]. Many trees can grow on land of marginal quality, have massive root systems, and their above-ground biomass can be harvested with subsequent resprouting without disturbance of the site. However, the cost for planting trees is high and the growth rate is low [18].

To achieve a stable persistent cover, it is important to use a mixed culture and combine grasses, shrubs, and trees in revegetation programs of mining soils because they represent two functional types of plants with different roles in the improvement of mine soils. For a longer duration, as considered for most phytoremediation processes, it cannot be expected to clean up the soil only by one plant species used exclusively in monoculture. Grasses, with their highly developed root system, can stabilize the soils and reduce erosion, while legumes can add nitrogen to the soil, preparing the establishment of other plant species typical of later stages of succession [19].

Perennial grasses develop a large plant biomass in a relatively short time and are recognized as heavy metal-tolerant biosystems, accumulating high levels of these elements. However, the shorter growing period of the seasonal flowering plants is a better option in phytoremediation over perennial plants, as it can be harvested yearly or seasonally, and the area can be replanted with subsequent seasonal flowering plants [20]

For phytoremediation, it is better to use plant species adapted to the climatic and soil conditions of the area to be de-polluted [18]. Use of indigenous plant species is generally favored because they show tolerance to imposed stress conditions, require less maintenance, and present fewer environmental and human risks than non-native or genetically altered species [17]. However, particular non-native plant may work best remediation of specific contaminant and can be safely used under circumstances where the possibility of invasive behavior has been eliminated [21].

15.7 Heavy Metal Removal by Phytoremediation

15.7.1 Heavy Metals in Soil

Heavy metals are the major environmental contaminants and pose a severe threat to human and animal health by their long-term persistence in the environment. The remediation of soils contaminated by heavy metals is a cost-intensive and technically complex procedure. Conventional remediation technologies are based on biological, physical, and chemical methods, which may be used in conjunction with one another to reduce the contamination to a safe and acceptable level. In spite of being efficient, these methods are expensive, time-consuming, and environmentally destructive [22].

15.7.2 Sources of Metal Pollution

Geological and anthropogenic activities are sources of heavy metal contamination. Sources of anthropogenic metal contamination include industrial effluents, fuel production, mining, smelting processes, military operations, utilization of agricultural chemicals, small-scale industries (including battery production, metal products, metal smelting, and cable coating industries), brick kilns, and coal combustion [23]. One of the prominent sources contributing to increased load of soil contamination is disposal of municipal wastage. These wastes are either dumped on roadsides or used as landfills, while sewage is used for irrigation. These wastes, although useful as a source of nutrients, are also sources of carcinogens and toxic metals. Other sources can include unsafe or excess application of (sometimes banned) pesticides, fungicides, and fertilizers [23]. Additional potential sources of heavy metals include irrigation water contaminated by sewage and industrial effluent leading to contaminated soils and vegetables [24].

15.7.3 Metal Toxicity

All plants have the ability to accumulate "essential" metals (Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, V, and Zn) from the soil solution. Plants need different concentrations for growth and development. This ability also allows plants to accumulate other "non-essential" metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Tl, and U), which have no known biological function [25]. Moreover, metals cannot be broken down, and when concentrations inside the plant cells accumulate above threshold or optimal levels, it can cause direct toxicity by damaging cell structure (due to oxidative stress caused by reactive oxygen species) and inhibit a number of cytoplasmic enzymes. In addition, it can cause indirect toxic effects by replacing essential nutrients at cation exchange sites in plants [26].

15.7.4 Soil Metal Groups

Metals are natural components in soil. Based on their role on physiological activities, they can be divided in two groups: (1) Essential heavy metals (Fe, Mn, Cu, Zn, and Ni) which are micronutrients necessary for vital physiological and biochemical functions of plant growth. They are constituents of many enzymes and other proteins and all plants have the ability to accumulate them from soil solution, (2) Non-essential metals (Cd, Pb, As, Hg, and Cr) have unknown biological or physiological function and consequently are non-essential for plant growth [27]. Both groups are toxic to plants, animals, and humans above certain concentrations specific to each element. High contents of both essential and non-essential heavy metals in the soil may inhibit plant growth and can lead to toxicity symptoms in most plants [28].

However, some plant species have the ability to grow and develop in metalliferous soils such as near to mining sites. Such plants can be used to clean up heavy metal-contaminated sites. Willow (*Salix viminalis* L.), maize (*Zea mays* L.), Indian mustard (*Brassica juncea* L.), and sunflower (*Helianthus annuus* L.) have been found to be highly tolerant to heavy metals. Vetiver grass (*Vetiveria zizanioides*) showed tolerance to Pb and Zn and it can be used for revegetating Pb/Zn mine tailings. Populus species are examples of plants widely used to remediate heavy metal-contaminated soils [29].

15.7.5 Heavy Metals

Heavy metals are natural constituents of the earth's crust. Their principal characteristics are an atomic density greater than 5 g cm^{-3} and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. From the geochemical point of view, trace elements are metals whose percentage in rock composition does not exceed 0.1%. The occurrence of heavy metals in soils can be the result of two main sources:

Natural source: Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000 mg kg^{-1}) and rarely toxic [30].

Anthropogenic sources: Human activities, such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations, are the main contributor to heavy metal contamination. Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence bioavailable than pedogenic, or lithogenic ones. The industry of mining and processing metals is a major source of farmland heavy metal contamination [31].

15.7.6 Heavy Metal Phytoavailability

Bioavailability and phytoavailability are terms used to describe the degree to which contaminants are available for absorption or uptake by living organisms that are exposed to them. Plants respond only to the fraction that is "phytoavailable" to them [32]. For heavy metal phytoremediation (and phytoextraction in particular), bioavailability of metals in contaminated soils is a crucial factor regulating heavy metal uptake by plant roots. However, metal phytoavailability is a complex phenomenon that is dependent on a cascade of related factors [33].

15.7.6.1 Soil pH

Soil pH directly influences the phytoavailability of metals as soil acidity determines the metal solubility and its ability to move in the soil solution. Metal cations are the most mobile under acidic conditions, while anions tend to be absorbed to oxide minerals in this pH range [18].

15.7.6.2 Soil Texture

Texture reflects the particle size distribution of the soil and thus the content of fine particles like oxides and clay [34]. Particle size distribution can influence the level of metal contamination in a soil. Fine particles (<100 μm) are more reactive and have a higher surface area than coarser material.

15.7.6.3 Soil Organic Matter

Soil organic matter is frequently reported to have a dominant role in controlling the behavior of trace metals in the soil. The organic matter is one of the factors that may reduce the ability of metals to be phytotoxic in the soil due to metal-organic complexation [35].

15.7.6.4 Redox Potential

The redox potential is one of the most soil properties that affect changes in metal speciation. Redox potential in soil is established by oxidation-reduction reactions resulting from microbial activity [36].

15.7.6.5 Root Zone

Plant root can influence heavy metal phytoavailability by modifying the soil properties in the rhizosphere. The plant enzymes exuded from the roots should play a key role in the transformation and chemical speciation of heavy metals in soils, which facilitate their uptake by plant [37].

15.8 Phytoremediation Technologies in Removing Soil Metals

15.8.1 Phytoextraction

This technology involves the extraction of metals by plant roots and the translocation thereof to shoots. The roots and shoots are subsequently harvested to remove the contaminants from the soil. Salt et al. [38] reported that the costs involved in phytoextraction would be more than ten times less per hectare compared to conventional soil remediation techniques. Phytoextraction also has environmental benefits because it is considered a low impact technology. Furthermore, during the phytoextraction procedure, plants cover the soil and erosion and leaching will thus be reduced. With successive cropping and harvesting, the levels of contaminants in the soil can be reduced [39].

15.8.2 Phytostabilization

Also referred to as in-place inactivation, it is primarily used for the remediation of soil, sediment, and sludges. It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. The plants' primary purposes are to (1) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, (2) act as a barrier to prevent direct contact with the contaminated soil, and (3) prevent soil erosion and the distribution of the toxic metal to other areas [8].

Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is useful for the treatment of lead (Pb) as well as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn). Some of the advantages associated with this technology are that the disposal of hazardous material/biomass is not required and it is very effective when rapid immobilization is needed to preserve ground and surface waters. The presence of plants also reduces soil erosion and decreases the amount of water available in the system [21].

Phytostabilization has been used to treat contaminated land areas affected by mining activities and Superfund sites. The experiment on phytostabilization by Jadia and Fulekar [40] was conducted in a greenhouse, using sorghum (fibrous root grass) to remediate soil contaminated by heavy metals and the developed vermicompost was amended in contaminated soil as a natural fertilizer. They reported that growth was adversely affected by heavy metals at the higher concentration of 40 and 50 ppm, while lower concentrations (5–20 ppm) stimulated shoot growth and increased plant biomass. Further, heavy metals were efficiently taken up mainly by roots of sorghum plant at all the evaluated concentrations of 5, 10, 20, 40, and 50 ppm. The order of uptake of heavy metals was: Zn > Cu > Cd > Ni > Pb. The large surface area of fibrous roots of sorghum and intensive penetration of roots into the soil reduces leaching via stabilization of soil and is capable of immobilizing and concentrating heavy metals in the roots.

15.8.3 Rhizofiltration

This technique is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations [41]. It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots [21]. Sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for their ability to remove lead from water, with sunflower having the greatest ability. Indian mustard has a bioaccumulation coefficient of 563 for lead and has also proven to be effective in removing a wide concentration range of lead (4–500 mg L^{-1}) [8]. The advantages associated with rhizofiltration are the ability to use both terrestrial and aquatic plants for either in situ or ex situ applications. Another advantage is that contaminants do not have to be translocated to the shoots.

An experiment on rhizofilteration by Karkhanis et al. [42] was conducted in a greenhouse with duckweed and water hyacinth (*Eichornia crassipes*) to remediate aquatic environment contaminated by coal ash containing heavy metals. Rhizofilteration of coal ash started from 0, 5, 10, 20, 30, 40%. Simultaneously, the physicochemical parameters of leachate have been analyzed and studied to understand the leachability. The results showed that pistia has high potential capacity of uptake of the heavy metals (Zn, Cr, and Cu) and duckweed also showed good potential for uptake of these metals next to pistia. Rhizofiltration of Zn and Cu in case of water hyacinth was lower as compared to pistia and duckweed. This research shows that pistia/duckweed/water hyacinth can be good accumulators of heavy metals in aquatic environment [43].

15.8.4 Phytovolatilization

This technique involves the use of plants to take up contaminants from the soil, transforming them into volatile forms, and transpiring them into the atmosphere [21]. Mercuric mercury is the primary metal contaminant that this process has been used for. The advantage of this method is that the contaminant, mercuric ion, may be transformed into a less toxic substance (that is, elemental Hg). The disadvantage to this is that the mercury released into the atmosphere is likely to be recycled by precipitation and then redeposited back into lakes and oceans, repeating the production of methyl-mercury by anaerobic bacteria.

15.9 Metal Uptake by Plants

This depends on the concentration of soluble and bioavailable fraction of metals in the soil solution. The bioavailable fraction of metal in the soil can be determined by the Potential Bioavailable Sequential Extraction (PBASE) procedure [18]. Even though chemical extraction won't extract metal from the soil in a manner identical to that of a plant root system, it can be used as a reliable method for assessing the bioavailability of metals bound to soil particles [44].

Plants extract and accumulate metals from soil solution. Before the metal can move from the soil solution into the plant, it must pass the surface of the root. This can either be a passive process, with metal ions moving through the porous cell wall of the root cells, or an active process by which metal ions move symplastically through the cells of the root. This latter process requires that the metal ions traverse the plasmalemma, a selectively permeable barrier that surrounds cells [10].

In a polluted soil, the concentration of bioavailable pollutants tends to reduce over time due to physical, chemical, and biological processes. Because of this reason, aged soils are more difficult to phytoremediate [10]. It is known that to enhance metal solubility, plants either excrete organic ligands or lower the soil pH in the rhizosphere. To improve metal solubility in the soil solution, synthetic chelates such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), pyridine-2-6-dicarboxylic acid (PDA), citric acid, nitric acid, hydrochloric acid, and fluorosilicic acid can be used in phytoremediation studies [45]. The addition of excess chelating agents may increase the chances of leaching the metals from the soil to groundwater. If the metal concentration in the soil is near to the phytotoxic levels, addition of lime or organic matter reduces the metal solubility [10].

15.9.1 Phytoremediation of As, Cd, Pb, and Zn

Arsenic pollution is one of the major concerns in the world due to its chronic effects on the health of human beings. Recently, it was proposed that phytoremediation could be an effective tool for arsenic clean up [46]. Research in this field has mainly concentrated on arsenic contamination in the aquatic environment. Studies have been done to remove arsenic from contaminated soil and revealed that Chinese brake fern (*Pteris vittata*) is an efficient As accumulator. This plant is not suitable for a region like Oklahoma, where the climate is too dry, even though it can be used with higher metal 20 concentrations. Also, the concentration of Zn affects the growth of *P. vittata*. A study has shown that a concentration of 1242 mg Zn kg^{-1} in soil causes phytotoxicity to the ferns [46]. Cadmium is present in most of the zinc-contaminated sites. Different plants such as indian mustard (*Brassica juncea*), willow clones (*Salix*), alpine penny-cress (*Thlaspi caerulescens*), sunflower (*Helianthus annus*), and corn (*Zea mays*) are able to accumulate Cd. *Brassica juncea* was able to accumulate cadmium from a soil with a concentration of 200 mg Cd kg^{-1} in soil. Experiments showed that *Thlaspi caerulescens* can be a good phytoremediator in a soil with 390 mg Cd kg^{-1}. *Helianthus annus* and *Zea mays* were also found as good accumulators in soil with a cadmium concentration of 90 mg kg^{-1} [47].

There are many plants that can accumulate lead in a very high concentration in its different parts. *Brassica juncea* can be effectively used as a phytoremediator for soils with lead contamination up to 500 mg Pb kg^{-1} of soil. *Helianthus annus* and *Zea mays* have been grown in a soil with a concentration of 16,000 mg Pb kg^{-1} [48]. Research using *Piptatherum miliaceum* (Smilo grass) has shown that this species can be used for remediating the metal contamination in a soil with 300–1500 mg Pb kg^{-1} concentration [49]. *Thlaspi praecox* is able to accumulate a considerable amount of Pb from soil with a concentration of 67,940 mg Pb kg^{-1} [50]. *Hemidesmus indicus* has been shown to remove 65% of the lead effectively from a soil having 10,000 ppm of lead concentration [51]. Most of the superfund sites in US are contaminated with zinc. Studies showed that *Piptatherum miliaceum* (Smilo grass) can be used for 21 phytoremediation in a soil with 100–600 mg Zn kg^{-1} concentration [49]. *Helianthus annus* and *Zea mays* have been grown in soil with a concentration of 75,000 mg Zn kg^{-1} and found to accumulate zinc in their harvestable parts [48].

15.9.2 Plants as Biosorbents for Heavy Metals Removal in Waste Water

Wastewater is a mixture of pure water with large number of chemicals (including organic and inorganic) and heavy metals, which can be produced from domestic, industrial and commercial activities, in addition to storm water, surface water, and ground water [52]. Due to the danger of the entry of chemicals into wastewater, it must be treated before the final disposal. Many physical, chemical, and biological methods have been developed for the treatment of wastewater. It is reported that biological methods are more interesting for wastewater treatment and one of the branches of biological method for wastewater treatment is phytoremediation [53]. The concept of this method is based on the using of plants and microorganisms in the same process as to remove the pollutants from environment [54].

Among phytoremediation techniques, artificial wetlands (AW) is known to be as the most effective technology to treat wastewater. The AWs can promote biodiversity via preparation of alarge habitat for a wide number of wildlife such as the reptiles, rodents, fishes, and birds. It should be noted that the selection of suitable species of plants is important for the implementation of phytoremediation [53].

The selected species must contain the following features: (1) high ability to uptake both organic and inorganic pollutants; (2) high ability to grow faster in wastewater; and (3) should be easy to control. It should be also noted that the ability of pollutant removal varies from species to species, plant to plant within a genus [55]. The rate of photosynthetic activity and plant growth have a key role during the implementation of phytoremediation technology for the removal of low to moderate amount of pollutants [56]. In addition to water hyacinth, plants like Water Lettuce (*Pistia stratiotes*), Duckweed (*Water lemna*), Bulrush (*Typha*), Vetiver Grass (*Chrysopogon zizanioides*), and Common Reed (*Phragmites australis*) have been successfully implemented for the treatment of wastewater containing different types of pollutant [57]. Nowadays, human health is being threatened with the release of polluted wastewater in presence of heavy metals into the environment.

Lasat [58] has shown that plants are successful in removing the heavy metals. The use of plants as biosorbents for the removal of heavy metals is considered to be inexpensive, effective, and eco-friendly technology. Phytoremediation can be considered advantageous if the plant is considered to be as solar-driven pump which can concentrate and extract particular type of elements present in the polluted wastewater. The root of the plant helps to absorb the pollutants existing in the wastewater, particularly the heavy metals and will help in improving the quality of water [59].

Water hyacinth has been widely studied in the laboratory at pilot and large scale for the removal of organic matter present in the waste water in comparison to other aquatic plants. Although water hyacinth is known to be a persistent plant all over the world, it is being widely used as a main resource for waste management and agricultural process [60]. Both the field and laboratory studies have shown that water hyacinth is capable of removing large number of pollutants present in the swine wastewater [61]. Duckweed and water hyacinth are being considered for the treatment of dairy and pig manure-based wastewater [59]. The treated wastewater in the presence of water hyacinth for the duration of 25 days resulted in the reduction of solids, calcium, magnesium, and total hardness. Wastewater from duck farm was treated by water hyacinth and resulted in 64, 23, and 21% removal of COD, TP, and TN, respectively [62]. In combination of water hyacinth and duckweed for treating dairy wastewater, it could remove 79% of total nitrogen and 69% of total phosphorus [57].

Chen et al. [63] demonstrated that 36% of nitrogen and phosphorus could be removed from swine wastewater using water hyacinth. Also reported among the different forms of nitrogen, ammonical nitrogen was found to be removed to a greater extent when compared to other forms of nitrogen.

Ismail et al. [64] showed the efficiency of water hyacinth and water lettuce for the uptake of nitrate, *ortho*-phosphate, nitrite and ammoniacal nitrogen. It was found that water hyacinth exhibited better performance for reducing nitrate in comparison to orthophosphate. Valipour et al. [65] in their latest study showed that the roots of water hyacinth are primarily involved in the transportation, where the shoots resulted in the accumulation of considerable amount of nutrients (N and P) in comparison to the root area.

Liao and Chang [66] ranked the heavy metal removal rate based on the ability of water hyacinth to remove (Cu > Zn > Ni > Pb > Cd) and showed that higher and lower removal efficiency belonged to Cu and Cd, respectively. Xiaomei et al. [67] used water hyacinth for the removal of Zn and Cd from wastewater and also measured the concentration of Cd and Zn absorbed in different parts of water hyacinth (stem, leaves, roots, flowers). It was observed for the presence of 2040 mg kg^{-1} of Cd and 9650 mg kg^{-1} of Zn accumulated in the roots of water hyacinth. According to Shaban et al. [68], to treat 1 L of wastewater contaminated with 1500 mg L^{-1} arsenic requires 30 g of dried water hyacinth root for a period of 24 h estimated chromium(III) removal from the aqueous solution and found the removal rate to be 87.52% with 10 mg Cr/1 solution. Gupta and Balomajumder [69] found that water hyacinth can uptake more than 99% of phenol in a single and twofold solution of Cr and Phenol (at 10 mg L^{-1}) in 14 and 11 days, respectively. Padmapriya and Murugesan [70], during their study for the removal of heavy metals in aqueous solution using water hyacinth, found Langmuir and Freundlich models fitted well for the biosorption of all the metal ions.

15.10 Fate of Absorbed Metals in Plant

The metals absorbed in a plant can accumulate in various parts of the plant. For an effective phytoremediation process, the metals should be accumulated in a harvestable part of the plant. Brake fern, one of the major plants for arsenic phytoremediation, accumulated almost 95% of arsenic taken up into the aboveground biomass. The arsenic concentration in the brake fern root was the least when compared to the other parts. The highest concentration was reported in old fronds followed by young fronds, fiddle heads, and rhizomes [71]. Arsenate usually enters the plant root through the phosphate uptake system, and to limit the toxicity, the plant chemically reduce As(V) to As(III) in the roots. In the case of Indian mustard, a large portion of absorbed As remains in the root itself and a small amount of arsenic is transported to the shoots; however, the addition of water-soluble As-chelators can increase this fraction [72]. In most plants, the major portion of absorbed Cd remains in the root of the plant and only some is translocated to the shoots [72].

Sunflower accumulates zinc mostly in the stem (437.81 mg Zn kg^{-1} dry weight) and lead in roots (54.53 mg Pb kg^{-1} dry weight). In the case of corn, lead and zinc were accumulated more in leaves (84.52 mg Pb kg^{-1} dry weight) (1967 mg Zn kg^{-1} dry weight) [48]. Hemidesmus indicus 22 accumulates lead in the shoots [51] and Smilo grass accumulates lead in roots and zinc in shoots [49]. Experiments on *Thlaspi praecox* revealed that Zn and Cd accumulate in the shoots and their concentration in the shoots is linearly correlated with total soil Zn and Cd concentrations, thus confirming that the plant can be used for the phytoremediation of soil contaminated with Zn and Cd. At the same time, 80% of the accumulated lead is immobilized in the roots [50].

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Chapter 16
Nutrient Management Strategies for Coping with Climate Change in Irrigated Smallholder Cropping Systems in Southern Africa

Davie M. Kadyampakeni, Isaac R. Fandika, and Lawrent L.M. Pungulani

Abstract Sound management of soil nutrients is critical for optimizing crop vegetative and reproductive development and realizing high yields in irrigated cropping systems. This paper discusses the work done in Africa and presents lessons from other parts of the world for improved nutrient management under irrigation. Considering the rising temperatures and erratic rainfall as a consequence of climatic change and depleted soil nutrients as a result of continuous cropping, this review offers remedial options for managing soil fertility while optimizing water use and crop yields. The paper intends to inform agricultural policy makers and help farmers and organizations in Africa to manage soil nutrient and water resources efficiently and achieve high yields. Importantly, this discussion should stimulate further research in nutrient and water management under varying ecological scenarios of southern Africa to provide a cogent basis for climate change adaptation interventions.

Keywords Irrigation management • Nutrient depletion • Nutrient use efficiency • Water use efficiency

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16.1 Introduction

Agricultural water management in southern Africa uses about 6% of cultivated area (Table 16.1). This is far below the irrigation potential pegged at 37% of cultivated land [1]. Average annual precipitation estimated at 9.07×10^{12} m^3 and annual agricultural water withdrawals of approximately 20.41×10^9 m^3 suggest increasing potential for water extraction and irrigated agricultural production [1]. One constraint for intensifying irrigated production is lack of site-specific nutrient guidelines for managing crops under irrigation. Farmers have been managing irrigated crop nutrients by trial and error, for example, using recommendations for crops grown during the rainy seasons due to lack of guidelines for irrigated nutrient management. Besides this, the recommendations that have been developed in the region are limited to a few crops such as sugar cane and rice. Considering the gross nutrient mining in sub-Saharan Africa, there is need for integrated sustainable agricultural systems of nutrient management by (1) applying modest amounts of mineral fertilizer according to site-specific recommendations, (2) improving water storage and nutrient retention through efficient use of manure and household waste, and (3) properly timed or split application of mineral fertilizers and appropriate tillage and conservation measures [2–5]. In view of lack of these guidelines, this paper documents the merits and demerits of nutrient management options that could be tested and adapted to specific agro-ecologies of southern Africa under irrigated conditions. This approach, together with judicious use of nutrient- and water-efficient and high yielding crop varieties, should lead to increased expansion of cultivated area and improved crop yields, water use, and food security, while helping farmers mitigate the effects of climate change. The paper discusses several nutrient management options for increasing crop yield, mitigating adverse climate change scenarios and conserving water quality.

16.2 Nutrient Management Strategies

16.2.1 Fertilization with Soluble Inorganic Fertilizer Sources Via Irrigation

The practice of applying water via irrigation is commonly called fertigation. It is a practice of applying macronutrients particularly N, K, and S in irrigation water [6]. This method is used extensively in commercial agriculture and horticulture (vegetables, fruits trees, and other high value crops) to supply additional nutrients or correct nutrient deficiencies detected in plant tissue analysis. Fertilizer injection during middle one-third or the middle one-half of the irrigation is recommended for fertigation using microirrigation to prevent the nutrients from accumulating near the soil surface or leaching beyond the root zone and terminating fertilizer application before irrigation completion.

Table 16.1 Water use patterns for southern Africa: agriculture water withdrawals, irrigation potential, irrigated area, and precipitation (FAO [1])

Country	Potential irrigation (ha)	Average annual precipitation (10^6 m^3)	Agriculture water withdrawals (10^6 m^3)	Total cultivated area (ha)	Total water-managed area (including wetlands and valley bottoms) (ha)
Angola	3,700,000	1,258,790	211	3,300,000	400,000
Botswana	13,000	241,825	80	380,000	7939
Burundi	215,000	35,460	222	1,351,000	104,430
Congo	7,000,000	3,618,120	112	7,800,000	13,500
Lesotho	12,500	23,928	0.6	334,000	2637
Malawi	161,900	139,960	810	2,440,000	118,290
Mozambique	3,072,000	827,161	550	4,435,000	118,120
Namibia	47,300	235,253	213	820,000	9573
Rwanda	165,000	31,932	102	1,385,000	102,500
South Africa	1,500,000	603,926	7836	15,712,000	1,498,000
Swaziland	93,220	13,678	1006	190,000	49,843
Tanzania	2,132,221	1,012,191	4632	5,100,000	184,330
Zambia	523,000	767,700	1320	5,289,000	255,922
Zimbabwe	365,624	256,729	3318	3,350,000	193,513
Total	19,000,765	9,066,653	20,413	51,886,000	3,058,597

16.2.2 Merits and Demerits

Benefits of fertigation over traditional broadcast or drop-fertilizing methods include (1) timely correction of in-season nutrient deficiencies, (2) application nutrients in synchrony with crop demand, (3) use a low volume of water, at low pressure, resulting in low energy costs, (4) improved nutrient and water use-efficiency due to increased root density and reduced nutrient leaching, (5) reduced incidence of pest and weed invasion, and other plant diseases, due to a reduced wetted area and drier soil surface [6–10].

Challenges associated with use of fertigation are ascribed to its requirement of properly designed irrigation system and skilled irrigation management, currently lacking in most parts of southern Africa, with the exception of South Africa. Thus, hands-on training and demonstrations of fertigated agriculture would improve Southern African farmer's skills in fertigation. In addition, fertigation may not work well with flood or furrow-irrigated systems because a lot of the nutrients may be deposited near the inlet. Also, application of fertilizers with high amounts of anhydrous NH_3 and Ca^{2+}, Mg^{2+}, and HCO_3^- may precipitate $CaCO_3$ and $MgCO_3$, causing emitter clogging. The invention of functional and cost-effective sand and screen filtration devices has helped to overcome the clogging problems and has ultimately resulted in an expanded use of micro-irrigation in areas with low-quality water [10].

16.3 Use of Organic Manures

Typical compositions of organic animal and plant manures are presented in Table 16.2 [11–14]. The composition of animal manure and crop residues and such typical composition ranges could be used as guidelines and provide the basis for calculating manure application rates in local smallholder cropping systems.

16.3.1 Merits and Demerits

Organic manures (1) increase plant-available N, P, and other micronutrients organic matter complexation, (2) improve soil organic matter content, (3) increase soil moisture retention, improve soil structure, and increase infiltration rate, and (4) reduce Al^{3+} toxicity in acid soils by complexation with organic matter [6].

The low nutrient content and bulkiness restricts greatly the distance manure can be transported, often no more than 10 km. Chemical composition of manure is highly variable; it is sometimes difficult to apply a specific amount of nutrients when manure is spread. Mineralization of manure is dependent on many factors and not well-controlled by the producer, thus there is potential for nitrate leaching [15].

Table 16.2 Typical composition for a number of organic wastes and manures

Nutrient source	N (%)	P (%)	K (%)	Mg (%)	C (%)
	Schumann [13]				
Animal manure	1–4	0.22–0.87	0.58–1.66	na[a]	na
Agricultural wastes	0.5–2.3	0.04–0.26	0.96–1.31	na	na
Agricultural industrial wastes	0.5–1.2	0.06–1.75	0.33–1.66	na	na
	Miller and Donahoue [12]				
Cattle	2–8	0.2–1.0	1–3	1–1.5	na
Poultry	5–8	1.0–2.0	1–2	2–3	na
Swine	3–5	0.5–1	1–2	0.1	na
Sheep	3–5	0.4–0.8	2–3	0.2	na
Cattle	2–8	0.2–1.0	1–3	1–1.5	na
Poultry	5–8	1.0–2.0	1–2	2–3	na
	Mafongoya et al. [11]				
Acacia karro	2.0	0.25	na	na	4.90
Acacia nilotica	1.1	0.46	na	na	3.80
Colososper mopane	1.0	0.24	na	na	3.76
Gliricidia sepium	0.9	0.43	na	na	4.15
Hay	2.2	0.17	na	na	4.32
Acacia karro	2.0	0.25	na	na	4.90
Manure (poultry)	2.3	1.5	2.7	na	15.2
Manure (Ruminant)	1.0	0.3	1.7	na	9.0
Solid waste	0.28	0.15	0.58	na	2.54

[a] Not applicable

16.4 Integrated Inorganic and Organic Fertilizer Sources Plus Irrigation

Several studies showed the importance of good irrigation (supplemental during the rainy season or full irrigation during the dry season) and nutrient management. For example, Kim et al. [16] demonstrated that maize plants responded to N and water simultaneously resulting in ~ 61 to 68% greater water use efficiency and 48–44% greater fertilizer use efficiency due to improved crop growth, evapotranspiration (ET), and N transport to the root zone. Other studies showed similar benefits in irrigated maize, rice, and vegetables, using either inorganic fertilizer sources or a combination of organic and inorganic fertilizers [14, 17–21]. Several cropping systems that have been tested and could be adapted to local conditions in southern Africa are presented in Table 16.3.

Table 16.3 Examples of fertilizer management options that could be tested and adapted for agro-ecologies in sub-Saharan Africa

Country	Rate (kg ha^{-1})			Irrigation rate	Cropping system	Irrigation system	Soil texture	Source
	N	P	K					
Malawi	120	na	92	40 mm very 3–4 days, 40 mm every week, 40% ASWD	Maize (*Zea mayz* L.)	Furrow	Sandy loam	Fandika et al. [17]
Malawi	120	na	92	40 mm very 3–4 days, 40 mm every week, 40% ASWD	Maize (*Zea mayz* L.)	Furrow	Sandy loam	Fandika et al. [17]
Iowa, USA	30, 140, 250	30	30	510 mm, 640 mm per season	Maize (*Zea mayz* L.)	Center-pivot	Sandy loam	Al-Kaisi and Yi [36]
UK	200	30	134	20 mm every day	Maize (*Zea mayz* L.)	na[a]	Sandy loam	Ogola et al. [37]
India	60, 120, 180	30	30	874 mm, 1274 mm of irrigation plus rainfall per season	Rice (*Oryza sativa*)	Flood	Loamy sand	Mahajan et al. [38]
Philippines	200	25	40	na	Rice-rice (*Oryza sativa*)	Flood	Clay and silty clay	Dobermann et al. [39]
Indonesia	120	17	33	na	Rice-rice (*Oryza sativa*)	Flood	Clay and clay loam	Dobermann et al. [39]
Vietnam	80	17	25	na	Rice-rice (*Oryza sativa*)	Flood	Clay	Dobermann et al. [39]
China	120	17	33	na	Rice-rice (*Oryza sativa*)	Flood	Clay loam	Dobermann et al. [39]
China	90	19	62	na	Rice-rice (*Oryza sativa*)	Flood	Silty clay loam	Dobermann et al. [39]
China	120	17	33	na	Rice-rice (*Oryza sativa*)	Flood	Silty clay loam	Dobermann et al. [39]
India	120	17	33	na	Rice (*Oryza sativa*)-wheat (*Triticum aestivum*)	Flood	Silty loam	Dobermann et al. [39]

India	100	21	41	na	Rice (*Oryza sativa*)-mungbean (*Vigna radiata*)-sesame (*Sesamum indicum* L.)	Flood	Clay	Dobermann et al. [39]
Colorado, USA	134, 179	112	Na	780 mm per season	Onion (*Allium cepa*)	Drip	Clay loam	Halvorson et al. [40]
Colorado, USA	134, 179	112	Na	2230 mm per season	Onion (*Allium cepa*)	Furrow	Clay loam	Halvorson et al. [40]
Canada	270	30, 60, 90	200	0.47 L h^{-1}, 1–2 h daily	Tomato (*Solanum lycopersicum*)	Drip	Sandy	Liu et al. [41]
Jordan	113, 170	55	183	381 mm per season	Potato (*Solanum tuberosum*)	Drip (including rainfall)	Clay loam	Mohammad et al. [42]
Jordan	79, 157, 236	39, 58	Na	343 per season	Squash (*Cucurbita* spp.)	Drip	Silt loam	Mohammad [43]
Colorado, USA	168, 224, 280	na	Na	735, 1064 mm per season	Maize (*Zea mayz* L.)	Furrow	Silty clay	Halvorson and Bartolo [44]
Nebraska, USA	197, 243	22, 80	Na	64–178 mm per season	Maize (*Zea mayz* L.)	Center-pivot	Silt loam	Djaman et al. [45]
North Dakota, USA	90, 135, 180, 225	57, 67	Na	106–136 mm per season	Maize (*Zea mayz* L.)	Center-pivot	Silt loam, silty clay	Derby et al. [46]
New Jersey, USA	560	336	504	152 mm per season (plus 620 mm rain)	Maize (*Zea mayz* L.)	Trickle	Sandy loam	Karlen et al. [47]
Oklahoma, USA	118, 236, 354	na	Na	na	Maize (*Zea mayz* L.)	Center-pivot	Loam	Freeman et al. [48]
North Dakota, USA	100, 200	34	140	na	Maize (*Zea mayz* L.)	na	Loam, sandy loam	Wienhold et al. [49]

(continued)

Table 16.3 (continued)

Country	Rate (kg ha^{-1})			Irrigation rate	Cropping system	Irrigation system	Soil texture	Source
	N	P	K					
Portugal	80, 160, 240	145	224	17, 98, 114, 256, 390, 527 mm of irrigation plus rain	Potato (*Solanum tuberosum*)	Sprinkler	Silt, fine sand	Ferreira and Carr [50]
China	240	53	100	258, 443 mm irrigation, plus 579 mm rain and 298, 414 mm irrigation plus 665 mm rain per season	Rice-rice (*Oryza sativa*)	Border	Silty clay	Ye et al. [51]
Italy	53, 105	26	Na	185–346 mm irrigation plus 169 mm rain, 190–373 mm plus 120 mm rain per season	Maize (*Zea mayz* L.)	Drip	Silty clay loam	Di Paolo and Rinaldi [52]
Burkina Faso	260, 353	49, 99	50, 60	320 mm irrigation plus 573 mm rain, 317 mm irrigation plus 180 mm rain per season	Tomato (*Solanum lycopersicum*)	na	Sandy loam	Sangare et al. [14]
Burkina Faso	405, 835	87, 156	237, 255	298 mm irrigation plus 558 mm rain, 155 mm irrigation plus 294 mm rain per season	Cabbage (*Brassica oleracea*)	na	Sandy loam	Sangare et al. [14]

Burkina Faso	128.5	39, 53	117, 220	406, 482 mm of irrigation per season	Carrotn (*Daucus carota*)	na	Sandy loam	Kangare et al. [53]
Burkina Faso	415, 468	79, 114	97, 440	302 mm irrigation plus 27 mm rain, 175 mm irrigation plus 402 mm rain per season	Lettuce (*Lactuca sativa*)	na	Sandy loam	Sangare et al. [14]
Burkina Faso	313, 453	63, 107	357, 438	412, 598 mm of irrigation per season	Lettuce (*Lactuca sativa*)	na	Sandy clay	Sangare et al. [14]
Burkina Faso	247, 396, 439, 486, 504, 601	58, 68, 90, 95, 120	285, 331, 383, 413, 496, 678	175–359 mm of irrigation and 29–538 mm of rain per season	Lettuce (*Lactuca sativa*)	na	Sandy clay	Sangare et al. [14]
Lebanon	240, 360, 480	na	na	490 mm by drip and 850 mm by sprinkler irrigation	Potato (*Solanum tuberosum*)	Drip, Sprinkler	Clay	Darwish et al. [54]
India	60, 120, 180	30	30	1279, 1679 mm of irrigation plus rainfall per season	Rice (*Oryza sativa*)	Flood	Loamy sand	Mahajan et al. [38]
China	240, 270	60, 75	75	412–425, 528–545, 529–547 mm of irrigation plus 538–802 mm of rainfall per season	Rice (*Oryza sativa*)	Flood	Sandy loam	Liu et al. [21]

(continued)

Table 16.3 (continued)

Country	Rate (kg ha^{-1})			Irrigation rate	Cropping system	Irrigation system	Soil texture	Source
	N	P	K					
Australia	150	15	na	310, 380 mm of irrigation	Wheat (*Triticum aestivum*)	na	na	Whitfield et al. [55]
Florida, USA	176, 220, 330	49	247	1.1, 2.6, 3.8, 4.6 mm per day of surface or subsurface drip irrigation	Tomato (*Solanum lycopersicum*)	Surface or subsurface drip	Sandy	Zotarelli et al. [19, 20]
Florida, USA	73, 82, 145, 164	na	na	0.9, 21.1 mm per day of surface or subsurface drip irrigation	Zucchini squash (*Cucurbita pepo* L.)	Surface or subsurface drip	Sandy	Zotarelli et al. [18]
South Dakota, USA	56, 112, 168	na	na	540, 480, 480 mm of irrigation plus rainfall per season	Maize (*Zea mayz* L.)	na	Silty clay loam	Kim et al. [16]

[a] Not available

16.5 Mixed and Rotational Cropping Systems

The mixed cropping and crop rotation will be important in managing fragile environments. Examples of mixed cropping systems would include cereal-legume or legume-vegetable, or cereal-vegetable arrangements. Use of legumes, in particular, would reduce the emission of greenhouse gases such as NO, CO_2, and N_2O due to their ability to symbiotically fix N compared with cereals [22]. Examples of legumes adapted to Africa include cowpea (*Vigna unguiculata* L.), common bean (*Phaseolus vulgaris* L.), soya bean (*Glycine max* L.), groundnut (*Arachis hypogaea* L.), pigeon pea (*Cajanus cajan*), chickpea (*Cicer arietinum*), bambara groundnut (*Vigna subterranea*), and lentil (*Lens culinaris*). They occupy well over 15 million hectares in Africa with yields in the order of 200–1400 kg ha^{-1} [23].

16.5.1 Merits and Demerits

The advantages of mixed and rotation cropping include increased productivity from the same piece of land through (1) better use of solar radiation, (2) increase in nutrient and water use efficiency, and (3) better control of weeds, pests, and diseases [24]. These cropping systems, particularly crop rotation, might be limited where land sizes per farm family are fairly small (0.5 ha or less).

16.6 Mitigating Greenhouse Gas Emissions in Irrigated Crop Production

Many environmentalists have been concerned with greenhouse gas (GHG) emissions in irrigated cropping systems due to N volatilization and denitrification losses (NO, N_2O) [25–27]. In southern Africa, Meixner et al. [28] found that NO fluxes are largely controlled by soil moisture. Thus, optimizing water management and fertilizer use would reduce the impact of N losses on GHG emissions. For example, in the various agroecosystems studied, the greatest NO emissions (27 ngN m^{-2} s^{-1}) were found in the agricultural plots. In China, researchers found that improved management of irrigation, timing of fertilizer applications, and split fertilizations increased maize yields and reduced N_2O and NO emissions by 7 and 29%, respectively, with 7 to 14% greater yield in irrigated maize [29, 30]. The remedial and mitigation options for reducing N-related GHG emissions include reduction in N fertilizer use through an increase in fertilizer use efficiency, preferential use of NH_4NO_3 instead of urea, improved timing of fertilizer application, the use of nitrification and urease inhibitors, improving the fertilizer uptake efficiency of crops in tropical agriculture, and intercropping cereals with legumes [22, 31–35]. Quantification of the effects of manures and fertilizers on GHG emissions in irrigated systems for development of climate change mitigation strategies is lacking in southern Africa. Thus, climate-change scenarios investigated in developed countries should provide important insights for developing and modifying nutrient management strategies for southern Africa's irrigated cropping systems.

16.7 Water Quality Monitoring in Irrigated Cropping Systems in Southern Africa

Systems for monitoring water quality in irrigation schemes are nonexistent in sub-Saharan Africa [14]. There is a need for developing guidelines for total nutrient loads for macro- and micro-nutrients because excessive application rates may negatively affect aquatic life and other important terrestrial organisms. While the primary goal of a nutrient management program is to increase crop yield, the corollary objective should be to conserve water quality for other ecological uses. Periodic monitoring of drainage ditches and in-field water sampling in irrigation schemes and 3–4 year experiments that compare various nutrient and water application rates would provide the requisite benchmarks for developing thresholds for fertilizer application rates for maximizing crop yield and water use while conserving environmental quality.

16.8 Conclusions

The paper presented selected options for improved nutrient management in irrigation systems for adaptation to southern Africa farming systems. Technologies that conserve water and increase nutrient use efficiency will be important in helping farmers realize high yields and greater farm incomes. Long-term goals for good nutrient management in southern Africa include (1) sustaining environmental quality by minimizing nutrient leaching to surficial and ground water sources, (2) replenishing soil fertility in nutrient-depleted soils, and (3) developing site-specific nutrient and water management recommendations for smallholder farming systems. The first necessary tasks will need on-farm adaptive-verification nationwide trials to validate and modify the elite technologies in concert with selected crop genotypes. Participatory technology selection by farmers, coupled with detailed financial analysis, will help in ensuring that water and nutrient management technologies identified are acceptable and economically feasible.

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Chapter 17
Phytoremediation of Landfill Leachates

Prasanna Kumarathilaka, Hasintha Wijesekara, Nanthi Bolan, Anitha Kunhikrishnan, and Meththika Vithanage

Abstract Municipal landfill leachate is a complex refractory wastewater which consists of extensive level of organic compounds, ammonia, and heavy metals. Contamination of water by landfill leachate has become a serious environmental concern worldwide due to its adverse impact on human health, aquatic organisms, and agricultural crop production. In recent years, constructed wetland (CW) has received promising attention in the treatment of landfill leachate, because of its cost-effective and eco-friendly nature and simplicity in operation, in addition to higher treatment efficiency. Hence, the present chapter is mainly focused on providing a concise discussion of the CWs and its phytoremediation attributes for the remediation of landfill leachate. Natural wetland plant species and short rotation coppice (SRC) have been introduced to remove contaminants from landfill leachate. Different processes such as phytoextraction, phytodegradation, phytovolatilization, rhizofiltration, phytostabilization, rhizo-redox reactions, sedimentation, adsorption, and complexation involve to remove nutrients (i.e., nitrogen and phosphate), heavy metal(loid)s, biological oxygen demand (BOD), and chemical oxygen demand (COD) to a great extent in CW systems. In addition, well-managed SRC systems save millions of dollars by eliminating the leachate transportation and treatment process which were earlier practiced. Further, there are a number of examples where phytoremediation has failed due to excessive leachate application and lack of management practices. Therefore, it is obvious that successful transfer of phytoremediation technologies from the laboratory to the field is a crucial step in terms of removal efficiency.

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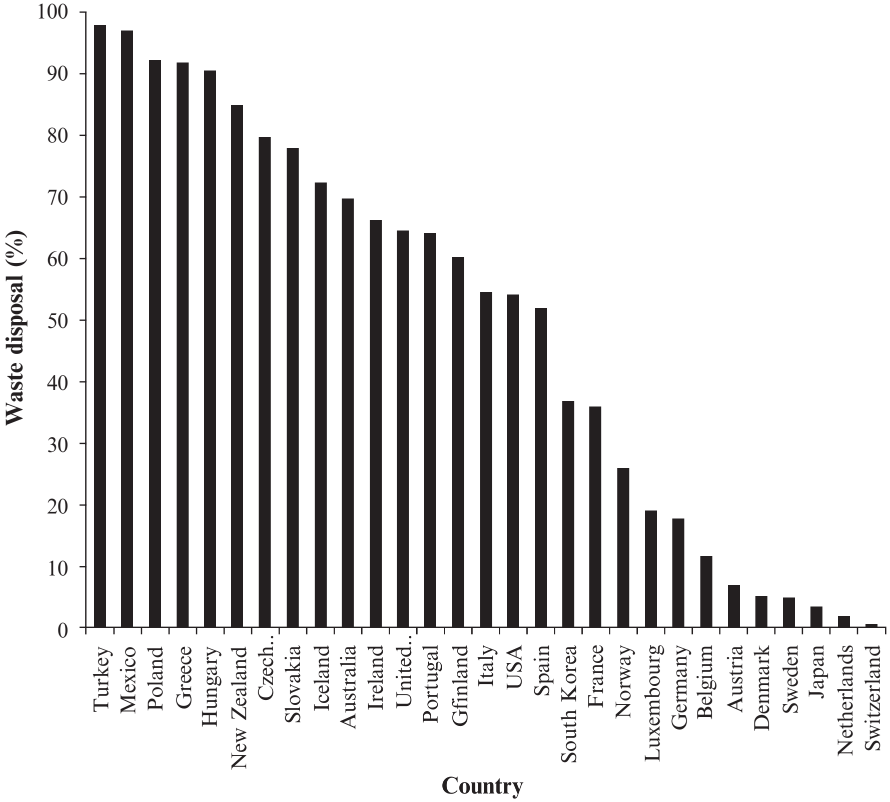

Fig. 17.1 Percentage of waste disposal in landfills in numerous nations. Reproduced from Bolan NS, Thangarajan R, Seshadri B, Jena U, Das KC, Wang H, Naidu R (2013) Landfills as a biorefinery to produce biomass and capture biogas. Bioresource Technol 135: 578–587 [3], with permission from Elsevier

Keywords Wetlands • Evapotranspiration • Macrophytes • Nutrients • Heavy metal(loid)s

17.1 Municipal Solid Waste Dumpsites

In many developing and developed countries, disposal of waste to open landfill sites is the most common method of waste management (Fig. 17.1). For instance, it is reported that approximately 90% of landfills in South and Southeast Asia are non-engineered open disposal facilities [1]. From an economic point of view, these open dump sites provide simple and cheap means of waste disposal. On the contrary, numerous contaminants including liquids, gases, and dusts may pollute the surrounding environment, if the open landfill sites are not properly managed [2, 3]. In this regard, soil and groundwater contamination, atmospheric pollution by gas emissions, and the consequent adverse health problems are particularly obvious in the open dump sites.

Depending on the source of landfill material, the contaminant type and the extent of deleterious effects vary significantly. Landfills could be divided into three main categories based on the waste materials received, namely (1) industrial waste, (2) municipal waste, and (3) a combination of industrial and municipal wastes [4]. As a result, different landfill sites play different role towards environmental contamination. For example, industrial waste is consisted of various kinds of hazardous materials including heavy metal(loid)s. These components can be easily leached from the landfill site to the groundwater and surface water bodies. On the contrary, municipal waste is mainly composed of variety of organic materials. Subsequent decomposition of these organic materials by microorganisms results in the release of leachate and emission of volatile organic compounds (VOCs) and greenhouse gases (GHGs) [5–7]. VOCs are generally more toxic at trace level than many other inorganic compounds and are implicated in carcinogenic and mutagenic effects in humans and animals. In addition, geomembranes are not effective in preventing the transport of VOCs, since they are able to diffuse readily through geomembrane polymers even in engineered landfills [8].

Non-biodegradable contaminants including heavy metal(loid)s are of significant concern in managing landfill sites, since these can be accumulated in soils and contaminated water bodies due to leaching and runoff. There have been a number of reports demonstrating the contamination of soil around the landfill site by various heavy metal(loid)s, such as Copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), and cadmium (Cd) [9, 10]. The microbial activity and soil quality can be seriously affected, consequently producing unfavorable conditions for plant growth. Deleterious effects of various heavy metal(loid)s depend upon not only the total metal concentration, but also the bioavailable fraction of a particular heavy metal(loid). However, owing to changes in soil properties, bioavailable fraction of heavy metal(loid)s can be increased, leading to potential toxicity to living organisms [4, 11].

17.2 Environmental Issues of Leachate

The main environmental aspect related to open dump sites is the discharge of leachate into the environment. More precisely, leachate contamination is the result of mass transfer process. Over its whole life-cycle process, the waste in landfill undergoes biological, chemical, and physical transformations. Primarily, three physical stages, solid phase (waste), liquid phase (leachate), and gas phase, can be identified in the landfill [12]. Since leachate contains high concentrations of contaminants including heavy metal(loid)s, xenobiotic organic compounds, dissolved and suspended organic matter, high biological oxygen demand (BOD), and chemical oxygen demand (COD), most detrimental concern associated with leachate discharge is that

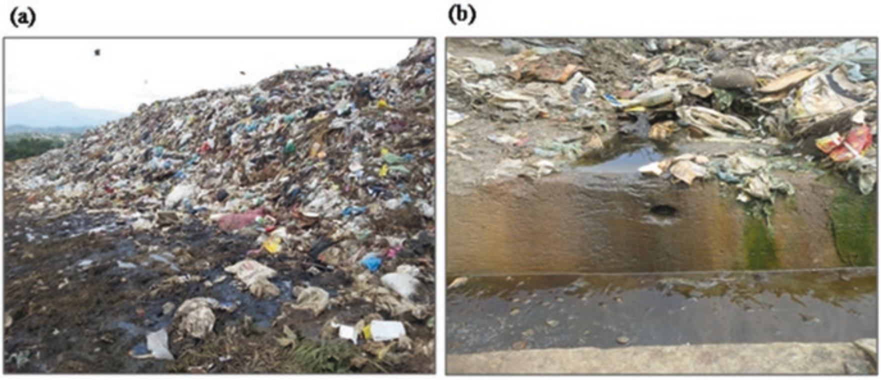

Fig. 17.2 Non-engineered landfill operation site (**a**) and subsequent production of landfill leachate (**b**)

of soil, groundwater, and surface water contamination [4]. However, leachate characteristics may vary due to the climatic variations, solid waste composition, and age of the landfill. In most climatic conditions, precipitation and snow accelerate the saturation of waste in the landfill with subsequent generation of leachate [13]. In addition, aerosols and malodors released during landfill handling and treatment is a serious concern. It is reported that some persistent pollutants such as dissolved carbon originate from household chemicals, industries, and co-deposited hazardous waste [14]. Leachate discharge into the soil and groundwater systems is likely to undergo attenuation processes including dilution, biodegradation, and other physico-chemical processes such as evaporation and adsorption [12]. Consequently, the interactions between these processes and the leachate load lead to the formation of leachate plume. The extent of leachate plume will determine the environmental risks to the surface water ecology and to human health via water supplies from groundwater and surface water [15]. Furthermore, terrestrial and aquatic plant species could be seriously damaged due to the uptake of contaminants with high concentrations.

It is obvious that the leachate impact on groundwater and surface water quality correlated with improper landfill management strategies in the past. Different factors such as landfill sitting, design, operation, maintenance, and cost mainly govern the discharge of leachate into the environment [16]. Similarly, large number of landfills is located on the ground or on a slope, hence accumulation of leachate could be a negative factor due to geotechnical stability [2]. Since there is no lining or leachate draining system in those open dump sites, the leachate problem persists for a quite long time, possibly many decades even after the landfill closure [17]. These cases showed that the importance of proper management practices is a must to control the effect of leachate. Figure 17.2 shows leachate generation and flow into the environment from non-engineered landfill sites or open dump sites.

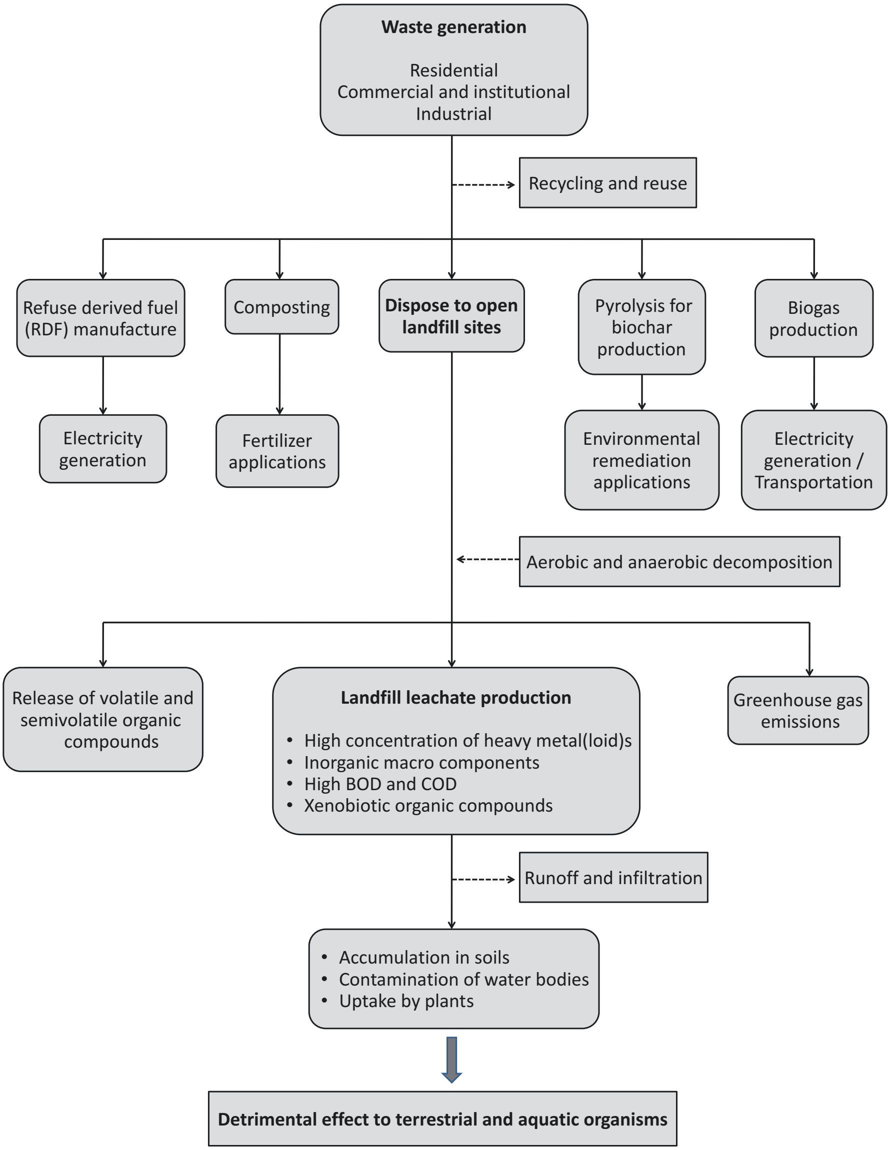

Fig. 17.3 Different waste management strategies and adverse effects of landfill leachate

17.3 Pollutants in Landfill Leachate

The landfill leachate has been identified as an intense pollutant causing severe contamination to the soil, water, and air ecosystems (Fig. 17.3). Due to the complexity of pollutants in landfill leachate, different ways are used to explain these pollutants. Basically, these pollutants are grouped into heavy metal(loid)s, xenobiotic organic compounds (XOCs), organic compounds, and inorganic compounds [5]. However, some of these groups are not mutually exclusive. On the contrary, most of the pollutants do not exist in the leachate as their unique fundamental molecules or ions, but present as complex matrices such as organic-metal(loid) derivatives. Microbial species and their toxins can be also counted as pollutants, since some of these species such as eubacteria and archaea are found to be dominated in landfill leachate [18–20].

The presence of alloys, paints (i.e., lead-based paints), automotive parts, lamp filaments, iron scraps, ceramics, and batteries (i.e., nickel–cadmium batteries) in a landfill contributes to the presence of heavy metal(loid)s in leachates [2, 21, 22]. Many researchers have reported a wide variation of heavy metal(loid) concentrations in leachates, thereby expressing the potential risk to the environment [2, 23]. Besides the sample handling techniques and protocols, the colloidal matter have a greater affinity to heavy metal(loid)s in leachate, therefore the heavy metal(loid) concentration may depend on the colloid content in leachate [5, 24]. The precipitation and sorption of heavy metal(loid)s should be uncounted to understand their fate and transport in the environment and in treatment plants.

Landfill leachate is one of the predominant wastewater types containing a wider range of XOCs such as benzene, phenol, trichloroethene, and chlorinated aliphatics. However, relatively low concentrations (i.e., <1 mg L^{-1}) of individual XOCs are reported in many cases [5, 25]. Most of these XOCs are derived from fire-retardants (i.e., tri(2-chloroethyl) phosphate), insect repellents (i.e., *N*,*N*-diethyltoluamide), and pharmaceuticals (i.e., fluoxetine and ibuprofen) [19, 26–28]. Some of the XOCs (e.g., toluene and phenol) are reported to cause serious carcinogenic and teratogenic effects to animals through chronic exposure [29].

Due to the decomposition of different types of wastes originated from slaughter houses, fish markets, and households, high concentrations of organics (e.g., recalcitrant substances such as humic and fulvic acids) and inorganic compounds (e.g., nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), sulfate (SO_4^{2-}), chloride (Cl^-), fluoride (F^-)) can be found in leachates from open dumpsites, thereby causing serious environmental pollution [2, 30]. Therefore, comprehensive characterization of landfill leachate is essential to design their appropriate treatment methods. In any case, temporal and spatial variation of the quantity and quality of landfill leachate is the most difficult issue in leachate management.

17.4 Conventional Treatment Methods for Landfill Leachates

Landfill leachate treatment technologies can be divided into three basic types, leachate recirculation, biological, and physicochemical. In most cases, combinations of these treatment technologies or integrated systems are used to design sustainable leachate treatment facilities. Additional environmental benefits such as energy crops and biogas production are also associated with some of these treatment methods [31].

17.4.1 Leachate Recirculation

Recirculation of landfill leachate into the landfill tip or into the landfill bioreactor is one of the cost-effective treatment methods. Many advantages have been reported with this technique such as reduction of leachate volume and time required for waste stabilization, enhanced biogas production, and improved leachate quality [32]. Combined treatment of landfill leachate with the domestic sewage is also identified as another type of leachate treatment method [31, 33]. A lab-scale study for treating a mixed substrate as landfill leachate and domestic sewage has been successful, concluding the importance of operative strategies such as pretreatment of leachate [34]. However, the leachate transfer methods do not behave as ultimate treatment methods, since they potentially pose disadvantages such as increment of effluent concentrations and inhibition of microbial activities during leachate recirculation [31].

17.4.2 Physico-chemical Treatment

A wide range of physico-chemical treatment methods are used for the treatment of landfill leachate. These include air or ammonia-stripping, adsorption, membrane filtration, coagulation–flocculation, chemical precipitation, chemical and electrochemical oxidation (i.e., ozonation), evaporation, reverse osmosis, photoelectrooxidation, and sedimentation–flotation [23, 31, 35, 36]. Basically, these physico-chemical treatment methods lead to a reduction of suspended solids, colloidal particles, color, pathogens, and toxic compounds including excess nutrients in the leachates. For example, activated carbon adsorption is used as a common method for the removal of dissolved organics and heavy metal(loid)s [37]. Further, waste materials such as waste steel scrap and slag are used in this type of treatment methods [38]. In most cases, a combination of these physico-chemical treatment methods has been used for achieving the targets and generally reflects better treatment abilities [31, 39]. However, the expensive nature for establishment and handling of these techniques limits their usage significantly and raises greater challenges for their commissioning.

17.4.3 Biological Treatment

Biological treatment methods are associated with the microorganisms (i.e., biodegradation through aerobic and anaerobic microorganisms) and plant species (i.e., phytoremediation). These techniques are mainly used for the treatment of organic pollutants and nutrients in leachate. Aerated lagoons, sludge processing reactors, biofilm reactors, and biofilters are examples of techniques that use aerobic biological processes [31]. Diffusers or mechanical aerators are generally used to supply aerobic conditions to these systems. Examples of anaerobic biological processes are anaerobic digesters, filters, and sequencing batch reactors [40]. Constructed wetland (CW) is a common type of biological treatment method [41]. Due to the inexpensive nature, reliability, and simplicity, these biological techniques are widely used in landfill leachate treatment. However, limited effectiveness of treatment processes with aging and low efficiency for treating old landfill leachates are identified as drawbacks associated with some of these biological treatment methods [31, 35].

17.5 Phytoremediation of Landfill Leachate

Advanced physico-chemical and biological leachate treatment technologies require continuous budget and energy supply and sufficient technical capabilities for the operation and maintenance of the equipments [16]. Therefore, high-tech solutions are not sustainable for many landfill sites, particularly in developing countries. Hence, a sustainable leachate treatment strategy with economical and technical feasibility and climatic compatibility is the only viable option in such regions. Over the last few decades, CW system has been recognized as an appropriate and practical alternative for landfill leachate treatment, making it safe to discharge into the surrounding environment [42–44]. Besides their small ecological footprint, CW systems possess similar aesthetic value as natural wetland systems.

Constructed wetland systems consist of different media types, and typically, same species of emergent plants. Constructed wetland system is mainly classified into free water surface system (FWS) and subsurface flow system (SSFS). In FWS (Fig. 17.4a), oxygen is prevalently introduced into the wetland via algal photosynthesis and atmospheric diffusion. In SSFS, leachate flows underneath and through the plant rooting media, and subsequently leachate level is maintained below the tip of the substratum. In other words, SSFS may act as fixed-film bioreactors [45]. In terms of fewer issues arising from odors, disease-related vectors, and public exposure, SSFS is highly recommended for landfill leachate treatment [46]. SSFS is of two types, horizontal and vertical (Fig. 17.4b, c). In the horizontal flow systems (HFS), the leachate is fed into the inlet and continues its way under the surface of the bed in a more or less horizontal path until it reaches the outlet zone. Conversely, in the vertical flow systems (VFS), landfill leachate is fed on the whole surface area through distribution system and passes the filter in a more or less vertical path. In VFS, greater oxygen transport is involved compared to the HFS [47, 48]. As a result, VFS is more efficient for removing ammoniacal nitrogen (NH_3-N) and organic matter from landfill leachate. The efficiency of landfill leachate remediation achieved by CW depends upon different factors including the type of media used (sand, gravel, clay, or silt), availability of microorganisms, and selectivity of plants (monoculture or mixed beds) [49].

In general, CW systems are receiving untreated or partially treated leachate [50]. For example, reed beds are considered unsuitable for primary treatment of high strength landfill leachate due to the toxicity of leachate to the reed. However,

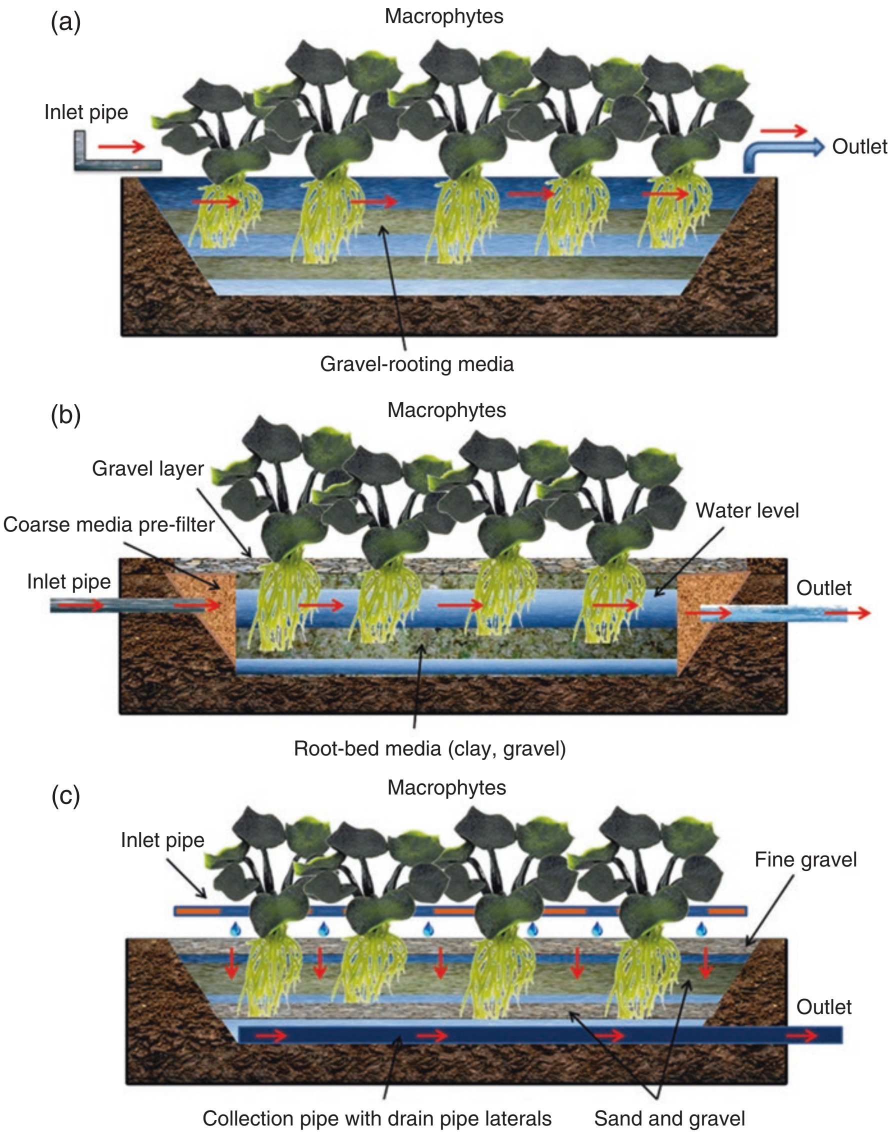

Fig. 17.4 Layout of different types of CW systems (**a**) surface flow (**b**) subsurface-horizontal flow (**c**) subsurface-vertical flow

aerobic biological pre-treatment of landfill leachate may lead to subsequent removal of contaminants effectively by reeds in CW system [51]. In the case of SSFS, sufficient mechanical pretreatment is required to remove excessive amount of suspended solids, since they may cause filtration-bed clogging and consequent surface flow. Aerated lagoons have been recognized as the prevalent pretreatment method for treating concentrated landfill leachate from landfill operations [52].

17.5.1 Physico-chemical Properties of Soil

Soil physico-chemical parameters play a major role for the purification capacity as well as longevity of CW systems. Physico-chemical parameters including pH, moisture content, bulk density, total organic carbon, and total nitrogen content could be effectively utilized to determine the status of soil quality [53]. More precisely, hydraulic conductivity (*K*) is a key factor that is closely correlated with the efficiency of landfill leachate treatment. On comparing different types of soils, sandy and gravelly soils possess high *K* values, whereas clay soils have low *K* values [51]. Therefore, the water movement through sandy and gravelly soils occurs rapidly, and hence, soil–water contact is decreased. Similarly, in clay soils also, rapid water movement and reduced soil–water contact take place. On the contrary, it is reported that silt or loamy soils mixed with sand and gravel may enhance soil–water contact at a great extent [51]. It has been experimentally observed that ammonia (NH_3), COD, total nitrogen, salinity, and conductivity were low when landfill leachate was percolated via clay and sandy soil column compared to the sandy soil alone. It can be attributed to adsorption of dissolved components including humic and fulvic acid onto soil particles, ion exchange, or precipitation [54].

Particle size and pore size distribution in a particular soil in CW systems has gained a significant attention among the soil physical parameters. Bruch et al. [55] assessed five different lava and one fluviatile operating sand filters for their differences in pore size distribution, specific inner surface area, and cumulative pore volume. The results revealed that these soil physical parameters had an influence on purification capacity and hydraulic conductivity. The alteration of soil hydraulic properties may influence the hydrology of CW system, thereby affecting the removal efficiency. During the wetland construction process, occurrence of soil disturbance including the mass grading of local landscape and redistribution of the upper soil horizons is observed [56]. Soil disturbance involves compaction and the vertical integration of soil horizons and regolith, which leads to new soil textures and change in bulk density. Additionally, loss of macrostructure and increment of clay amount may change the way water is held within the soil matrix. A study by Campbell et al. [57] observed that soils in CW consisted less organic matter, greater bulk density, and increased rock fragments. It is reported that compaction removes all macro structure within the soil matrix in terms of long-term operation basis [56].

17.5.2 Importance of Rhizosphere Microbiology

Since the microorganisms are the first organisms which deal with the pollutants in landfill leachate, they should be having an own mechanism to grow and overcome extremely toxic conditions in CW systems [58]. The health of soil ecosystems basically depends on the biological processes including decomposition of organic matter and nutrient cycling. Soil microorganisms involve transformations of organic matter and mineralization of nitrogen (N), phosphorus (P), and sulfur (S) [59]. Additionally, microbes play a crucial role in the degradation of complex chemical compounds into simpler components, which can be easily absorbed by plant species. Wetland plant species are capable of growing in environments where their root system is submerged. These plants transport air towards the root system by diffusive and/or convective mechanisms via specialized gas channel tissues called aerenchyma [60]. Subsequent leakage of air from the root system may provide aerobic conditions within the water-saturated soil instead of anaerobic conditions. Hence, soil rhizosphere provides habitats for both aerobic and anaerobic microorganisms. On the contrary, organic compounds such as sugars, alcohols, and acids which are released by the plant species into the rhizosphere act as a carbon source for microorganisms [61]. In this way, soil itself facilitates appropriate habitats for different types of microorganisms such as bacteria, fungi, and yeasts [49].

Assessing soil microbial parameters such as microbial biomass and basal respiration and biochemical parameters such as enzyme activities can predict the response of microbes to environmental changes including temperature and moisture, as well as pollution [62]. It is obvious that changes in microbial biomass lead to detrimental impacts in soil health. Therefore, different microbial testing techniques can be used to investigate soil quality with reliable and accurate measurements [51]. For instance, fluorescence in situ hybridization (FISH) technique can be utilized to recognize the number and relative distribution of bacterial species and their strains. Thus, this technology facilitates insights into the diversity of microorganisms in CW systems. A study by Sawaittayothin and Polprasert [63] revealed that the predominance of bacteria such as heterotrophys and autotrophs is responsible for BOD_5 removal from the landfill leachate, and FISH technique was used for evaluating phylogenetic identity, morphology, and number of microorganisms.

17.5.3 Selection of Plant Species for Leachate Remediation

Typical natural wetland plant species such as cattail (*Typha latifolia* L.), reed (*Phragmites australis*), rush (*Juncus effuse* L.), yellow flag (*Iris pseudacorus* L.), and mannagrass (*Glyceria maxima*) are used in CW [64]. Table 17.1 summarizes various plant species used in CW systems in different countries around the world and Fig. 17.5 shows successful utilization of different plants for the treatment of reverse osmosis rejected concentrate at Medawachchiya, Sri Lanka. The inherent features of wetland plant species such as extremely high transpiration rates, frost resistance, disease resistance, and tolerance to high heavy metal(loid) concentrations make them successful for remediation purposes. Additional characteristics including ease of rooting, fast establishment, quick growth, extensive rates of photosynthesis, and elevated usage of water make them successful in CW systems [42]. Further, the clear advantage of using vegetation species in a CW is provision of supporting media for biological activities. Moreover, ecological advantages

Table 17.1 Use of different plant species for the removal contaminants in landfill leachate

Landfill leachate location	CW configuration	Plant species	Operational parameters of CW	Reference
Wola Pawlowska, Poland	1 m high lysimeter of 0.6 m in diameter and 0.28 m^3 in volume	Willow (*Salix amygdalina L.*)	Hydraulic loading rate (HLR)—1, 3 and 5 mm day^{-1}	Białowiec et al. [69]
		Reed (*Phragmites australis*)		
Rhinelander, USA	Trees were planted at a spacing of 3 m within rows and 4 m between rows	Hybrid poplar (*Populus nigra* L. × *P. maximowiczii* A. Henry 'nM6')	Thermal dissipation probes were used to measure sapflow	Zalesny et al. [42]
Vrhnika municipal centre, Slovenia	Length of 25 cm long cuttings were planted in 12 L pots	Poplar (*Populus deltoids* Bartr. cl. I-69/55 (Lux),	Compost-soil mixture in the range of 1:2 on a volume:volume basis was utilized	Justin et al. [44]
		Two willow species (*Salix viminalis* L. and *Salix purpurea* L.)		
Da-Liao MSW site, Taiwan	Each system consisted of two types of basins in series, free water surface, and subsurface flow microcosm	Reed (*P. australis*)	HLR was 0.014 m^3 m^{-2} day^{-1}	Yang and Tsai [101]
		Cattail (*Typha orientalis*)		
		Virens (*Dracaena sanderiana*)		
Oneida country landfill, USA	Cuttings were planted in a split plot design with eight blocks at a spacing of 1.2 × 2.4 m	Poplar (*Populus maximowiczii*)	Drip irrigation was used to apply treatments and application rate was 22.7 L $tree^{-1}$	Zalesny et al. [102]
Lamby way, Poland	Three glass tanks (80 L × 5 W × 55 H (cm)) were used	Reed (*P. australis*)	Tanks were subjected to continuous recirculation as in a horizontal flow CW	Bialowiec et al. [64]
		Willow (*Salix viminalis* × *burjatica*)		
Sanitary landfill site, Slovenia	Constructed as a vertical flow–horizontal flow system. Vertical beds covered 41 m^2, 22 m^2 (5 m × 4.4.m), and 19 m^2 (5.3 m × 3.6 m) with a depth of 0.8 m. The horizontal bed covered 270 m^2 (13.5 m × 20 m) with a depth of 0.4 m	Reed (*P. australis*)	HLR—0.5 cm day^{-1}	Bulc [103]
		Cattail (*T. latifolia*)		

Landfill leachate location	CW configuration	Plant species	Operational parameters of CW	Reference
Solid waste transfer station, Thailand	Constructed horizontal subsurface flow system. Four ponds of 1 m wide, 3 m long and 1 m depth	Cattail (*T. augustifolia*)	Different HLR—0.01, 0.028 and 0.056 $m^3\ m^{-2}\ day^{-1}$	Chiemchaisri et al. [104]
Dragonja landfill site, Slovenia	Consisted of two interconnected beds with a horizontal subsurface flow. The first bed was 21 m long, 10 m wide and 0.9 m deep. The second bed was 23 m long, 10 m wide and 0.8 m deep.	Reed (*P. australis*)	HLR—3 cm day^{-1}	Bulc et al. [105]
Solid waste dump site, Slovenia	Consisted of six interconnected beds with a horizontal and vertical subsurface water flow	Reed (*P. australis*)	After the pretreatment process, pumps all the water in 3 h on the landfill cover where willows and reeds are planted	Justin and Zupancic [74]
		Willow (*S. purpurea*)		
Saginaw Township, USA	System consisted of aeration, settling, intermittent vertical sand filtration and surface flow wetland treatment	Cattail (*T. latifolia*)	The total system detention time was 180 days	Kadlec and Zmarthie [106]
Wilmington, USA	FWS [14.3 m × 4.1 m × 31 cm (L × W × H)] was used	Sweet flag (*Acorus calamus*)	Flow volume for the wetland was 11 m^3 and detention time was 10–12 days	Kozub and Liehr [83]
		Soft rush (*Juncus effuses*)		
		Arrow arum (*Peltandra virginica*)		
		Pickerel weed (*Pontederia cordata*)		
		Lizard’s tail (*Saururus cernuus*)		
		Softstem bulrush (*Scirpus validus*)		
		Burreed (*Sparganium androcladum*)		

(continued)

Table 17.1 (continued)

Landfill leachate location	CW configuration	Plant species	Operational parameters of CW	Reference
North-western region, Bulgaria	Consisted of VFS, 123 mm in diameter and 900 mm in height	Reed (*P. australis*)	Used three different flow rates, 40, 60, and 82 mL min^{-1}, respectively	Lavrova and Koumanova [45]
Iowa, USA	Subsurface flow dimension was 15.5 × 6 m; L × W	Stiff goldenrod (*Solidago rigida*)	Other local plant species including curly dock (*Rumex crispus*), bull thistle (*Cirsium vulgare*), stinging nettle (*Urtica dioica*), willow (*Salix spp.*) and cannabis (*Cannabis sativa*) were dominated with time	Nivala et al. [79]
Nonthaburi province, Thailand	Horizontal subsurface flow and free water surface flow systems were examined	Cattail (*T. latifolia*)	Bed was filled with mixed sand and clay	Ogata et al. [43]
Upstate New York, USA	Subsurface flow dimension was 33 × 3 × 0.6 m; L × W × H	Reed (*P. australis*)	Leachate application rate was 1.4 m^3 day^{-1} and residence time of 15 days	Sanford et al. [107]
Bangkok, Thailand	Subsurface flow dimension was 4 × 0.5 × 0.5 m; L × W × H	Cattail (*T. angustifolia* L.)	Different hydraulic retention (HRT), 1, 3, 5 and 8 days, were studied	Sawaittayothin and Polprasert [63]
Northeastern part of Ankara, Turkey	Operated three subsurface wetland systems. Two of them were vertical flow mode and one being horizontal flow mode. The dimensions were 1 L × 0.5 W × 0.4 H (m)	Cattail (*T. latifolia*)	Leachate application rate was 10 L day^{-1}	Yalcuk and Ugurlu [47]

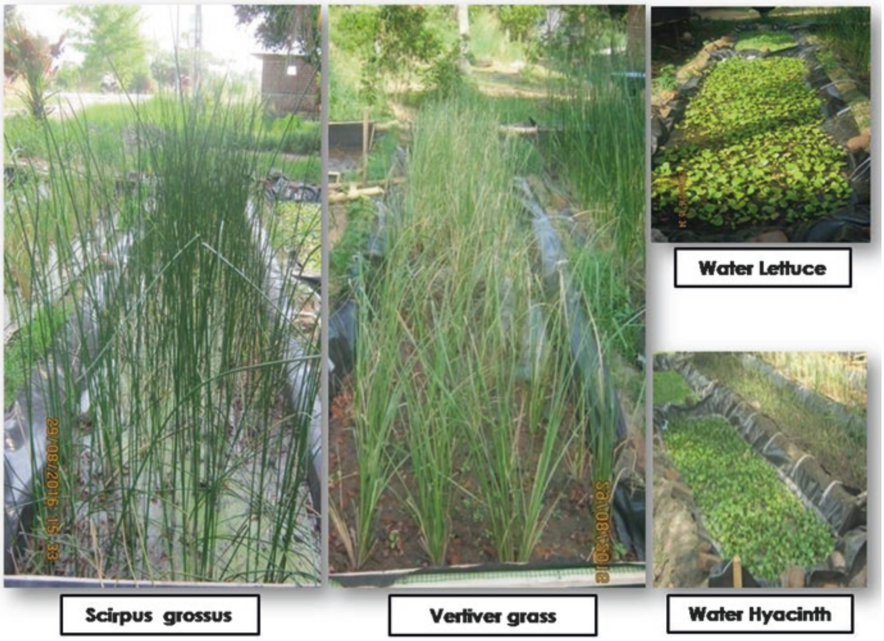

Fig. 17.5 Reed (*Phragmites* sp.) plants in constructed wetland systems

including carbon sequestration, erosion control, pollution prevention, and enhancing landscape appearance are some of the prevailing benefits of wetland plant species [44].

In the last couple of decades, short rotation coppice (SRC) has been introduced to remove contaminants from landfill leachate [51]. In this management strategy, plant species which possesses multiple shoot growth are cut down particularly on a 3-year rotation period. Consequently, harvested biomass could be used as a sustainable energy source as well as a CO_2 neutral fuel. It has been identified that willow (*Salix* sp.), poplar (*Populus* sp.), and eucalyptus (*Eucalyptus* sp.) are the most prevalent species in SRC systems. For instance, in Sweden, 14,500 ha of willow coppice are grown commercially [65]. Both willow and poplar possess a rapid growth rate, 1–3 m year^{-1}, in addition to high plant densities and high biomass yields [51]. Some studies indicated that planting densities, between 12,000 and 25,000 trees ha^{-1}, have been successfully established [66]. Additionally, a study by Mitchell et al. [67] revealed that biomass yield from willow SRC laid between 2.2 and 13.5 oven dried tonnes (odt) ha^{-1} year^{-1}. It appears that SRC can be successfully utilized for treating not only landfill leachate, but also different sources of pollutants such as municipal wastewater, sewage sludge, and agricultural effluents, since SRC possesses extensive transpiration rates. For example, *Salix cinerea* possesses high evapotranspiration rates ranging from 16.4 to 27.4 L m^{-2} [51].

Table 17.2 Phytoremediation processes in wetland plant species

Process	Importance
Phytoextraction	Contaminants are taken up from rhizosphere, transported and translocated to above ground shoots.
Phytodegradation	Plants take up, store, and metabolize or convert toxic contaminants to non-toxic by-products.
Phytovolatilization	Plants extract volatile metal(loid)s and organic compounds and release them into the atmosphere.
Rhizofiltration	Plant roots grown in aerated water precipitate and concentrate toxic components and pollutants are broken down by soil microorganisms.
Phytostabilization	Plants stabilize the pollutants rendering them harmless and control soil erosion and water infiltration, as well as humidification and lignification of organic compounds.
Rhizo-redox reactions	Plants alter the speciation of heavy metal(loid)s, thereby affecting their bioavailability and mobility.

17.5.4 Application of Vegetation Filter

Vegetation filter is a term commonly applied to explain the soil–plant treatment system in CW. More precisely, different phytoremediation processes are involved to remove detrimental substances from contaminated water and soil with the help of wetland plant species [11, 48]. Different types of phytoremediation processes and their significance in the removal of contaminants are summarized in Table 17.2.

In terms of hot and dry climatic conditions, evapotranspiration can be utilized in soil–plant systems for treating landfill leachate. It seems obvious that greater amount of leachate volume decreases due to evapotranspiration of soil–plant systems [68]. It has been experimentally observed that USEPA recommended plant species such as willows, poplars, and reeds have been successfully utilized in recent years for landfill leachate evapotranspiration [69]. It is well-known that higher transpiration rate sometimes exceeds the annual rainfall of willow stands leading to reduced groundwater level [70]. For instance, Agopsowicz [71] observed that introduction of willows for landfill leachate removal enhanced the evapotranspiration efficiency by 1.28–5.12 folds more than the evaporation efficiency from the plantless soil surface. This study also found that evapotranspiration of 3-months old willow sprouts was 1.6–1.8 fold greater than an average rainfall rate in Poland. Similarly, Białowiec et al. [72] noticed that transpiration of 3-months old sprouts of *Salix amygdalina* L. leads to evapotranspiration between 80 and 90%. Nevertheless, landfill leachate evapotranspiration efficiency is basically dependent on the physico-chemical characteristics of landfill leachate, as well as the plant species used [73, 74]. For instance, various dissolved compounds in landfill leachate may induce negative impacts on plant species in two ways. Firstly, biomass growth rate is inhibited and results in the reduction of evapotranspiration [69]. Transpiration efficiency coefficient (β^{-1}—ratio of biomass transpiration; unit—g d.m. mm^{-1}) may provide a clear view related to landfill leachate influence on plants [75]. If harmful compounds are available to plants, transpiration coefficient value reduces significantly. For example, although β^{-1} values typically lies between 1.9 and 4.9 g d.m. mm^{-1} for willow, Białowiec et al. [69] have reported β^{-1} values in the range 0.12 and 0.45 g d.m. mm^{-1}. Secondly, it is known that dissolved substances are responsible for reducing the difference between soil-water tension and soil-water tension at air entry. As a result, evaporation of landfill leachate could be remarkably decreased [70].

It is obvious that when designing a soil–plant system for landfill leachate treatment, linear relationship between biomass growth and transpiration is a particular concern. Further, proper management strategies and fertilization may stimulate the plants' growth and increase its biomass [69]. For example, the dark color of the sand at the top of waste heap increases soil temperature, which enhances evaporation. Additionally, inherent characteristics of organic soil types such as higher water retention and capillary suction may increase evaporation in landfill site. Additionally, since some wetland plant species belongs to non-food crop category (i.e., willow), transformation of disease-related pathogens via food chain does not occur.

Despite leachate treatment difficulties, its characteristic features including high concentrations of plant macro- and micro-nutrients (N, K, Mg, Ca, Zn, and B) facilitate the potential of landfill leachate reuse as a valuable fertilizer for growing energy crops such as short rotation willow coppice [73]. There are several examples in literature regarding the use of landfill leachate irrigation on tree growth, exhibiting its fertilizing capability. For instance, Justin et al. [44] detected a positive relationship in the biomass production of willow and poplar plantations treated with landfill leachate possibly due to the fertilization properties of landfill leachate. The usage of leachate showed up to 155% increment in the aboveground biomass compared to the control which was treated with water. Further, poplar was observed as the most efficient plant in biomass production owing to its high leaf production [44].

Tree sap flow has been recognized as a good surrogate indicator with respect to water usage of a particular plant Smith and Allen [76]. In general, heat is utilized as an indicator to measure sap flow in trees, and heat pulse, heat balance, and thermal dissipation methods are commonly used for measuring sapflow [76]. A study by Zalesny et al. [42] demonstrated that sap flow of hybrid poplars (*Populus nigra* L. × *P. maximowiczii* A. Henry 'NM6') showed a negative relationship with temperature, wind speed, rainfall, and vapor pressure deficit. In addition, they observed an increase in sap flow as sapwood area increased from 43.8 to 122.3 cm^2. Further, exploitations to the stand were reported as 2.8 and 11.3 mm d^{-1} for two consecutive years, respectively, exhibiting great capacity for reducing landfill leachate amount.

It is known that the tips of major roots as well as lateral roots of plant species may release oxygen (O_2) into the rhizosphere [77]. As a result, a layer of O_2 around the roots ranging from 1 to 3 mm thickness is formed, whereas the thickness of the layer is determined by the actual redox status of the rhizosphere [60]. Accordingly, redox conditions in the subsurface may determine the aerobic processes such as nitrification and anaerobic processes such as methanogenesis and denitrification. Membrane inlet mass spectrometry (MIMS) can be utilized to monitor dissolved gases such as O_2, CO_2, and CH_4 within the treatment bed of a vegetation filter treating leachate at a particular landfill site [78]. Williams et al. [60] observed a positive correlation between the dissolved oxygen profile throughout the CW bed and the distribution of willow roots in the soil. Therefore, it seems obvious that depth distribution of roots in a vegetation filter bed provides important information with respect to oxygenation potential.

17.6 Pollutant Diminution in Landfill Leachates by Phytoremediation

17.6.1 BOD and COD Removal

In a CW, the biological reactions are controlled by microorganisms via biofilm formation on the bed material [49]. Photosynthetically produced O_2, which helps in the growth of microbes, may transport to the root zones in CW systems. It seems obvious that relatively low COD removal efficiency at the start-up time in CW system could be attributed to the formation of active microorganisms. Additionally, leachate treatment performance enhances when the CW system matures [47]. The BOD_5/COD ratio is an important parameter which explains whether organic compound of landfill leachate is biodegradable or not. For example, if the BOD_5/COD ratio in landfill leachate is quite low, it means a majority of the organic compounds are non-biodegradable and vice versa [15]. Moreover, a low organic loading rate may lead to low COD removal efficiency in CW systems. In case of later stage of leachate, a sizeable fraction of COD flow along the CW systems leads to intermittent and poor COD removal efficiency [79]. In terms of removal efficiency, temperature does not affect significantly. Nevertheless, hydraulic retention time (HRT) is apparently responsible for greater COD removal rates. In this context, HFS gains higher COD removal than VFS, possibly due to the higher HRT values [47]. Moreover, aeration may increase BOD_5 and COD removal efficiencies in CW systems. Several demonstrations of BOD and COD removal efficiency from landfill leachate in CW systems are presented in Table 17.3. A study by Nivala et al. [79] found higher BOD_5 efficiencies, up to 97%, with supplemental aeration. However, in the absence of aeration, the BOD_5 removal rate was between 75 and 81%.

17.6.2 Nitrogen and Phosphate Removal

It is known that different forms of N such as nitric oxide (NO), NO_3^-, and NH_3 are highly soluble in water and can enter the water bodies along with landfill leachate discharges. Thus, the removal of excess N from landfill leachate is particularly important in terms of water quality. It is well-established that numerous removal processes including nitrification, denitrification, uptake by plants and microorganisms, NH_3 volatilization, and adsorption onto cation exchange are involved in the removal of N in CW [46]. Table 17.3 summarizes the N and phosphate (PO_4^{3-}) removal efficiency from landfill leachate.

Table 17.3 Raw leachate characteristics and treatment efficiency of CW systems

Plant species	Metal(loid)	Raw leachate concentration (mg L^{-1})					Leachate treatment efficiency (%)					Reference
		NH_3-N	Total P	COD	BOD	Metal(loid)	NH_3-N	Total P	COD	BOD	Metal(loid)	
Reed (*Phragmites australis*)	Cr	198	5.5	2800	204	0.27	100	100	96	92	70	Lavrova and Koumanova [45]
	Zn					0.25					100	
	Al					0.33					77	
Stiff goldenrod (*Solidago rigida*)	NA	253	NA	873	177	NA	98	NA	60	97	NA	Nivala et al. [79]
Cattail (*Typha latifolia*)	NA	29	NA	1650	NA	NA	38	NA	7	NA	NA	Ogata et al. [43]
Cattail (*T. angustifolia* L.)	Cd	25	3.5	385	130	1	96	93	81	91	99	Sawaittayothin and Polprasert [63]
Cattail (*T. latifolia*)	Pb	122	NA	212	NA	8.45	67	83	42	NA	>90	Yalcuk and Ugurlu [47]
	Ni					1.71					30	
Reed (*P. australis*)	Fe	496	2.3	485	76	3.90	51	53	50	59	84	Bulc [103]
Reed (*P. australis*)	Fe	88	NA	1264	60	10	81	NA	68	46	80	Bulc et al. [105]
Cattail (*T. augustifolia*)	NA	NA	NA	500	17	NA	NA	NA	63	44	NA	Chiemchaisri et al. [104]
Reed (*P. australis*)	Cr	327	2.37	1508	193	0.66	42	38	41	65	33	Justin and Zupancic [74]
Willow (*Salix purpurea*)	Mn					1.05					12	
	Cu					0.15					18	
	Zn					0.34					33	
	As					0.04					34	
Cattail (*T. latifolia*)	Zn	382	2.8	NA	NA	19	99	89	NA	NA	16	Kadlec and Zmarthie [106]
	As					7					29	
	Cr					24					67	
Cattail (*T. latifolia*)	Cd	94	NA	72	12	0.003	NA	NA	2	53	33	Martin et al. [108]
Reed (*P. australis*)	NA	230	NA	2000	300	NA	NA	NA	88	91	NA	Mæhlum [109]
Cattail (*T. latifolia*)												

NA not available

Temperature plays a major role in CW systems and has an effect directly or indirectly on the nutrient uptake by plants and microbial activity [80]. From the previous studies, it is demonstrated that nitrification efficiency in CW systems becomes inhibited at a water temperature of 10 °C and further decline rapidly at about 6 °C [80]. Contrarily, at relatively higher ambient temperatures, significantly higher total Kjeldahl nitrogen (TKN) and NH_3 removal were achieved. A study by Akratos and Tsihrintzis [81] has shown that TKN and NH_3 removal were higher at temperatures above 15 °C. Similarly, Yalcuk and Ugurlu [47] demonstrated that the highest ammonia-N (NH_3-N) removal of 62.3% was achieved at higher ambient temperatures.

Furthermore, organic matter oxidation and transformation of N by microorganisms depend upon the ambient temperature [81]. It is known that oxygen concentration is also temperature-dependent. In general, the solubility of oxygen increases with decreasing temperature and vice versa [47]. However, it is important to maintain aerobic conditions in CW systems to avoid denitrification. During the day time, photosynthesis results in oxygen generation which facilitates oxygen requirement for stabilization of organics and nitrification. Consequently, plant rhizosphere aeration enhances aerobic decomposition processes including nitrification and gaseous losses of N through denitrification [82]. In general, autotrophic nitrification involves two successive reactions. Initially, ammonium is converted to nitrite by ammonium oxidizing bacteria. Following that, NO_3^- oxidizing bacteria converts nitrite to NO_3^-. The influence of different flow rates and recirculation ratios for removal of NH_4-N has been successfully investigated by Lavrova and Koumanova [45]. They revealed that lower flow rates achieved higher NO_3-N concentrations, whereas the effect of recirculation ratios was opposite. It is well-established that 1 mg L^{-1} of dissolved oxygen (DO) is sufficient for the oxidation of ammonium. According to the above study, the DO values during nitrification experiment ranged between 5.2 and 8 mg L^{-1}.

In CW systems, denitrification mainly depends on the availability of NO_3-N and organic C. Additionally, denitrification also depends on environmental conditions including pH, ambient temperature, the amount of DO, and the availability of substrates for microbial attachment [83]. More precisely, mean N loss in CW systems can be assigned to denitrification. It is obvious that lack of organic substrate may inhibit denitrification process possibly due to the minimization of synthesis and activity of denitrifying enzymes [47]. Nevertheless, CW systems are unable to remove C completely, since plant litter and plant/root may contribute increment of C to the system [84]. Therefore, low amount of C in CW is not a huge problem, since it is vital for anaerobic respiration and denitrification process.

The possible processes for PO_4^{3-} loss in CW system include sedimentation, adsorption, and biological transformations [85]. Low concentration of P in effluent can be attributed to the uptake by plants and microorganisms [47]. Moreover, insufficient P level in leachate may adversely affect biomass growth, and consequently, the treatment capability in CW system. For this reason, denitrification rate, in particular, is reduced to a great extent. Typically, landfill leachate consists of low levels of minerals with reactive iron (Fe) or aluminium (Al) or calcium (Ca) hydroxide, which are capable of stimulating PO_4^{3-} precipitation to a great extent [86]. A study by Sakadevan and

Bavor [87] explained that while P removal in a long-term basis is mainly dependent on the substratum, litter, and Al/Fe component, plant uptake is less involved. It has been experimentally observed that phosphorous removal in a CW system is a seasonal dependence and particularly correlated to plant growth and consequent PO_4^{3-} uptake. Nevertheless, it appears that PO_4^{3-} removal is less linked with temperature because it is apparently governed by adsorption process [47]. As previously mentioned, flow rates and recirculation ratios are responsible for PO_4^{3-} removal efficiency. A study by Lavrova and Koumanova [45] demonstrated that higher retention time and higher recirculation ratio eliminated a greater concentration of total P from the leachate.

17.6.3 Heavy Metal(loid) Removal

From the previous studies, it is well-understood that heavy metal(loid) removal in CW systems is mainly governed by various biological and physico-chemical factors including microbial activity, uptake by plant species, sedimentation, flocculation, precipitation, adsorption, complexation, oxidation, and reduction, and cation and anion exchange [88, 89]. Typically, it is impossible to remove heavy metal(loid)s; however, their physico-chemical characteristics can be modified by (im)mobilization and subsequently managed [47]. Higher amounts of heavy metal(loid)s are removed due to the binding processes in CW systems. Typically, heavy metal(loid) ions possess positive charge; hence, they are rapidly adsorbed, complexed, and bound with suspended particles. In terms of long-term removal, heavy metal(loid)s are precipitated as their insoluble salts such as sulfides, hydroxides, carbonates, and bicarbonates and subsequently deposited within the wetland substrate [41]. Additionally, algae and microorganisms are able to take up heavy metal(loid)s available in the dissolved form in CW system [88].

The symplastic and apoplastic pathways provide a route towards absorption of heavy metal(loid)s into the roots of plant species. Symplastic pathway, an energy-dependent process, is mediated by specific or generic metal(loid) ion carriers or channels. But in apoplastic pathway, it is the opposite; the metal(loid) ions of metal(loid)-chelate complex penetrate the root via intercellular spaces [58]. More precisely, plant roots are able to solubilize soil-bound heavy metal(loid)s by acidifying the soil environment. Some enzymes (i.e., reductases) bound to the plasma membrane can also reduce the soil-bound heavy metal(loid) ions. Additionally, mycorrhizal fungi and root-colonizing bacteria increase the bioavailability of heavy metal(loid)s in CW systems [90]. Hence, rhizospheric microorganisms enhance the plant uptake of heavy metal(loid) ions.

In hyperaccumulator plants, heavy metal(loid)s absorbed by roots efficiently transport to the shoots via xylem system and the xylem loading process is governed by membrane transport proteins. Therefore, in metal(loid) accumulators, xylem loading process and translocation to shoot are stimulated by the complexation of metal(loid)s with low molecular weight chelators such as organic acids, and the metal(loid)s are converted into a less toxic form at any point of the transport pathway [58]. There are several examples in the literature of the use of wetland plant species for removing heavy metal(loid)s from landfill leachate and are summarized in Table 17.3.

17.7 Advantages and Disadvantages of Phytoremediation of Landfill Leachate

17.7.1 Economic Benefits

There is little information on detailed economic analysis related to feasibility of landfill leachate treatment systems. Nevertheless, from the limited studies, it is well-understood that SRC treatment systems provide economically feasible disposal options for landfill leachates, if managed properly. In other words, on-site utilization of landfill leachate treatment in CW systems saves millions of dollars at each site where it is implemented [73]. In Sweden, short-rotation willow coppice is grown over an area of 14,500 ha as a commercial crop. In the last few decades, about 30 landfill leachate treatment systems on restored caps or adjacent to the landfill are operated using willow plantations in Sweden [91]. It is well-known that, due to high evaporation, willow plantations are able to reduce greater amounts of leachate formation, and therefore, recycling of landfill leachate back to establish willow plantation leads to near zero net discharge of leachate [73]. It is reported that short-rotation willow coppice can be harvested every 3–4 years and approximately 6–10 t dry matter ha^{-1} $year^{-1}$ will be produced [65]. Subsequently, harvested dry matter could be used to generate electricity and produce heat.

A number of case studies in the USA indicated that on-site phytoremediation strategies save transportation and disposal costs significantly. For example, around 2100 hybrid poplar trees were planted over an area of 5.5 acres in Jeffco landfill, name of the state, and subsequently, 14 million gallons of leachate have been processed, thus saving a total amount of $810,000 [92]. Similarly, a republic landfill in Chicago consisting of more than 4000 hybrid poplar trees over a 7.5 acre processed greater than 2.7 million gallons of landfill leachate, thus saving $350,000 in leachate disposal costs in 2 years [92]. Furthermore, 3 acres of vetiver (*Chrysopogon* sp.) were installed in a landfill and processed three million gallons of leachate per year. As a result, the disposal cost dropped from $0.13 to $0.015 per gallon [92].

Apart from energy production, the usage of SRC for landfill leachate treatment enhances the economic competitiveness of renewable energy systems. In other words, landfill leachate facilitates a viable option for producing SRC biomass, since approximately 20–30% of the SRC production cost required for irrigation and fertilization could be saved [51]. Hence, the leachate treatment cost could be offset by selling the biomass. For instance, SRC treatment system in Sweden possesses US$ 13–18 compared to the conventional leachate treatment plant, which costs about US$ 10–27 [93]. So, there is an increasing trend to utilize energy crops for treating landfill leachate in the world, since this strategy addresses not only an environmental issue but also energy production. More precisely, energy conversion technologies such as spark ignition gas engines can be applied to convert SRC biomass to generate electricity [94].

Phytoremediation strategy can also be integrated with other novel treatment technologies, thereby stabilizing or enhancing their existing functions simultaneously and avoiding individual drawbacks. Integrating CWs with other emerging technologies such as membrane bio-reactor, electrochemical oxidation, and microbial fuel cells has been studied over the last few years and has been proven to be efficient for treating pollutants and for sustainable energy recovery [95, 96].

17.7.2 Phytotoxicity of Landfill Leachate

It is especially important to study the responses of plant species to landfill leachate stress, since phytoremediation strategies for landfill leachate is gaining an increasing focus in recent years [97]. As previously mentioned, landfill leachate possesses toxic components including organics and heavy metal(loid)s and may outweigh the beneficial effects of nutrients. Additionally, the high concentration of chloride and sodium leads to high ionic strength in landfill leachates [73]. The toxicity of landfill leachate may damage plant species when it is utilized for irrigation. However, the plant toxicity depends on different factors such as plant species, soil type, irrigation rates, and climatic conditions. Some of the deleterious impacts of landfill leachate are premature leaf senescence, leaf damage, less biomass production, and poor survival rates [73, 98].

There are several examples in the literature on the effect of landfill leachate on plant species. A study by Sang et al. [99] monitored several physiological changes of maize (*Zea mays* L.) such as growth chlorophyll content, lipid peroxidation, protein oxidation, and activities of antioxidant enzymes. The results revealed that landfill leachate affected the growth and chlorophyll level of maize seedlings. In addition, lipid peroxidation and protein oxidation in leaf tissues were increased to a great extent indicating plant stress landfill leachate [99]. Phytotoxicological tests play a major role in designing phytoremediation technologies for treating landfill leachate [100]. Such tests are able to indicate the maximum dose of treated leachate, which does not affect the plant species negatively. Dimitriou et al. [73] conducted a pot experiment to quantify the growth responses of five different willow clones with different leachate mixtures. The results showed that plant growth rates were reduced with leachate irrigation. Additionally, they have suggested that leaf length could be a useful stress diagnostic tool for use in situ showing a high correlation to growth [73].

In spite of detrimental effects of landfill leachate on plant species, many wetland plant species and SRC are tolerant of high level of heavy metal(loid)s; however, there is a concern about the risks of bioaccumulation of toxic heavy metal(loid)s via food chain. Therefore, proper disposal and harvesting practices are necessary for plant biomass which accumulates heavy metal(loid)s [48]. Similarly, accumulation of heavy metal(loid)s in wetland substrates could have long-term implications. Due to the oxidation and disturbance of wetland substrate, sedimented heavy metal(loid)s can be released into the system [41].

17.8 Summary and Conclusions

In the last couple of decades, an on-site treatment of landfill leachate with the help of CW is widely practiced in numerous nations in the world. It is obvious that CW could be the ideal technology for landfill remediation due to its cost-effective and eco-friendly nature. Additionally, establishing vegetation in landfill sites will facilitate erosion and hydraulic control by reducing infiltration of rainfall. It appears that the degree of success in terms of contaminant removal efficiency by CW systems varies depending upon the plant species selected, availability of microbial community, climatic conditions, physico-chemical properties of soil, and CW configuration. It is well-established that nutrients (i.e., N and PO_4^{3-}), heavy metal(loid)s, BOD, and COD can be successfully removed to a great extent by CW systems. Additionally, well-managed SRC systems save millions of dollars by eliminating the transportation and treatment process which were earlier practiced. Nevertheless, some of the deleterious impacts of landfill leachate may adversely influence the treatment efficiency of wetland plant species.

Phytoremediation of landfill leachate is still new and has to be developed. There are a number of examples where phytoremediation has failed. Basically, this failure can be attributed to excessive leachate application and lack of management practices due to poor understanding of the plant–soil system. The current knowledge and understanding on the limitations may enhance future investigations in respect to phytoremediation of landfill leachate. For this reason, experience, investigations, and field trials are vital to forecast and certify that treated leachate as well as harvested wetland plants has almost detoxified attaining minimum risk to human beings and the environment. Additionally, residual management is quite necessary to overcome problems arising from the public. Fundamental investigations based on phytoremediation of landfill leachate is not sufficient to solve the problem; hence, enough attention is to be given for developing large scale investigations with new strategies and approaches together with integrated technologies. There have been several investigations regarding landfill leachate remediation by using SRC, though results found to date have been promising. Even though landfill leachate is utilized as a means of fertilization for SRC to enhance yields, further investigations are necessary to establish the full potential of this strategy. Also, assessing the microbial interactions and their symbiotic activities on landfill leachate treatment is also an urgent necessity. Genetic engineering technology can be applied to enhance existing traits or confer novel capabilities of plant species which are used for treating landfill leachate. Overall, it is obvious that successful transfer of phytoremediation technologies from the laboratory to the field is a crucial step for the future of landfill leachate phytoremediation to be more efficient.

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Chapter 18
Phytomining of Rare and Valuable Metals

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Abstract The exponential growth of low-grade mining ores and metal-polluted soils around the world during the last decades is expected to continue at a higher rate in the foreseeable future. Yet, the strategic and commercial importance of some elements found in those sub-economic ores and soils, their elevated market prices, and the corresponding environmental concerns have opened a window of opportunity for phytomining. This phytoextraction-based technology uses the ability of certain plants to uptake valuable metals, producing a bio-ore from the harvested biomass that allows metal recovery through smelting. Once applied at large scale, phytomining may either function as a standalone operation to retrieve the desired element or jointly with phytoremediation, financing the costs of the latter. This chapter reviews the advances of phytomining since its inception in the 1990s, focusing on the results obtained to date, with gold, nickel, thallium, and rhenium.

Keywords Phytoextraction • Phytoremediation • Hyperaccumulation • Gold • Nickel • Thallium • Rhenium

18.1 Introduction

The uptake of valuable metals by plants has fascinated scientists for nearly three centuries. Ever since Beccher and Kunckel ascertained the presence of gold in plants during the 1700s [1], they were promptly followed by illustrious

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contemporary chemists including Berthollet, Sage, Rouelle, Darcet, and Deyeux, who also claimed to have found small amounts of the precious metal in vegetables [2]. Then, after Lungwitz suggested the possibility of using plant tissue analysis for gold bioindication in 1900 [3], several researchers have reported the occurrence of this much sought-after metal in many plant species from different locations throughout the twentieth century [4, 5]. These studies marked the beginning of biogeochemical exploration, highlighting the utility of plants for prospecting of gold, silver, or even uranium, along with other less valuable elements [6]. In the late 1970s, Jaffré et al. coined the term hyperaccumulation to characterize plants that uptake nickel to concentrations surpassing 1000 mg kg^{-1} [7], while in 1983, Chaney suggested the use of hyperaccumulator plants for the reclamation of metal-polluted sites, a process known as phytoremediation [8]. Phytoremediation is a low-cost, solar-driven and environment-friendly alternative to conventional solutions that are often impractical due to their prohibitive costs, unworkability, and detrimental side effects [9–11]. Two phytoremediation categories are especially relevant for the mitigation of metal pollution in soil: phytostabilization and phytoextraction. Phytostabilization is a management strategy to restrain metals in the rhizosphere of metal-tolerant plants (known as metallophytes), averting their migration through the soil and into aquifers [12, 13]. On the other hand, phytoextraction involves the uptake of a considerable amount of a given metal by the root and its translocation into the shoot. This method reduces the concentrations of metals in the soil, allowing their safe disposal after harvest [9, 12]. Phytoextraction requires metallophytes presenting fast growth-rate, high biomass yield, hyperaccumulation (or at least elevated metal levels in the shoots combined with high biomass production), and bioconcentration and translocation factors greater than 1 [12, 14].

Still, it was not until the 1990s that phytomining, the use of phytoextraction to recover valuable metals from waste substrates, was proposed [15–18]. In recent years, the ever-rising price of rare and valuable elements, the incapacity of conventional mining to extract the totality of metals from mineral ores, and the build-up of billions of tons of mine waste around the world have strengthened the importance of phytomining, prompting its development [5, 19, 20].

18.2 Hyperaccumulation

The uptake and accumulation of metals in plants generally depends on the bioavailability of these elements in their growth substratum. Hence, metal availability is highly influenced by the pH, oxygen content, nutrient balance, and coexistent inorganic and organic compounds [21, 22]. For some metals, low solubility and strong interactions with the organic or silicate matrix result in partial or complete unavailability. Metals are also influenced by a series of root mechanisms that encompass cation exchange, exudation of low-molecular weight organic acids, chelating compounds and enzymes, and the acidification of the rhizosphere through H^+ secretion. These processes, as well as symbiotic associations between plants and mycorrhizal fungi and bacteria [22, 23], have the potential to increment metal bioavailability and promote their entry into root cells over passive or active absorption [21, 24, 25]. When in the root, metals can be stored or translocated into the aboveground parts, usually via the xylem in a process mediated by membrane transport proteins, for detoxification and sequestration in the vacuoles [25, 26].

Certain plants have the uncommon capacity of hyperaccumulating metals, both essential and/or non-essential for their growth and development, to levels that can surpass 2% of their dry biomass weight [26]. Hyperaccumulators are classically described as plants that accumulate metals to concentrations10 to 100-fold higher than those normally measured in the shoots of other plants growing in the same environment [9, 27]. Thus, the threshold concentrations in aboveground plant tissue of hyperaccumulators should be at least 100 mg kg^{-1} for Cd, 1000 mg kg^{-1} for Co, Cu, Ni, As, and Se, and 10,000 mg kg^{-1} for Zn and Mn, to mention a few metals [28, 29]. Recently, the definition of metal hyperaccumulation has been revamped by Van der Ent et al. [30], who propose that the onset concentrations should be at least one order of magnitude and 2–3 orders of magnitude greater than the typical levels found in plants growing on metalliferous soils and normal soils (not metal-enriched), respectively. Hence, the thresholds for Cu, Co, and Cr should be lessened to 300 mg kg^{-1} dry shoot weight, and the criterion for hyperaccumulation of Zn should be lowered to 3000 mg kg^{-1} dry shoot weight [30, 31].

Furthermore, hyperaccumulators must also exhibit a bioconcentration factor (BF) and translocation factor (TF) greater than 1. The BF denotes the competence of a plant to extract metals from the growing media and accumulate them. The TF expresses the plant's aptness to translocate metals from the root to the shoot. A TF higher than 1 indicates that the plant is capable of transporting metals from roots to its aboveground parts. The BF and TF are calculated according to Eqs. (18.1) and (18.2), respectively [9, 32].

$$BF = \frac{C_{\text{Plant}}}{C_{\text{Soil}}} \tag{18.1}$$

$$TF = \frac{C_{\text{Shoot}}}{C_{\text{Root}}} \tag{18.2}$$

where C_{Plant} is the metal concentration in the plant (mg kg^{-1}), C_{Soil} is the metal concentration in the soil (mg kg^{-1}), C_{Root} is the metal concentration in the root (mg kg^{-1}), and C_{Shoot} is the concentration of metal in the shoot (mg kg^{-1}).

Plants can also be divided into obligate or facultative hyperaccumulators, depending on whether or not they are restricted to metalliferous environments [31]. Hyperaccumulators have been identified in numerous studies and may occur in nearly 500 species of vascular plants from 45 families of angiosperms, including species pertaining to Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae [10, 33, 34]. From these species, 85–90% are obligate endemics to metalliferous environments [31].

18.2.1 Chelant-Assisted Phytoextraction

When metals are not sufficiently available for plant uptake, chelating agents can be used to increase their bioavailability in soil. Chelants promote the formation of strong water-soluble complexes with metals desorbed from the soil solid phases by decreasing the free-metal activity, which in turn causes the dissolution of previously unavailable metals. When the chelant saturates, the solid phase becomes deprived of metals, or the metal solubility equilibrium is reestablished, the chelation process halts [35, 36]. The characteristics of the chelant and the soil matrix regulate the quantity of bioavailable metals in the soil solution. The efficacy of a chelant in the mobilization of metals is generally related to the stability constants of the corresponding metal complexes [37, 38]. Stability constants can be utilized to classify chelating agents according to their general effectiveness, but not regarding the value of a specific chelant on different metals, due to the influence of metal speciation in a given soil [36]. Moreover, the biodegradation of metal complexes is highly dependent of the metal type and is not correlated to the stability constant of the chelate complexes [38]. Upon metal complexation by the chelating agent, plants may uptake the metals through a number of mechanisms that include: (a) absorption of free metals following their separation from the chelant (known as the split-uptake mechanism); (b) absorption of the intact chelant-metal complexes; or (c) metal exchange between plant metabolic ligands and the chelants [35, 36].

Natural and/or synthetic chelants have been broadly used in phytoextraction trials, to increase metal bioavailability and, consequently, the extraction and translocation of metals to the shoot [10]. Chelating agents can be divided into two major groups, inorganic and organic. The latter can be subdivided into synthetic aminopolycarboxylic acids (APCAs), natural APCAs, and low molecular weight organic acids (LMWOAs) [39]. A large number of synthetic APCAs have been employed to enhance phytoextraction: cyclohexylenedinitrilotetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediamine di-o-hydroxyphenylacetic acid (EDDHA), ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), hydroxyethylenediaminetriacetic acid (HEDTA), and hydroxyethyliminodiacetic acid (HEIDA) [10, 36, 39]. Among these, EDTA has gained recognition as an efficient chelant to promote the uptake of a wide range of metals by plants. Nevertheless, the reduced biodegradability, leaching risk, and toxicity of synthetic APCAs led to the study of less hazardous chelating agents [35, 36]. In this context, two natural APCAs, ethylenediaminedisuccinic acid (EDDS) and nitrilotriacetic acid (NTA), have been suggested as viable alternatives to synthetic APCAs, due to their elevated biodegradability, reduced toxicity, and chelating potential [36]. Moreover, experiments with LMWOAs have also yielded promising results for numerous metals. In comparison with APCAs, LWMOAs have the advantage of avoiding excessive metal mobilization effects and leaching risks, given their higher biodegradability and consequent lesser persistence in soils [35]. In addition, considering that the roots naturally release many of these LMWOAs, they pose very little phytotoxicity threat [35, 39]. Contrarily to their organic counterparts—with the exception of thiourea (CH_4N_2S)—the use of inorganic chelants in phytoextraction has been generally restricted to enhance the bioavailability of a single element: gold [5, 19]. In its natural form, Au(0), it is not readily available for plant uptake due to low solubility in soil. Hence, a number of inorganic chelants, most of them cyanide-derived, are applied in order to chelate Au(0) and transform it into Au(I) or Au(III). Gold is then ready for root absorption, although once in plant tissue it is promptly reduced to Au(0) (over 90%), and only small quantities remain as Au(I) and Au(III) [21]. However, the addition of inorganic chelants like ammonium thiocyanate (NH_4SCN), ammonium thiosulfate ($[NH_4]_2S_2O_3$), potassium bromide (KBr), potassium cyanide (KCN), potassium iodide (KI), sodium cyanide (NaCN), and sodium thiocyanate (NaSCN) has raised concerns due to their persistence in soil, detrimental impact on soil microbiota, and potential to mobilize undesirable amounts of elements such as As, Cu, Fe, Ni, or Zn into the groundwater [37, 40]. Table 18.1 summarizes the list of most organic and inorganic chelating agents used in phytoextraction studies.

18.3 Phytomining of Rare and Valuable Metals

The procedure for a phytomining operation comprises a number of standard steps, including: (1) locate a site (mine tailings, mineralized or polluted soils) with subeconomic levels of the target metal; (2) plant a high biomass yield species with aptitude to accumulate elevated amounts of the target metal (ideally a hyperaccumulator) and tolerate other coexisting metals; (3) where necessary, apply a chelating agent near plant maturity in order to increase the bioavailability of the target metal; (4) harvest the plants when these reach maximum biomass production or exhibit symptoms of decay; and (5) incinerate the harvested plant biomass to retrieve the bio-ore, from which the target metal can be recovered through smelting. In addition to these stages, several approaches and variables may influence the outcome of a phytomining trial of a given element.

18.3.1 Gold

In spite of numerous studies reporting the ability of plants to accumulate gold throughout the twentieth century [4, 64, 65], the first true gold phytomining experiment was only conducted in the late 1990s [18]. In that pioneering greenhouse trial, *Brassica juncea* plants accumulated gold in their aboveground tissues to a concentration of 57 mg kg^{-1}, from silica sand artificially spiked with gold chloride to achieve a gold concentration of 5 mg kg^{-1}. Prior to harvest, the substrate was treated with NH_4SCN to increase gold's bioavailability and its uptake [60]. Another study using the same auriferous substrate basis and a gold concentration of 3.8 mg kg^{-1} tested the potential of five root crops to extract gold upon treatment with ammonium

Table 18.1 List of chelating agents that have been reported to increase metal bioavailability in soil

Type	Chelating agent	Element	Reference
APCAs	CDTA^s	Pb	[41]
	DTPA^s	Cd, Cu, Ni, Pb, Zn	[42, 43]
	EDDHA^s	Pb	[44]
	EDDS^n	Cd, Cr, Cu, Pb, Ra, U, Zn	[38, 45–48]
	EDTA^s	Cd, Cr, Cu, Ni, Pb, Ra, U, Zn	[42, 43, 46, 47, 49, 50]
	EGTA^s	Cd, Pb	[41, 51]
	HEDTA^s	Cd, Cr, Ni, Pb	[43, 50, 52]
	HEIDA^s	Cu	[53]
	NTA^n	As, Cd, Cr, Cu, Pb, U, Zn	[45, 48, 49, 51, 53]
LMWOAs	Acetic acid	Pb	[41]
	Citric acid	Cd, Cr, Cu, Ni, Pb, Ra, U, Zn	[42, 45–47, 54]
	Fumaric acid	Cd	[55]
	Gallic acid	Cd, Cu, Ni, Pb, Zn	[42]
	Oxalic acid	Cd, Cr, Cu, Ni, Pb, U, Zn	[42, 45, 56]
	Succinic acid	U	[56]
	Vanillic acid	Cd, Cu, Ni, Pb, Zn	[42]
Gold-Specific	Ammonium thiocyanate^i	Au	[57–60]
	Ammonium thiosulfate^i	Au	[57, 58]
	Potassium bromide^i	Au	[61]
	Potassium cyanide^i	Au	[59, 61]
	Potassium iodide^i	Au	[61]
	Sodium cyanide^i	Au	[57, 59, 62]
	Sodium thiocyanate^i	Au	[61]
	Thiourea^o	Au	[57, 63]

^{s} synthetic; ^{n} natural; ^{i}inorganic; ^{o} organic

thiocyanate and ammonium thiosulfate [58]. The results exhibited an average gold concentration of 113 mg kg^{-1} in the roots of *Raphanus sativus* ‘oriental radish’ and 89 mg kg^{-1} in the roots of *Daucus carota* treated with NH_4SCN (1 g kg^{-1}) and $(NH_4)_2S_2O_3$ (2 g kg^{-1}), respectively. Assuming a biomass yield of 18 tons ha^{-1} (tops + roots), and the subsequent gold yield of 1450 g ha^{-1} for *D. carota* after treatment with ammonium thiosulfate, a profit of US$ 7,550 would be expected.

Lamb and colleagues [61] employed an artificial substrate prepared from silica sand finely disseminated with gold to a concentration of 5 mg kg^{-1}, to study the ability of *B. juncea*, *Berkheya coddii,* and *Cichorium intybus* to uptake gold under the effect of ammonium thiocyanate, ammonium thiosulfate, potassium bromide, potassium cyanide, potassium iodide, and sodium thiocyanate. The data showed average gold levels of 97 mg kg^{-1} and 326 mg kg^{-1} in *B. coddii* and *B. juncea*, respectively, when hyperaccumulation was induced via KCN.

A 2003 greenhouse study evaluated the accumulation of gold in canola (*Brassica* sp.), growing in soils presenting increasing concentrations of the metal: 1.25, 2.5, and 5.0 mg kg^{-1} [66]. The media was treated with potassium cyanide at a rate of 0.2 g kg^{-1} and induced the accumulation of approximately 60, 120, and 150 mg kg^{-1} of gold in the plants, according to each increment in the substrate gold levels. In addition, the authors made an economical assessment estimating a potential gross profit of US$ 6,437 per hectare.

In 2005, a field trial was carried out in Brazil to assess the feasibility of using *B. juncea* and *Zea mays* to extract gold from oxidized ore containing 0.6 mg kg^{-1} of the precious metal [59]. One week before harvesting, the trial plots were treated with ammonium thiocyanate (0.3 g kg^{-1}), potassium cyanide (0.15 g kg^{-1}), and sodium cyanide (g kg^{-1}), to induce gold hyperaccumulation. The highest concentrations, 30 and 39 mg kg^{-1}, were found in *B. juncea* after the application of KCN and NaCN, respectively. Centered on these results, a later appraisal was made to determine the profit obtained at the end of the process [67]. It was calculated that a profit of approximately US$ 20,000 ha^{-1} could be taken.

One year later, Rodriguez et al. [68] spiked soil with $KAuCl_4$ to attain concentrations of gold in the substrate of 5 and 10 mg kg^{-1}. *Chilopsis linearis* was allowed to grow for 4 weeks after germination, until utilizing thiourea and ammonium thiocyanate as chelants (both as 0.76 mg kg^{-1}). Average gold levels in the stems of plants developed in the soil holding 5 mg kg^{-1} gold reached 296 mg kg^{-1} with CH_4N_2S and 197 mg kg^{-1} with NH_4SCN.

In 2007, a new pot study screened some Australian native plants and exotic agricultural species for their prospective use in cyanide-induced gold phytoextraction [62]. The chosen plant species comprised *Eucalyptus polybractea*, *Acacia decurrens*, *Sorghum bicolor*, *Trifolium repens*, *Bothriochloa macra*, *Austrodanthonia caespitosa*, and *Microlaena stipoides*. Crushed ore from the Davis stockpile at the Stawell Gold Mine in Victoria (Australia), presenting mean gold levels of 1.75 mg kg^{-1}, was picked as substrate. Following standard practice, 1 week before harvesting, the ore was treated with sodium cyanide at the rates of 0.1 and 1 g kg^{-1}. The most significant results were obtained with *B. macra* and *T. repens,* which presented shoot gold concentrations of 24 and 27 mg kg^{-1}, respectively, under the effect of 1 g kg^{-1} NaCN.

A greenhouse experiment used mine tailings featuring 2.35 mg of gold per kg of ore, collected from an active mine (El Magistral) in the state of Sinaloa, Mexico [69]. To evaluate the potential of *Sorghum halepense* for gold phytoextraction, the plants were allowed to grow for 10 weeks after germination, and 2 weeks before harvest, their respective pots were treated with different rates of thiourea (0.0076, 0.015 or 0.030 g kg^{-1}); sodium cyanide (0.5, 1, or 2 g kg^{-1}); ammonium thiosulphate (1, 2 or 4 g kg^{-1}); and ammonium thiocyanate (0.32, 0.64 or 1.28 g kg^{-1}). The most efficient chelating agent was NaCN at a dose of 1 g kg^{-1}, inducing the accumulation of 23.9 mg kg^{-1} of gold in the aboveground dry matter of *S. halepense*.

Entering the current decade, Wilson-Corral et al. [70] reported the results of an earlier gold phytoextraction study. The authors used gold-enriched silica sand (3.8 mg kg^{-1}), ammonium thiocyanate at a rate of 1 g kg^{-1} [19], and the plants *Amaranthus* spp., *S. halepense*, *Helianthus annuus*, *Sesamum indicum*, *Gossypium hirsutum*, *Brassica campestris,* and *Amoreuxia palmatifida*. Although average concentrations of gold in plant tissue were not disclosed, levels above 304 mg kg^{-1} were found in *B. campestris*. Subsequently, the same research team carried out two new field and greenhouse experiments [57]. In the field trial, average gold concentrations of 19, 22, and 15 mg kg^{-1} were attained in the leaves, stems, and roots of *H. annuus*, grown in a 50 m^2 plot constructed over the tailings of the aforementioned Magistral Mine in Mexico. These results were obtained inducing gold hyperaccumulation with sodium cyanide at a rate of 1 g kg^{-1} of ore. Concerning the greenhouse study, *Kalanchoe serrata* plants were cultivated in pots containing the same tailings, but 2 weeks before harvesting gold bioavailability was increased through the application of sodium cyanide (1 g kg^{-1}), ammonium thiocyanate (1.24 g kg^{-1}), ammonium thiosulphate (2 g kg^{-1}), and thiourea (0.03 g kg^{-1}). Average gold levels in the aerial parts of the plant reached 9 mg kg^{-1} and 10 mg kg^{-1}, when treated with NH_4SCN and $(NH_4)_2S_2O_3$, respectively. Based on the results of biomass yield and gold levels in plant tissue from these studies, Wilson-Corral et al. suggested earnings of US$ 15,098 ha^{-1}.

A 2014 pot experiment assessed the viability of three plant species (*Lindernia crustacea*, *Paspalum conjugatum*, and *Cyperus kyllingia*), to extract gold from cyanidation tailings (1.68 mk kg^{-1} gold) located in the Sekotong District of West Lombok Regency, Indonesia [71]. Ammonium thiosulfate (2 g kg^{-1}) and sodium cyanide (1 g kg^{-1}) were applied to the corresponding pots to induce hyperaccumulation. Though the addition of the chelants enhanced the uptake of gold to the shoots by 106% ($[NH_4]_2S_2O_3$) and 30% (NaCN), the average concentrations have only reached a maximum value of 0.6 mg kg^{-1} in *P. conjugatum* under the effect of ammonium thiosulfate.

Table 18.2 summarizes some of the most relevant gold phytoextraction results obtained to date.

18.3.2 Nickel

Conventional nickel mining usually requires ores with a cut-off grade above 30,000 mg kg^{-1} to be economically feasible. However, few ore bodies present those concentrations and the existing ones are becoming depleted [72]. On the other hand, the weathering of ultramafic rocks has generated large areas of serpentine soils that are widely scattered around the globe. Nickel levels in serpentine soils are usually within the range of 1000–7000 mg kg^{-1} [24, 72], making them impractical for commercial mining. The discovery of numerous nickel hyperaccumulators growing on serpentine soils across the planet, has impelled the assessment of their potential to

Table 18.2 Concentration of Au in plants from different phytomining trials

Media	Plant species	Chelant	Au (mg kg^{-1})		Reference
			Plant	Media	
Tailings	*Bothriochloa macra*	NaCN	24	1.75	[62]
	Brassica juncea	KCN	30	0.64	[59]
	Brassica juncea	NaCN	39	0.64	[59]
	Helianthus annuus	NaCN	19	2.35	[57]
	Kalanchoe serrata	$(NH_4)_2S_2O_3$	10	2.35	[57]
	Sorghum halepense	NaCN	24	2.35	[69]
	Trifolium repens	NaCN	27	1.75	[62]
	Zea mays	NaCN	20	0.64	[59]
Silica sand	*Berkheya coddii*	KCN	97	5	[61]
	Brassica campestris	NH_4SCN	304	3.8	[70]
	Brassica juncea	NH_4SCN	57	5	[60]
	Brassica juncea	KCN	326	5	[61]
	Daucus carota	$(NH_4)_2S_2O_3$	89	3.8	[58]
	Rapahanus sativus	NH_4SCN	113	3.8	[58]
Soil	*Chilopsis linearis*	CH_4N_2S	296	5	[68]
	Chilopsis linearis	NH_4SCN	197	5	[68]

phytomine this metal [16, 17]. Table 18.3 exhibits some plant species known to hyperaccumulate nickel.

Nicks and Chambers carried out the first phytomining experiment in 1995, using the nickel hyperaccumulator *Streptanthus polygaloides*, an endemic serpentinophyte from California [17]. Results obtained in a serpentine soil presenting nickel levels of 3,340 mg kg^{-1} showed an average nickel concentration of 5,300 mg kg^{-1} in the shoots and a biomass yield of 4.8 tons ha^{-1}. The authors suggested that upon plant selection, an optimized strain could generate a biomass of 10 tons ha^{-1}, containing 10,000 mg kg^{-1} of nickel. Considering the price of nickel at the time, US$ 7.65 kg^{-1}, and a 50% return to the grower [84], the crop would be worth US$ 382, plus US$ 131 relative to energy generation from biomass incineration.

In 1997, Robinson and colleagues conducted two separate studies to determine the potential of *Alyssum bertolonii* and *B. coddii* for nickel phytomining [85, 86]. To evaluate *A. bertolonii*, in situ experimental plots were prepared over serpentine soils in Murlo (Tuscany, Italy), presenting nickel concentrations of 1,600 mg kg^{-1}. These plots were fertilized with different N + P + K regimes during a 2-year period. The best fertilizer treatment generated biomass and nickel yields of 9 tons ha^{-1} and 72 kg ha^{-1}, respectively. Those results translated to an economic return of US$ 539 according to the nickel price of the period, in addition to US$ 219 from energy production [84, 85]. The field trials with South African nickel hyperaccumulator *B. coddii* resulted in a biomass yield of approximately 22 tons ha^{-1}. Assuming a maximum concentration of 7,880 mg kg^{-1} (shoots), 168 kg of nickel would be extracted per hectare and a return of US$ 1,260 plus US$ 288 (from energy generation) would be obtained. Nevertheless, given that nickel concentrations in plants grown in

Table 18.3 Some nickel hyperaccumulators from different locations across the planet

Plant species	Concentration (mg kg^{-1})	Location	Reference
Alyssum bertolonii	13,400	Italy	[73]
Stackhousia tryonii	41,260	Australia	[74]
Sebertia acuminata	11,700	New Caledonia	[7]
Rinorea niccolifera	18,000	Philippines	[75]
Psychotria costivenia	38,530	Cuba	[76]
Phyllanthus insulae-japen	38,720	Indonesia	[74]
Jatropha sp.	13,500	Brazil	[77]
Alyssum murale	13,160	Serbia	[78]
Bornmuellera baldaccii	12,115	Albania	[79]
Berkheya coddii	11,600	South Africa	[80]
Alyssum heldreichii	11,800	Greece	[81]
Thlaspi apterum	21,500	Bulgaria	[81]
Phyllanthus nummularioides	26,560	Dominican Republic	[74]
Psychotria cf. *gracilis*	10,590	Malaysia	[74]
Streptanthus polygaloides	14,800	California, U.S.	[82]
Alyssum pintodasilvae	9,000	Portugal	[83]

experimental studies are lower than those found in wild plants, the authors suggested a more conservative estimate. Hence, considering average shoot levels of 5,000 mg kg^{-1}, the production of nickel would be 110 kg ha^{-1}, worth US$ 670 plus US$ 288 from energy revenue [84, 86].

Subsequently, a 2003 study with *Alyssum murale* Waldst. & Kit. and *Alyssum corsicum* Duby announced maximum shoot nickel concentrations of 22,000 mg kg^{-1} and biomass yield up to 20 tons ha^{-1} [72]. Based on these figures, the authors predicted nickel extraction of 400 kg ha^{-1}, worth US$ 1,749 (after deducting production and land rental costs, and 25% of the nickel value to support the expenses of metal recovery and license and royalty fees).

Recently, Bani et al. [87] undertook field trials at two ultramafic vertisol sites from Albania, to assess the influence of plant density on nickel phytomining with *A. murale*. In the first site, two different plots presenting soil nickel levels of 3,100 and 2,060 mg kg^{-1} were planted with *A. murale* at a density of 1 and 6 plants per square meter, respectively. In the second site (3,300 mg kg^{-1} nickel), *A. murale* was planted at a rate of 4 seedlings per square meter. The results showed that plant densities of 1, 6, and 4 plants per square meter generated biomass yields of 10, 5, and 10 tons ha^{-1}. Accordingly, nickel production was of 77, 41, and 112 kg ha^{-1}. Assuming the current price of nickel, these results would provide an economic revenue of US$ 728, 388, and 1,059, respectively, suggesting that a plant density of 4 plants per square meter would be the most profitable strategy.

Still in 2015, another study evaluated the biomass yield of *Alyssum serpyllifolium* Desf. susbp. *lusitanicum* T.R. Dudley & P. Silva (also known as *Alyssum pintodasilvae*), growing under natural conditions in the Portuguese massifs of Morais and Bragança [88]. The results depicted average biomass production of 6.3 tons ha^{-1} in the Morais massif and 8.1 tons ha^{-1} in the Bragança massif. The correspondent nickel yield of 27.7 kg ha^{-1} (Morais massif) and 27.4 kg ha^{-1} (Bragança massif) would be worth US$ 512 and 506, respectively.

18.3.3 Thallium

In light of its scarcity [24], high bioavailability for plant uptake [89, 90], and elevated price [91], thallium has also been the target of some phytomining-related studies.

In 1999, an investigation conducted over the tailings of a lead/zinc mine at Les Malines (Les Avinières, France) discovered thallium concentrations up to 3,070 mg kg^{-1} in the shoots of *Iberis intermedia* [92]. Although average thallium levels on aboveground tissues surpassed 1,000 mg kg^{-1} and the biomass yield pointed to 15 tons ha^{-1}, the authors assumed 800 mg kg^{-1} and 10 tons ha^{-1}, respectively, to ensure a conservative economic assessment. Thus, the resulting 8 kg of thallium per hectare would be worth US$ 2,400 (considering the price of thallium at that time, US$ 300 kg^{-1}). The same study has also analyzed the feasibility of thallium phytomining with *Biscutella laevigata*. With a biomass production of 4 tons ha^{-1}, *B. laevigata* would need an average shoot thallium concentration of 425 mg kg^{-1} to reach the proposed $US 500 ha^{-1} necessary to make phytomining viable. The results showed that approximately 39% of the plants topped this concentration threshold [89, 92, 93].

Other studies have also presented additional thallium hyperaccumulators that could be suitable for phytomining. Escarré et al. [94] found average shoot thallium concentrations of 250 mg kg^{-1} (maximum levels reached 1,500 mg kg^{-1}) on *Silene latifolia*, growing on the aforementioned Les Avinières region, France. Jia and colleagues collected samples of *Brassica oleracea* var. *capitata* growing in long-term thallium-contaminated sites from Lanmuchang, West Guizhou Province, China [95]. Shoot thallium levels up to 1,503 and 818 mg kg^{-1} were determined in plants harvested in soils of mining sites and alluvial soils, respectively.

18.3.4 Rhenium

Rhenium is one of the scarcest (7×10^{-8}%) and most broadly dispersed elements on Earth's upper crust. Because of its rarity and distinguishing physicochemical properties, rhenium is also one of the most costly metals [96, 97]. Rhenium is usually found in soils as perrhenate (ReO^{-}_{4}), its most stable form, presenting great mobility and solubility [98, 99].

Following different accounts of rhenium accumulation, both on field [100, 101] and laboratory trials [102], two rhenium phytomining experiments were carried out to date [14, 103]. The first, published by Bozhkov et al. [103], is actually the corollary of a series of earlier communications by the same authors [104–106], in which besides reporting the concentrations of rhenium in plants growing around the Asarel mine in Bulgaria, the results of two pot experiments are also presented. One of the pot trials employed *Atriplex hortensis*, *Polygonum fagopyrum*, *Medicago sativa*, and *T. repens* growing on soil spiked with an aqueous $KReO_4$ solution 2-weeks after seed germination, in order to attain a soil rhenium concentration of 128.72 mg kg^{-1}. A week after, plants were harvested and rhenium levels up to 3,150; 9,130; 46,586; and 35,090 mg kg^{-1} were found in *A. hortensis*, *P. fagopyrum*, *M. sativa*, and *T. repens*, respectively, denoting their capacity to hyperaccumulate this metal. The other pot test [106] used soil collected at the Asarel mine (5 mg kg^{-1} rhenium) and the plant *M. sativa*. Plant tissue was analyzed 10 and 35 days after sowing, exhibiting maximum rhenium levels of 2,780 and 4,870 mg kg^{-1} ash. Based on rhenium's price at the time, the authors suggested a revenue of US$ 21,915 $tons^{-1}$, which would be roughly US$ 74,617 per hectare based on their biomass yield projections. However, these results are extremely optimistic, for they are calculated on top of maximum rhenium concentrations in plant tissue (not average values), and do not consider any process expenses. Furthermore, their studies fail to provide crucial methodological details and results (such as number of replicates used or biomass production), experimental consistency (the pot experiment design is poorly explained), and statistical analysis (inexistent), averting to duplicate their work.

In 2015, a pot trial developed under greenhouse conditions assessed the potential of *B. juncea* and *Equisetum hyemale* growing on organic substrate spiked with $KReO_4$ to obtain rhenium concentrations of 5, 10, 20, 40, and 80 mg kg^{-1} [14]. The plants were harvested 45 and 75 days after sowing. According to increasing substrate rhenium levels, *B. juncea* presented shoot rhenium concentrations that ranged from 1,553 to 22,617 mg kg^{-1} at 45 days, and 1,348 to 23,396 mg kg^{-1} at 75 days, whereas in *E. hyemale* the concentrations varied between 74 and 925 mg kg^{-1}at 45 days, and between 87 and 714 mg kg^{-1} at 75 days. The authors estimated that, considering production and bio-ore extraction costs, a profit of US$ 3,906 ha^{-1} could be feasible.

18.4 Perspectives

In spite of the technological progresses of the mining sector, current solutions are incapable of extracting the totality of metals from mineral ores. Thus, billions of tons of toxic mine waste with residual metals are scattered throughout the 0.4×10^6 km^2 of land estimated to be affected by mining activities around the world [107]. Many of these metals are much in demand for their high market prices or strategic significance [108, 109], opening a clear window of opportunity for phytomining. The auspicious results obtained during the last two decades reveal that it may be a feasible and inexpensive alternative to exploit low-grade ores and mineralized or polluted soils. Moreover, as a still infant technology, phytomining could benefit from a series of scientific advances and approaches to improve the process profitability and sustainability, and stimulate its commercial application, including: (i) the use of genetic manipulation to enhance metal hyperaccumulation [26, 27]; (ii) the utilization of plant growth promoting bacteria to reduce metal phytotoxicity and increase biomass yield [23, 110]; (iii) where necessary, restrict chelating agents to highly biodegradable compounds in order to avoid collateral effects on the soil microbiota and leaching of metals into groundwater [37]; (iv) the selection of native or naturalized non-invasive plant species to prevent environmental damage; and (v) the development of target-specific phytomining to meet precise and highly lucrative market needs—such as in vivo gold nanoparticles desired for industrial, chemical, electronic, and medical applications [111, 112].

Phytomining is an exciting plant-based technology that after further improvement may be successfully applied at commercial scale. The work carried out so far suggests that phytomining is viable either as a standalone operation to recover valuable metals, or as a tool to finance the expenses of concomitant processes like phytoremediation.

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Chapter 19
Air Phytoremediation

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Abstract Air pollution presently is a challenge fo-r many areas of the world. Plants are higher organisms that can best deal with this problem despite the fact often in the air is a mixture of pollutants of different origin and toxicity. The world of plants is very diverse and well adopted to changes in the environment, including air. This large biodiversity allowed to select species with a very high tolerance, which are the base for the discipline known as phytoremediation. All plants during their presence in the environment run the process of phytoremediation, but some species tolerate a very high concentration of selected pollutants. Moreover, they are able to uptake/accumulate and next to degrade/detoxify in order to make them less harmful. Tolerant plant species can be found in very extreme conditions but for phytoremediation are useful plant species which besides being cultivatable, produce a large leaf area and biomass. Urban areas often contribute in creating high polluted sites as street canyons, road crossing, bus stops, and surrounding of heavy traffic freeway. In all these places, air pollution can be mitigated by the presence of selected plant species. Additionally, agronomic practices allow to maintain them on a polluted site and to form them in configuration for optimal deposition of pollutants. Air phytoremediation in urban areas, where at present men spend most of the time, is strongly desired and hard to overestimate if environment and human health and well-being are the prospect.

Keywords Air pollution • Anthropocene • Bioremediation • Effective air phytoremediants • Green infrastructure

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19.1 Introduction

Air pollution nowadays is among the biggest challenges in urban ecology having negative impact on human health, well-being, and the health of the environment as a whole and remains a major issue in many parts of the world. Changes in the economy and society started with the industrial revolution referred to as the beginning of the Anthropocene [1] have led to significant changes in the environment, and we now know for sure in the atmosphere as well. These changes either cover appearance or increase the level of various often very toxic pollutants, among which as the most important are particulate matter (PM), volatile organic compounds (VOCs) with most often listed in this group polycyclic aromatic hydrocarbons (PAHs), ozone (O_3), nitrogen oxides (NO_X), in some cities sulfur dioxide (SO_2), and also heavy metals. When pollutants are inhaled, they act as potential carcinogens, mutagens, allergens, and teratogens causing a number of diseases, mainly in the respiratory and cardiovascular systems including lung cancer that consequently increases morbidity and mortality. WHO reports that in 2012, around seven million people died—one in eight of total global deaths—as a result of air pollution exposure. The organization estimates and confirms that air pollution is now the world's number one environmental health risk [2].

Current knowledge and public awareness of the negative impact tends to seek effective measures to reduce the negative impact and as far as possible "repair" already degraded environment. A strategy for reducing emission and remediating already emitted pollutants is urgently needed. Once pollutants are already in the ambient air, with few exceptions, the only option to clean up the air is to use a nature-based, environmental-friendly biotechnology called phytoremediation. In this technology, appropriately designed or selected higher plant species together with their microbiome are employed to remove pollutants from the air and to degrade or detoxify them in a sustainable, conscious, and controlled manner.

It is important to keep in mind that raked leaves litter and mown, grass as containing impurities, should be treated as pollutants.

19.2 Air Pollutants and Their Origin

In most cases, these pollutants are of anthropogenic origin, such as fossil fuel combustion, non-exhaust vehicular emissions, household, industrial production, demolition of old buildings, burning of tropical forests and slash-and-burn agricultural technique, and climate change. All of the above points lead to endanger the environment. Fossil fuel combustion pollutes air with a number of contaminants, among which, as the most often and dangerous are listed: particulate matter (PM), volatile organic compounds (VOCs) with the most often listed in this group polycyclic aromatic hydrocarbons (PAHs), heavy metals (HMs), carbon oxides as the toxic CO and major greenhouse gas CO_2, oxides of sulfur (mainly SO_2) and various oxides of nitrogen (NO_X), ozone (O_3), and black carbon (BC) as not so dangerous. The SO_2 and NO_X are main gases responsible for acid rains.

19.3 Particulate Matter

The number one air pollutants on a global scale are particulate matter. They are one of the most harmful air contaminants, especially for infants and elderly [3]. A characteristic feature of the PM is the ability to stay in the air for quite a long time, i.e., from hours to several weeks. They are able to move on long distances from the source of their emission as, for example, from Asia to the West Coast of the United States [4]. Chemically, PM is a complex, heterogeneous mixture of different chemicals with some of them characterized as very toxic. Besides, they themselves are toxic; they also serve as the nuclei collecting on their surface other contaminants. Based on their aerodynamic diameter, there are several classifications of PM. Most often in literature, the classification with four fraction sizes is cited: large (10–100 μm), coarse (2.5–10 μm), fine (0.01–2.5 μm), and ultrafine (below 0.01 μm). Particulate matter of smaller diameter than 2.5 μm ($PM_{2,5}$) and 10 μm (PM_{10}) serves as an indicator of air quality. The recently published study showed, based on available information, that traffic (25%), combustion and agriculture (22%), domestic fuel burning (20%), natural dust and salt (18%), and industrial activities (15%) are the main sources of PM which constitute to cities' air pollution. However, there are significant differences in these numbers, between various regions of the world [5].

Usually their occurrence is promoted by an incomplete combustion. Globally, the main sources of PM emissions are the burning of fossil fuel and biomass for industrial purpose, food preparation, and homes' heating. However, now in many parts of the world, in the urban environment, a major source of pollution is undoubtedly considered transport. In the case of air pollution by motor vehicles and traffic-related PM, pollution from exhaust and non-exhaust sources is estimated to contribute almost equally. It is not always realized that about 50% of traffic-related pollution comes from non-exhaust sources. It is generated by brake, clutch, tire, and road surface wear and due to picked up materials by vehicle traffic from roadside. Approximately 50% of the wear debris generated during braking will be in size of PM_{10} and will stay in the air, while the rest as bigger and heavier becomes soon deposited on road surface or nearby roadside [6]. There is probability that some part of pollutants greater than PM deposited on the road under the vehicles of heavy weight becomes crushed to PM size. Brake wear can contribute up to 55% by mass to total non-exhaust PM and more than 20% to total traffic-related PM pollution. It should be noted that once pollutants are emitted into the atmosphere, our opportunities to remove them are very limited. In cleaning up the air from pollutants undoubtedly participate rain but, also plants especially those with large leaf area play very important role [6].

19.4 Organic Pollutants

Organic pollutants are formed during incomplete combustion as a result of burning wood or peat that takes place at lower temperatures and subsequently are emitted to the air. The list of organic pollutants opens polycyclic aromatic hydrocarbons (PAHs) which can exist in over 100 different combinations. The US Environmental Protection Agency has compiled a list of 16 priority PAHs, as the most common and harmful to humans and environment. In other countries this list might slightly differ. They are present in the atmosphere from primary sources and from reactions of the parent PAHs that can be chlorinated and nitrated [7, 8]. PAHs are a problem because they are very persistent in the environment, which increases with the number of rings. If they enter into the soil (washed off by rain from air, plants, or other surfaces), they will be strongly retained by the soil sorption complex, and the more dry the soil is, the PAHs will be bound more strongly. Washed off PAHs from other sources are transported with the storm to the pond or river. Individual PAHs vary in behavior. Those with three and four rings in the air very easily turn into a vapor form. The PAHs with five or six rings are solid and are very persistent in the environment. Their degradation processes are performed by wood-rotting fungi and go very slowly. N-PAHs are about ten times more carcinogenic compared to the unmodified, parent PAHs. Both N- and Cl-PAHs are long lasting in environment. Plants have great difficulties in uptake of PAHs and their degradation. Gaseous three- and four-ring PAHs, as all of them, are hydrophobic, but they can pass through the stomata to the interior of the plants, while those with five and six rings probably partly penetrate in by process of diffusion through the lipophilic wax layer. Most of the plants are not able to degrade PAHs for this purpose; they “employed” some microorganisms of microbiome or accompanying phyllobacteria and endobacteria living on the surface of leaves or inside the host cells, respectively [9].

19.5 Heavy Metals

Combustion of fossil fuels releases into the environment quite a few of heavy metal list of which open very toxic arsenic (As), lead (Pb), mercury (Hg) and chromium (Cr) VI, cadmium (Cd), recently joined antimony (Sb), and in high concentration zinc (Zn) and copper (Cu). Toxicity of all of the above is well recognized and described. However, in the last years, it has been demonstrated that the behavior of metals emitted during the combustion can be more complicated, and the new structures are formed by them which are much more toxic. During the combustion of fossil fuels in high temperature, metals will transfer to gaseous form and in part can be easily oxidized. In this form metals became extremely reactive with organic compounds forming products known as environmentally persistent free radicals (EFPRs) [10]. Formation of EPFR occurs when oxidized metals as CuO_2, ZnO_2, and FeO_2 are reduced by the delivery of electron from organic compounds [10]. The new structures become free radicals, lasting from days for phenoxy radicals to months and even years for semiquinone radicals [11]. It is worth noting that present in significant quantities in the air, nontoxic form of iron oxide after the entry into reactions with organic gaseous pollutants becomes very reactive pollutant as well as forming EPFR. But the question is open: how much of the Fe is in the reactive toxic form of EPFR. This as can be assumed depend on whether Fe is also accompanied by contamination with organic compounds. From deposits on the leaf surface, substantial amounts of Fe [12] plants probably use part by themselves [13]. Both metals and organic compounds of high molecular weight slowly, on the base of physical diffusion, penetrate through the wax into the first living cells of the epidermis where they are sequestered [14]. The penetration of heavy metals into the leaf via the epidermis is confirmed by a presence of higher level Pb and Cr in the leaves, because these metals are uptaken from the soil only in small quantities [15].

Zinc is a very important element for plants, very easily taken up from the soil even in high quantities if available, but in site heavily polluted by vehicles, it is also deposited on leaves from polluted air. Its presence in the air is also deposited on leaves due to abrasion of tires, brake linings with Zn, and corrosion of crash barriers. Manganese is added to the steel (0.8%) as a coolant to lower the temperature. It is difficult, however, to judge on its level in plant tissues whether its presence is an effect of pollution and if so in what proportion, because this element can be taken up in high quantities when plants are irrigated with water from deep wells.

Copper as an essential element for plants is very desirable; however, in higher concentrations, it is toxic to them. In the soil Cu is a medium bioavailable, but in polluted air, it also is coming from brake lining wear and alloy bearings. In brakes manufactured Cu is used because it is an excellent conductor of heat; during braking temperature, it can reach up to 1000 °C [6]. The high number of cars currently in use release to the environment large amount of Cu that some of the countries intend to limit content of this element in brakes to be no higher than 0.5%. Elements as Pb, Ni, Cr, V, and generally also Fe are poorly uptaken from soil by the plants and in small quantities translocated to the aboveground part, so their presence in leaves and twigs indicates on air pollution as a main source [16, 17].

Iron is emitted by transport vehicles in large quantities to the air [6], and probably substantial part of it is in oxidized form. The oxidized form of Fe probably reacts with secreted organic contaminants by vehicles and forms highly reactive EPFR. The phenomenon is a subject of study, but it is possible that we should change our opinion that this element is not so toxic as is commonly believed. At present it is assumed with high probability that Fe in form of EPFR is very toxic.

Lead is not well uptaken by plants, and its distribution to the upper part of the plants is very limited. Pb presence depends more on the air-contaminated exhaust fumes (even now in small amount from unleaded gasoline), from brake linings with Pb, and with wind picked up particles of soil contaminated with this element in previous years.

Nickel and vanadium are frequent additions to the alloy steel, and the level of both in the air increases in sites polluted by transport. A good example is Norway where the level of vanadium in the soil on clean sites was higher than in polluted ones, but the leaves of the plant growing on polluted sites by transport were more contaminated with V [17].

19.6 Black Carbon

Black carbon (BC) consists of pure carbon as a product of incomplete combustion of fossil fuel and biomass and is commonly referred to as soot. Toxicological study indicates that the BC is not pollutant, which directly harms human health as a component of the fine PM, but it plays a role of a carrier for a wide variety of toxic compounds on its surface. BC is the most strongly light-absorbing components of PM. BC suspended in the air absorbs sunlight and generates heat in the atmosphere, when deposited on snow and ice, which are light in color; thus, it facilitates increased melting and diminishes their otherwise substantial reflective capacity [18]. Therefore, BC contributes to climate change [19] and thus has a strong direct influence on global warming. Control of BC might slowdown this process [20], and it is supposed that it will be more effective than our efforts to reduce CO_2 emissions. We can assume that BC similarly affects also temperature of the plant surface in addition to reduction of the access of light to the photosynthetic apparatus. Inhibition of photosynthesis recorded in plants growing in the vicinity of coal mine partially can be explained by the negative impact of BC on plants [21]. In the cocktail of air pollutants, sometimes it is difficult to determine exactly the negative action of BC itself since it is also a carrier of many others, often more toxic compounds. Also during forest fires, BC is one of the predominant components of the aerosol with its negative impacts on photosynthesis [22]. Coniferous compared to deciduous plant species better withstand the stress conditions as in the first smaller reduction of photosynthesis is noted [22].

19.7 Gaseous Pollutants

Dominant route in the penetration of gaseous contaminants to the plants is stomata, cell structure that controls the plant gas exchange, but non-stomatal entering of gases takes place too. This way gets into the plant: CO_2, CO, NOx, SO_2, O_3, and gaseous two- and three-ring PAHs.

The CO_2 released into the atmosphere is a major greenhouse gas, undoubtedly considered as the main causative factor of climate change, but for plants, it is one of the nutrients and promotes their better growth. A huge global surface of the plants makes them a very important player in the removal of the gaseous pollutants from the atmosphere. Biologically active surfaces that are most exposed to contact with penetrating contaminants from the air as plasmalemma of plants' or lung epithelium have protective mechanisms. The contact layers are reaching in four major low molecular mass oxidants: ascorbate (vitamin C), uric acid, reduced glutathione, and alpha-tocopherol.

There are many different reactive forms of nitrogen present in the air environment. In urban areas, the main source of emissions of oxidized form of nitrogen (NOx) is transport, with NO_2 present in high quantities. The further fate of NO_2

depending on environmental conditions can be very different. A high temperature in the presence of ultraviolet promotes the formation of O_3, reactive radicals oxidizing most of biological molecules, and NO. This process is reversible, and when environmental conditions change, it may proceed in the opposite direction forming again NO_2 and O_2. In the presence of H_2O, other transformations create a mixture of nitric and nitrous acids (HNO_3, HNO_2), both are of acid rain components. The third possible direction of NO_2 transformation is process of nitration, which in recent years has devoted more attention. It was experimentally confirmed that NO_2 in polluted urban areas can promote the nitration of protein and peptide molecules. The process of posttranslational modification of proteins by nitration, in an environment polluted with NO_2, runs very smoothly as shown in a study by Franze et al. [23], where in a period from several hours to several days, 20% of the analyzed samples were modified. For a long time, results of observations and studies indicated that asthma and allergic diseases are enhanced by traffic-related air pollution, and it is already confirmed that nitration enhances allergic responses [24]. Most studies of this phenomenon were conducted on the protein Bet v 1, birch pollen, and it was confirmed, on molecular level, as a posttranslational modification of this protein by NO_2 [25]. In a polluted environment, higher allergenicity and changes in the protein composition of the pollen of *Platanus orientalis* was noted, one of the most important species in urban forestry of the warmer area of the world [26]. Also, pollen of invasive weed *Artemisia artemisiifolia* is more potent in allergenicity at elevated levels of NO_2 [27]. Crown evidences are samples of birch pollen collected in the natural conditions, which showed that pollen from urban areas had a higher allergenic potential than pollen from rural areas, despite the fact that content of allergen in analyzed samples was the same [28].

Nitrogen is the second most important element after carbon for plants, primarily for protein synthesis. Not all forms of this important element for life are available for plants including the most abundant atmospheric N_2. In this situation plants, if it is possible, uptake nitrogen in any available form if they only can do that, even in the form of ammonium, which when uptaken in larger quantities can be toxic. In light of the above question arises whether plants are able to use nitrogen from NO_2. Answers to this question were a subject of study by a team led by H. Morikawa [29]. They studied 217 of herbaceous and woody plant species in terms of their ability to use NO_2 as nitrogen source. Authors reported that plants are using nitrogen from NO_2 and found out that between tested species, huge differences exist in the capacity of using NO_2. They highlighted the group of species best in the ability to uptake ^{15}N-labeled NO_2 and named them as NO_2-philic. The most efficient in the uptake and assimilation of NO_2 within woody species proved to be *Magnolia kobus*, *Eucalyptus viminalis*, *Populus nigra*, *Robinia pseudoacacia*, *E. grandis*, *E. globulus*, *Populus sp.*, *Sophora japonica*, and *Prunus cerasoides*; within herbaceous cultivated plant, efficient was *Nicotiana tabacum*, and from herbaceous plants, the naturally growing along roadsides was *Erechtites hieracifolia.* Further research in this laboratory evaluated the uptake of NO_2 by 70 woody species (present in the surroundings of a road) in a concentration 0.1 $\mu l\ l^{-1}$ and compared with concentration 4 $\mu l\ l^{-1}$ which was already inhibiting assimilation of NO_2. The most valuable result of this experiment was identification of a group of species that tolerate high level of NO_2 and lead efficient assimilation at both high and low concentration of this pollutant, among which valuable in this context appear *Robinia pseudoacacia*, *Sophora japonica*, *Populus nigra*, and *Prunus lannesiana* [30]. It is interesting that these species are also listed as good phytoremediants of heavy metals from soil.

A more dangerous and toxic to hemoglobic organisms (including humans) is the carbon monoxide (CO), which naturally is secreted in small amounts by all living organisms, from bacteria to humans. It is also one of the products from the combustion appearing in the large quantities when oxygen is limited. Although for man it is extremely toxic but plants tolerate higher levels of CO, what is confirmed by their presence noted nearby sites where combustion of fossil fuels and biomass takes place? The amount of CO detained by plants from the downloaded to the air was assessed already many years ago. In the 1980s of the last century, one of the most extensive experiments was performed [31], in which ability of 35 plant species to accumulate ^{14}CO and its further fate was studied. Several fold interspecies differences in ^{14}C content in the leaves of tested species and differences in metabolism were found. Absorbed CO in the corn (C_4 plant species) was oxidized to CO_2, while in the bean plants (C_3 species), part of the CO was also oxidized to $CO_{2,}$ but the other part was reduced and incorporated into the amino acid serine. The list contains 17 surveys of woody species, among which stand out the following species: *Ficus variegata*, *Acer saccharum*, *A. saccharinum*, *Gleditsia triacanthos*, *Pinus resinosa*, *P. nigra*, and *Fraxinus pennsylvanica*. Also two shrub species *Syringa vulgaris* and *Hydrangea sp.* are characterized by their high ability to fix CO [31].

19.8 Organic Carbon

Organic carbon (OC) creates structures of organic compounds which are emitted by plants directly into the air but also can be formed from organic precursor gases emitted from anthropogenic and natural sources. Particles containing OC may also pose some risk to human health [32]. Some plant species release significant amounts of organic compounds consisting OC called biogenic volatile organic compounds (BVOCs) to defend themselves against herbivores, pathogens, and other stress factors. To this group of compounds, what belong mainly are isoprene, monoterpenes, sesquiterpenes, and other C_{10}-C_{15} BVOCs [33]. These stressful conditions cause the creation in the tissues of leaves ROS against which the plant defends itself leading oxidation of isoprene [34]. The phenomenon is still being investigated, but there is evidence that high level of BVOC contributed ozone and EPFR formation. Ozone and EPFR forming potential of plants should be taken into account in increasing of the green infrastructure. Taha [35] suggests limit from the cultivation quantity of the tree species that emit more than 2 $\mu g\ g^{-1}\ h^{-1}$ isoprene (isoprene of micrograms per gram of dry-leaf mass per hour) and 1 $\mu g\ g^{-1}\ h^{-1}$ of monoterpenes. According to Curtis et al. [33], *Aesculus glabra* exceeds this value considerably and *Corylus colurna* and *Tilia americana* slightly. Similar studies previously were conducted by

Benjamin and Winner [36] who assessed the ozone-forming potential (OFP) of 308 species of trees and shrubs dividing them into three groups: low-OFP contributing less than 1 g ozone d^{-1}, medium between 1 and 10 g ozone d^{-1}, and greater than 10 g ozone d^{-1}. The results of evaluation of so many species grown in urban areas are valuable source of information till present. In the group of low-emitting BVOC, there are popular genera such as *Malus*, *C. camphora*, *orange*, and *pear* and species *Ginkgo biloba* and *Juglans nigra*. The group of high-emitting BVOC belong to the following genera: *Salix*, *Quercus*, *Populus*, *Pinus*, and *Liquidambar*. Similar assessment in Europe to carry out Karl with coauthors [37] also noted the high emissions of BVOC by species belonging to the previously mentioned genera. According to Baraldi et al. [38], BVOC emissions are conducive to both high radiation and temperature.

19.9 Plant Species for Air Phytoremediation

As already mentioned, when pollutants are emitted into the outdoor air, our opportunities to remove them are limited, and besides rain, only plants are participating in this. At this point, it should be remembered that in parts of the world with higher precipitation, substantial part of PM deposited on leaves is washed off from them into the soil or on sealed urban area with every rainfall. Plants as autotrophic organisms during photosynthesis are carrying out gas exchange, uptake of CO_2, and release of water vapor process fundamental for plants' life importance. However, simultaneously, pollutants present in the ambient air, list of which is very long, also enter into the plants' tissues. For the efficient running of this crucial process for plants, they developed a series of defense mechanisms against uptaken air pollutants. In consequence, plants, by the way, act as an efficient biofilter removing from the air, at the same time, many pollutants with varying actions of toxicity. In that context trees and shrubs deserve special attention, Leaf Area Index (LAI) of which is relatively high. LAI indicates how many times 1 m^2 of the ground, occupied by plant(s), is covered by them. In this role the best are trees, LAI of which reaches ten; for shrub, it usually ranges from five to seven but also herbaceous plants like grass lawns with the index around two plays a positive role.

The presence, in environment, of plants accumulating significant amount of PM enables them to act as a biological filter [39, 40]. In several studies significant differences between plant species in the level of PM accumulation were reported [41–43] with some of them perfectly acting as a biological filter. Penetration of contaminants by a biofilter and stopping them depends on the turbulence around plants' organs and increases with faster air movement. Also leaves folding and the presence of various formations on their surface as, for example, trichomes or hairs play positive role in PM accumulation [41, 42]. Besides, metabolic uptake by plants, the stickiness of the leaf surface, and their aerodynamic properties are involved in higher PM accumulation [43], and electrostatic forces between heavy metal ions settling on PM and leaf blades cannot be ruled out [44]. In the deposition of PM on the leaf surface, very important is the presence of the epicuticular wax, which the main role is to protect plants against negative external factors. In fulfilling this protective role, important are the thickness of the wax layer, its structure, and chemical composition. Plant physiologist distinguished 23 major forms of wax [45]. Accumulated on the surface, PM in part remains on it, while the other parts of PM penetrate deeper and stuck in the wax layer (hereinafter designated as surface PM, sPM and in wax PM, wPM, respectively) [41]. sPM can be easily washed off by rain, while wPM stays in the wax for much longer time [46, 47].

In the temperate zone falling in the autumn, leaves contain significant amount of both sPM and wPM weight of which per 1 cm^{-2} often is around or even exceeds 40 μg. It is not worthy that 40 μg m^{-3} of air is designated as a norm according to European Union standard. Comparison of these values shows the potential of what vegetation has in cleaning of the air. Rain washes off PM both from the air and from the surface of the leaves and thus cleans up the air, but it should be noted that between rainfalls, just plants accumulate PM on their surface and in wax, thus ensuring continuous removal of pollutants from the air. Although part of PM that stuck in wax is removed together with wax as leaves are getting older, a new wax is produced and layered on the leaves' surface into which another part of PM from the air will penetrate. Therefore, it can be assumed that in older leaves, wax layer already developed remains in similar amount through the vegetation time; in spite of that, wax is entrained by wind and rain [48]. Torn off by the wind, wax together with wPM as heavier than just PM much faster falls at a certain distance from the tree/shrub, while the wax with $_s$PM is washed off by the rain, which gets into the soil under the plants. In recent years, several research centers evaluate plant species in order to recommend them for cultivation in areas with PM-polluted air. Position of a given species on the lists drawn up is variable and depends on location and local pollution and the weather conditions as wind and rain [49]. However, there are species which are located at the top part of the list, despite growing in different locations. The following tree species are listed as tolerant to the pollution and accumulating significant amount of PM, so they can be considered as potential phytoremediants: *Pinus sylvestris*, *Betula pendula*, *Pyrus calleryana*, *Sorbus intermedia*, *Populus sp.*, *Alnus spaethii*, *Robinia pseudoacacia*, *Elaeagnus angustifolia*, *Sophora japonica*, *Fraxinus pennsylvanica*, *F. excelsior*, *Quercus ilex*, and *Tilia x europaea* "Pallida" [41, 50–54].

In the cities shrubs play also an important role as phytoremediants. Due to their smaller size, they can be planted closer to the edge of the road, and, therefore, they would be the first objects on which emitted from vehicle engines pollutant are directed. Proposed for PM phytoremediation shrubs are *Pinus mugo*; *Syringa meyeri*; *Spiraea sp.* and *Stephanandra incisa*; *Taxus x media*, *T. baccata*, and *Hydrangea arborescens*; *Acer campestre* and *Physocarpus opulifolius*; *Sorbaria sorbifolia*; and *Forsythia x intermedia* [50, 51]. An interesting group of plants for phytoremediation is climbers, which occupy little land but produce a large leaf area directed vertically. They can, therefore, be planted in downtown of old cities where land is usually extremely limited as well as on screens of the highways. List of recommended species for temperate zone is short and comprises *Hedera helix*, *Parthenocissus tricuspidata*, *P. quinquefolia*, and *Vitis riparia* ([55] and authors not published data). One of the most tolerant climbers in polluted urban environment is *Polygonum aubertii* [56]. Although it is a little less decorative species but as fast growing and very tolerant to pollution, the species is recommended for places where garbage-collecting containers are located as excellent for camouflage of this place.

In phytoremediation, natural herbaceous vegetation plays an important role in colonizing almost any available soil surfaces in urban areas and providing therefore a surface for PM deposition. LAI of these species is much smaller but still on some species like *Achillea millefolium*, *Berteroa incana*, *Polygonum aviculare* and *Brickellia veronicifolia*, *Flaveri trinervia*, and *Aster gymnocephalus*, much greater amounts of PM are deposited than in other species [57]. In case of *Aster gymnocephalus*, additionally accumulation of significant amounts of heavy metals was recorded [58].

19.10 Green Infrastructure Directed to Phytoremediation

The presence of any plant promotes phytoremediation, but its significant level is important for the environment and can be achieved only in properly planned green infrastructure. Each technology has its own niche. In the case of phytoremediation, it is very effective in mitigation of pollution but not from very highly polluted air (as far as plants as living organisms can endure it); at the same time, it is a cheap technology and can be used on large scale. At present, our task is to recognize how this process is run, what kind of mechanisms are involved, and which species are carrying out phytoremediation most efficiently. Once this technology is introduced in practice, questions will always be raised on how to make it more efficient and cost-effective in comparison to other technologies.

We should of course remember that general principle in environmental protection strategies is elimination or reduction of both the source and level of pollutants emission. These concerns are also underlined by WHO [2] in recommendations of a series of measures for mitigation of the negative impact of polluted air on humans and environmental health and men's well-being. Many of these actions are already in the hands of city authorities, who carry them through introduction of several legislations as, for example, restriction of wood and low-quality coal burning. Also recommendations proposed in California like improving traffic flow, enforcing speed limit, reducing number of stops and sharp turns, imposing vehicles' weight limits, and rerouting tracks are quite easy to implement [59]. All these can be applied via administrative decisions. These actions play crucial role in prevention/reducing pollutants emission, but unfortunately they do not eliminate pollutants that are already being emitted into the atmosphere.

Having in mind the current status of air pollution, in many parts of the world, strategies/technologies of pollution mitigation are urgently needed. When pollutants are already in the atmosphere, the only option where men are able to lower the pollution is to use environmental-friendly biotechnology called phytoremediation where plants are used as a tool for cleaning up the air.

Plants as organisms leading sedentary type of live possess several defense mechanisms against stresses including negative impact of air pollution. Plants possess large ability to change or even create the environment in order to make it optimal or close to optimal for them. They would achieve this goal much faster if they exist in greater amount of what is confirmed by the environmental conditions prevailing in the forest or park. The presence of plants in an appropriate number ensures and improves several environmental parameters as increased humidity level, lowered temperature, infiltrated storm water, reduced wind and noise, and aesthetic values.

The team under the leadership of Dr. Nowak estimated that green infrastructure in the United States saves the death of 850 people and 670,000 from the problems of respiratory system (per year) as a result of exposure to air pollution [60]. Other detailed evidence on the positive greenery effects obtained in New York is reported by Lovasi et al. [61], who showed that children living in the streets with trees get sick much less on asthma in their early childhood as compared with those who were living nearby the streets without trees. Focusing green infrastructure on air purification should simultaneously also build health-supporting role of the ecosystem, like the improvement of city climate, and create space for physical activity and health conditions of the residents [62]. The efficient removal of the pollutants from the outdoor air via phytoremediation is challenging especially that this technology is in the early stages of its development. However, the ability of plants to change a climate in the city is building a hope to use them for effective removal of air pollutants as well. Therefore, one of the first tasks is to increase the green infrastructure, especially with trees since they have a large surface and thus are able to perform cleaning the air on greater scale, and additionally below them, there is a space for shrubs and herbaceous plants. A good example of such activities is New York, where campaign of planting of one million new trees by 2017 has become a model for copying by many cities in the world. The city authorities have taken a number of other initiatives to increase green infrastructure, of which the most spectacular was/is to build a linear High Line Park on the viaduct defunct railways of the city. On the 2.5 km long park, more than 160 species of plants from different habitats were gathered, making this place the biggest and unique attraction in Manhattan [63].

It is obvious that the most important role in greenery plays larger areas with trees as parks and forests surrounding the city as well as green belts along roads with extensive traffic. This raises the question of how wide should be the belt of vegetation needed to perform effective biofilter function. During examination of plants' efficiency as a biofilter, a significant reduction in particulate matter on leaves of *Quercus ilex* was noted at a distance from 0 to 20 m from the edge of the road. This reduction was stronger in the lower part of the crown, at a height of 1.5–2 m, which on average is at the height of a man's face [53]. A similar pattern of PM accumulation on the leaves (at the same height) of the *Tilia cordata* grown in the park was recorded by Popek et al. [64]; the greatest accumulation of PM was on trees grown 15 m from the edge of the road with heavy traffic. In further distances significant decrease (around 50%) in the amount of PM was recorded. Interesting is the fact that in the first measuring point (3 m from the roadway), a little less of PM was accumulated on the leaves, that was probably due to their entrainment by moving vehicles and PM pumping into the biofilter, which pose some resistance to pollutants' penetration [64]. The process of pollutants movement through biofilter consists of two interdependent phenomena which take place (1) deposition and (2) dispersion. The effectiveness of these processes is demonstrated by results of Al-Dabbous and Kumar [65], which showed 37% reduction in PM on the sidewalk separated from the roadway with coniferous plants. This evergreen plant as a barrier for PM is demonstrated by the fact that on its part facing to the road, 11% higher amount of PM was noted when compared to the area without vegetation. Interesting results on the evaluation of various barriers, i.e., vegetation barrier and solid barrier, and of combined applications of both were presented by Tong with colleagues [66]. The vegetation barrier consists of conifers plants that, as evergreen, performed phytoremediative role all year round. Authors evaluate the ability of the plants in the barriers to mitigate the pollutant emitted by vehicles as referred to trees' height and distance on which PM is moving. Both the height of the barrier (6 and 9 m) and its distance from the road did not have significant effect on PM movement on further distances. On the other hand, however, when they compared the width of the vegetative barrier (6 m, 12 m, and 18 m), the results confirmed and expected positive impact of the greater width. Authors of this interesting and very useful planning of roads and surrounding studies recommend two options. The first consists of a wide vegetation barrier with high canopy density with two rows having tall plants conducive to falling particles between the two rows and the second row that would be a barrier for deflection of pollutants. The second option is a combination of solid barrier (6 m high) with vegetation barrier (9 m high) located behind the solid ones. It can be assumed that, in addition to accumulation of pollutants, the solid barrier would better protect against noise.

Vegetation should constitute a sort of sieve that allows to penetrate pollutants through, but it should not be too dense to prevent increasing of pollution concentration. Additionally, if possible, architecture of the vegetation should direct pollutants toward the ground in order to avoid deflection of polluted airflow over the biofilter [67]. In many cities, buildings are close to streets that form a kind of canyon, where the dynamics of the air movement depending on the wind direction differently behaves and the presence of trees in canyons might increase pollutant concentrations. This is confirmed by measurements carried out in such conditions, and authors suggest to consider the possibilities of the negative impact of urban forestry in canyon street in the city [68]. Although the positive impact of urban forestry in reduction of air pollution is widely known, but in this situation, it is necessary to develop an optimal status of greenery. The results of Jin et al. [69] with *Platanus x acerifolia* and *Cinnamomum camphora*, very common tree species on the streets in China, indicated the optimal range for these species as 50–60% and 1.5–2.0 for canopy density (CD) and leaf area index (LAI), respectively. These optimal parameters can be obtained through plant species selection, their planting pattern, and pruning. Additional reduction of air pollution by urban forest in the canyon street can be achieved by creation of green walls on the buildings, which are able to reduce NO_2 and PM_{10} by 40% and 60%, respectively, as well as, although less effectively, green roofs [70].

The evaluation of the reduction of pollution by urban forest in the Leicester City Center (UK) demonstrates that trees reduce concentration of ambient vehicular pollutants, at pedestrian height, on average by 7% [71]. To know the dispersion of air pollutants, a group of researchers from the Aarhus University developed Operational Street Pollution Model (OSPM) which elaborated the behavior of air masses in the street canyons, taking into account the impact of wind resulting in higher concentrations of pollutants in the leeward side than in windward ones [72]. The model in the following years has been further developed for urban areas [73]. The program has been proved as very useful, and now, it is used by many companies from the area of planning and urban lands management on different continents.

To assess the phytoremediation potential of urban forestry, iTree and UFORE models that refined and took into account general parameters like crown volume, species-specific effects, plant health conditions, different designs, etc. were developed [74].

19.11 Agronomic Recommendation Supporting the Air Phytoremediation

Air pollution is usually a mixture of a number of pollutants; therefore, it is necessary to grow the species known as highly tolerant to adverse environmental conditions. Plants developed a number of defense mechanisms including creating a barrier on their surface to stop pollutants from entering inside of the plants and, in case when pollutants would get in, to transform and maintain them in the nontoxic form. In the polluted air dominated with contamination such as PM (vehicle and household emission) and NO_2 (heavy track diesel emission), it is recommended to cultivate species tolerant to these pollutants or with some risk for the success, species of the same genus. Trees growing in the canyon streets and in front of the biofilter surrounding roads should be some kind of sieve, through which the air flows to encounter small obstacles and deposit the pollution on subsequent encountered leaves, twigs, and branches. In the case of schools, kindergarten and playground pollutants should well penetrate into the biofilter (to avoid deflection of airflow) and then must be stopped inside the biofilters to ensure the greatest possible safety for the children. Since cultivated plants possess characteristics for a given species/cultivar architecture, crown size, shape, and canopy density, these parameters must be taken into consideration in planning greenery in the specific sites.

In places with high pollutant emissions as crossroads, bus stops, and uphill streets, it should be preferred that species are not only tolerant to air pollution but also are good phytoremediants accumulating pollutants, that along with mown grass, falling in autumn litter, can be raked and burned in an incinerator. Trees and shrubs growing in urban areas are often formed by pruning for better fulfilling aesthetics goals, health, or rejuvenation purposes; however, in light of the obtained results, we should form them also for optimal phytoremediation canopy density. More resistant to pollution are deciduous trees, but on the autumn, they lose their foliage meaning that during winter they do not play a phytoremediative role. It can be, to some extent achieved, by planting together with them with evergreen climbers as, for example *Hedera helix*. It is worth noting also that young oak (usually up to 10 years) and hornbeam formed as a hedge do not shed their leaves until spring; therefore, all this time, the phytoremediation functions are fulfilled.

Man uses plants as a building material, source of food, and energy and has recently "employed" them as green liver for helping in cleaning up air in the humanosphere.

Nowadays, men are trying to take advantage of this by "harvest" of plant biomass saturated with pollutants via an operation called phytoremediation which contributes in the purification of air of the humanosphere in order to make it more safe both at present and for the incoming generations.

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