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Editors

Phytoremediation

Management of Environmental Contaminants

Volume 1

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Preface

Come forth into the light of things. Let Nature be your teacher.

William Wordsworth

Phytoremediation technologies use green plants and their associated microbial communities to remove, degrade, or stabilize inorganic and organic contaminants entering the air, water, and soil of a multitude of ecosystems. In some cases phytoremediation applications can serve as one of several useful components in the overall management and control of contaminants using relatively low-cost solar-driven physiological/biochemical mechanisms common to most plants. Many phytoremediation applications have the added value of providing a remediation option that offers a minimum disruption to the ecosystem or habitat under repair.

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

The chapters in this book provide a diverse account of selected phytoremediation research projects and case histories from specific sites and/or laboratories on four continents around the world. Volume 1 provides a diverse global perspective and includes observations and data collected from multiple sites in fifteen countries in Africa, Asia, South America, Europe, and the USA. Organic and inorganic contaminants covered include BTEX, PAHs, RDX, hydrocarbons including petroleum, and heavy metals/metalloids. Chapters in Volume 1 also discuss the influence of key factors on the general management of contaminants in soil and water such as bioavailability, landscape design, and process amendments including biochar.

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

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Part I

Overview of Phytoremediation Applications

Giant Reed (*Arundo donax* L.): A Green Technology for Clean Environment

1

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1.1 History of *Arundo donax* L.

Many literatures stated that *Arundo donax* L. is indigenous to the Mediterranean region, but different sources suggest that *Arundo* was introduced to the Mediterranean region and southern USA from subtropical parts of the world like in India, in China, and in southern USA, and some genotypes are also adapted to cooler climate conditions. Under natural site conditions *Arundo donax* L. is usually found along river banks, creeks and generally in moist soils but it grows also successfully on relatively dry and infertile soils such as roadsides and is used to mark field sites (Tucker 1990; Sharma et al. 1998; Günes and Saygin 1996). *Arundo donax* L. was known by different names such as Giant cane, Carrizo, Arundo, Spanish cane, Colorado River reed, and Wild cane. The science community has adopted the common name, giant reed (Bell 1997).

The uses of giant reed have been dated back to 5,000 BC when the Egyptians used giant reed leaves as lining for underground grain storage. In the fourth century giant reed was used for medicinal purposes such as a sudorific, diuretic, and the treatment of dropsy. Also, it has been stated that mummies were wrapped with giant reed leaves.

It has been well documented that giant reed was introduced intentionally to California in the early 1820s by the Spanish for erosion control. Also the Spanish used giant reed as building material, firewood, and fodder (Frandsen 1997). In addition, governmental agencies over time have encouraged farmers to plant giant reed for erosion control in drainage canals (Boose and Holt 1999). *Arundo donax* L. was first introduced to Australia in 1788, by the first fleet bringing the British to Australia for colonization (Lee 2009). In Australia, it has been used especially for windbreaks around horticulture crops, to stabilize sand drifts and the immature canes are cut when shorter than 1.8 m for use as a drought reserve grass for ruminants (Spafford 1941; Williams and Biswas 2010).

1.2 Characteristics and Production of Giant Reed

Giant reed (*Arundo donax* L.) is a robust erect perennial grass species reaching up to 14 m height under optimal growth conditions, growing in many clumps. Individual tough and hollow stems, 3–5 cm in thickness, have a cane-like appearance similar to bamboo with alternate leaves, 30–60 cm long and 2–6 cm broad, tapered tips and hairy tuft, at the base. Stems produced during the first growing season are unbranched and photosynthetic (Bell 1997).

Giant reed has a widespread network of rhizomes under the soil surface, 5–30 cm in depth (Fig. 1.1). The fibrous roots originating from the rhizomes are able to grow into the soil to 5 m in depth in certain moist soils, whereas most roots are more than 100 cm long. The rhizomes (3–8 cm wide and 10–25 cm in length) grow tough, fibrous, and long tap roots (Bell 1997; Frandsen 1997; Sharma et al. 1998). The giant

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Fig. 1.1 Rhizomes of giant reed plant grow around drains (*left*), several culms grow from the rhizome buds (*right*) (photographs were taken in September 2012 from Kafr El-Sheikh, Egypt)

Fig. 1.2 Giant reed plantation in the demonstration garden of Debrecen University, Hungary, 2012 (compared to Prof. Fari M.) (*Left*), giant reed plants grown in pure red mud sample collected from Veszprém County, Hungary, October 2010 (*right*)

reed stem is a hollow, segmented culm that measures from 1 to 4 cm in diameter and is able to branch. The culms' walls range from 2 to 7 mm in thickness, and the internodes can reach 30 cm in length. This stem structure is able to support the erect position of such a tall plant, as its mechanical stability is not dependent on turgor pressure (Spatz et al. 1997). Several stems grow from the rhizome buds during all the vegetative season, forming dense clumps. In the Mediterranean basin, where the warm, temperate climate is characterized by mild, wet winters and hot, dry summers, giant reed new shoots emerge from buds on rhizomes (Fig. 1.1) in late winter/early spring, achieving maximum growth rates in midsummer. Under optimal conditions stems can grow 10 cm day^{-1}, placing it among the fastest growing plants (Perdue 1958; Bell 1997). The culms carry alternate leaves that originate from the nodes and are very hard and brittle with a smooth glossy green surface that turns golden yellow at the end of the growing season (Perdue 1958; Frandsen 1997; Spatz et al. 1997). Giant reed spreads from horizontal rhizomes below the soil and forms dense stands on disturbed sites, sand dunes, wetlands, and riparian habitats.

Giant reed has the ability to survive in a wide range of soils, including apparently inhospitable and marginal land (Fig. 1.2). It can grow in heavy clays to loose sands and gravelly soils. After the first year of growth, it is also able to survive in sites of high moisture and salinity, including marshes (Perdue 1958). Sandy soil is the most common type of soil in which it is found (Frandsen 1997). Giant reed can grow year round but optimal growth occurs between February and October on the Northern hemisphere. It grows well when the water table is close or at the soil surface but will be retarded if there is lack of moisture in the first year of growth. Droughts have little effect on the established stands that are in the second or third year of growth. Giant reed can survive extended droughts because of the drought-resistant rhizomes and roots that can reach water supplies. It also can survive very low temperatures in the dormant winter season but is subjected to possible damage with frost events after the start of the spring growth. Its vigor makes giant reed an effective potential competitor for other plant species. Once established, giant reed tends to cover large areas with dense clumps, compromising the presence of native vegetation not able to compete (Bell 1997; Coffman et al. 2010).

Indeed, although giant reed is a C_3 grass, it shows high photosynthetic rates and unsaturated photosynthetic potential compared to C_4 plants (Papazoglou et al. 2005; Rossa et al. 1998). Despite being a C_3 species, the biomass yield of giant reed was higher than the other C_4 species tested by Angelini et al. (2005) and Kering et al. (2012). Directly after plantation, several rhizome buds are activated and up to ten stems per rhizome may emerge until the end of the first growing period. In southern France giant reed produced 20–25 t ha^{-1} dry matter (Toblez 1940). Other authors reported dry matter (DM) yields of 35 t ha^{-1} in northern Italy (Matzke 1988), while also 53 t ha^{-1} dry matter were measured in wild stands of giant reed in Turkey (Günes and Saygin 1996). First results from field plots in southern Germany showed in the years 1989 and 1990 dry matter yields between 7 and 26 t ha^{-1} (Oster and Schweiger 1992) (Fig. 1.2).

The water requirement of giant reed is about 300–700 mm in the vegetation period and a transpiration coefficient of approximately 200 L kg^{-1} DM was calculated (Chiaramonti et al. 2000). These data are comparable with Miscanthus growth requirements (transpiration coefficient: 250 L kg^{-1} DM, water requirement 500–700 mm per vegetation period). For plant cultivation not more than 100 kg nitrogen per ha and 150 kg P_2O_5 as well as 200 kg K_2O per hectare are recommended for fertilization (Dalianis 1996).

Furthermore, in giant reed stems and leaves, there are several noxious chemicals such as triterpenes, curare-mimicking indols, and hydroxamic acids (Bell 1997); these compounds together with the thickness of the tissues can protect them from the attack of herbivorous insects and other grazers (Bell 1997; Spencer et al. 2010).

1.3 Propagation of Giant Reed

Giant reed reproduces vegetatively by rhizomes and also by stems, which will root at the nodes along the stalk. Rhizomes in perennial grasses are underground stems that produce adventitious roots and shoots. Stems produce adventitious roots and shoots near the tips of branches or from sprouts at the stem base or stump. It flowers in late summer and produces a plume-like inflorescence. Giant reed does not produce seed in Europe (Lewandowski et al. 2003; Mariani et al. 2010) or Asia (Bhanwra et al. 1982; Popov and Belyaeva 1987) nor in the USA (Balogh et al. 2010) or Australia (Williams et al. 2008). So, conventional breeding through sexual hybridization cannot be performed. Since it is an asexually reproducing species due to sterility, its genetic variability and the chances for finding new genotypes or varieties are low (Lewandowski et al. 2003). Since it is a sterile plant without viable seeds, and vegetative propagules such as rhizomes and divisions are bulky, large-scale propagation of giant read for plantations requires in vitro micropropagation. In the USA, Márton and Czakó (2004, 2007, 2011) developed a universal cell culture procedure which established embryonic cell cultures, micropropagation, and genetic engineering protocols for successful use in many species of monocots such as giant reed.

1.4 Phytoremediation Potentials of Giant Reed

Giant reed (*Arundo donax* L.), a perennial plant, which is widely used as energy material and paper pulping, has received considerable attention for remediation soils polluted by multi-metals due to its capacity of thriving in various range of adverse conditions with rapid growth and high yields (Lewandowski et al. 2003). The adaptability to extreme soil conditions combined with rapid and vigorous growth makes giant reed an interesting candidate for environmental studies on phytoremediation treatments (Table 1.1). The use of plants to remove contaminants from polluted water and soil can be an advantageous strategy, which can also be used to remove metals which cannot be biodegraded.

The potential of giant reed to serve as a phytoremediation plant is dependent on the condition that it meets the general characteristics of phytoremediation plants.

A plant is a hyperaccumulator, if it meets the following criteria:

1. Concentrations of heavy metals in plant shoots should reach hyperaccumulation, which is different for different heavy metals, e.g., it is more than 1,000 mg kg^{-1} for Pb and Cu (Baker and Walker 1989; Brown et al. 1994), for As (Ma et al. 2001), for Ni and Co (Brooks 1998), and for Cr (Lombi et al. 2001) and more than 10,000 mg kg^{-1} for Zn (Brown et al. 1994).
2. Concentrations of heavy metals in shoots should be 10–500 times greater than those in normal plant (Shen and Liu 1998).

Table 1.1 Summary for studies on phytoremediation potentials of *Arundo donax* L.

Contaminant(s)	Type of experiment	Experimental conditions	Growth medium	Experimental duration	Used concentrations	Summary	Reference
Bauxite-derived red mud	Pot experiment	Greenhouse conditions	Soil	3 months	–	*A. donax* is able to remediate red mud-contaminated soils, as well as pure red mud	Alshaal et al. (2013)
Cr	Pot experiment	Greenhouse conditions	Nutrient solution	3 weeks	50, 100, 200, 400, 600, and 900 μg L^{-1}	*A. donax* can remediate the moderately Cr contaminated environments (up to 500 μg L^{-1})	Shabana et al. (2012)
Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn	A 2-year sampling was conducted in an urban watercourse of Catania (Sicily, Italy)	Field conditions	Water and sediment	2 years	–	*A. donax* can act as a potential biomonitor of trace element contamination in water and sediment	Bonanno (2012)
Salinity	Constructed wetland (CW) beds	Open environment	Soil	1 year	Electrical conductivity of wastewater was 8.9 mS cm^{-1}	Using *A. donax* in CWs for wastewater remediation	Idris et al. (2012a, b)
As, Cd and Pb	Pot experiment	Greenhouse conditions	Soil	3 months	Pots were homogeneously sprayed with aqueous solutions containing 80 mg As, 10 mg Cd, and 500 mg Pb per kg soil	*A. donax* has ability to decontaminate As-, Cd-, and Pb-contaminated soil, and using acetic acid, citric acid, and sepiolite could be considered optimum soil amendments for *A. donax* remediation system	Miao et al. (2012)
NO_3^- removal	–	–	Aqueous solution	–	–	The utilization of *A. donax* reed based anion exchange resin (ALR-AE resin) in actual water samples indicated that the ALR-AE resin could be used for the treatment of many ionic polluted wastewaters	Xing et al. (2012)
Cd and P	Pot experiment	Laboratory conditions	Hydroponic culture	2 months	1, 2 mg Cd L^{-1} and 1.4, 1.7, 1.8, and 2.0 mg PL^{-1}	*A. donax* has high potential for cadmium removal in hydroponic culture. The effect of cadmium on phosphorus removal was not observed in this condition	Sagehashi et al. (2011)
As	Pot experiment	Open environment	Soil	3 weeks	50, 100, 300, 600, and 1,000 μg L^{-1}	*A. donax* can be used for the remediation of arsenic contaminated soils	Mirza et al. (2011)
As	Pot experiment	Greenhouse conditions	Nutrient solution	3 weeks	50, 100, 300, 600, and 1,000 μg L^{-1}	*A. donax* plants may be employed to treat contaminated waters containing arsenic concentrations up to 600 μg L^{-1}	Mirza et al. (2010a)

As and Hg	Pot experiment	Greenhouse conditions	Soil	2 months	As (150, 250, and 350 mg kg^{-1}) Hg (2,500, 3,000, and 3,500 mg kg^{-1})	As and Hg significantly increased propagation and biomass in *A. donax*. Thus, the growth of *A. donax* is recommended for soil contaminated with As and Hg	Mirza et al. (2010b)
As, Cd, and Pb	Plastic tackle	Greenhouse conditions	Soil	70 days	As (13.7–414 mg kg^{-1}) Cd (1.10–126 mg kg^{-1}) Pb (52.4–2,552 mg kg^{-})	*A. donax* is a promising, naturally occurring plant with strong metal tolerance, which can be cultivated in soils contaminated with multiple metals for ecoremediation purposes	Guo and Miao (2010)
Cd and Ni	Pot experiment	Field conditions	Soil	2 years	The plants were irrigated with aqueous solutions of Cd and Ni in concentrations of 5, 50, and 100 mg L^{-1}	High soil Cd and Ni concentration were not stress factors, as they did not inhibit stomatal opening and did not affect the function of the photosynthetic machine of *A. donax*	Papazoglou (2007), Papazoglou et al. (2005, 2007)
Sewage effluents	Closed gravel hydroponic system	Open environment	Pig's waste as nutrient solution in gravel bed	3 years	–	*A. donax* is useful system for recycling wastewaters	Mavrogianopoulos et al. (2002)

–, not available

3. Trace metal concentration in shoots should be invariably greater than in roots (Brown et al. 1994).
4. An enrichment coefficient (the ratio of metal concentration in shoot to that in soil) should be greater than one (Brown et al. 1994).

Many studies (Table 1.1) indicate that giant reed may have a potential use for phytoremediation purposes (Alshaal et al. 2013; Shabana et al. 2012; Miao et al. 2012; Mirza et al. 2010a, b; Papazoglou 2007; Papazoglou et al. 2005, 2007). Through the extremely high evapotranspiration, giant reed can remove large amounts of nutrients and water from wastes and has a potential for treating saline wastewaters or pollutants (bioremediation) (Idris et al. 2012a, b).The plant is able to efficiently transfer arsenic (As), absorbed from the growing medium and accumulate it into the shoots, showing good tolerance to the presence of the metal (Mirza et al. 2010a, b). Giant reed is a plant only slightly affected by the presence of metals such as cadmium (Cd), nickel (Ni), arsenic (As), and lead (pb) in the rhizosphere, and because of this trait, it can be a potential crop for contaminated soil, capable of high biomass production in polluted areas (Papazoglou et al. 2005, 2007; Guo and Miao 2010). Giant reed is able to grow well in trace metal-polluted soils, since these trace metals were not stress factors, they did not inhibit stomatal opening and did not affect the function of the photosynthetic apparatus of giant reed (Papazoglou et al. 2007).

Miao et al. (2012) investigated the effects of different five amendments such as acetic acid (AA), citric acid (CA), ethylenediaminetetraacetic acid (EDTA), sepiolite, and phosphogypsum on growth and trace metal uptake of giant reed grown on soil contaminated by As, Cd, and Pb. They found that the concentrations of As, Cd, and Pb in giant reed shoots were extraordinarily increased when 2.5 mmol kg^{-1} AA and CA, 5.0 mmol kg^{-1} EDTA, and 4.0 g kg^{-1} sepiolite were applied to soil as compared to the control. Furthermore, the accumulations of As and Cd were also significantly increased under the above condition, while the shoot Pb accumulation was clearly enhanced by adding 4.0 g kg^{-1} sepiolite and 8.0 g kg^{-1} phosphogypsum to soil, respectively. Based on above data, we can conclude that AA, CA, and sepiolite could be used as optimum soil amendments for giant reed remediation system.

It is well documented that increased soil trace metal content resulted in toxic effects on biochemical and physiological plant processes and on growth reduction (Dalla Vecchia et al. 2005; Das et al. 1997; Parida et al. 2003; Ryser and Sauder 2006; Sheoran et al. 1990). Such extremely high cadmium contents have been reported in surface soils near metal processing industries, e.g., 3.2–1,781 mg kg^{-1} Cd in Belgium and 26–1,500 mg kg^{-1} Cd in the USA (Kabata-Pendias and Pendias 1992). Ni concentrations of up to 10,000 mg kg^{-1} have been reported in soils derived from some ultrabasic igneous rocks (He et al. 2005). Papazoglou (2007) studied the stress tolerance of giant reed plants under irrigation with trace metal aqueous solution. A 2-year pot experiment was carried out in the field and an automated drip irrigation system was established to provide daily the plants with aqueous solutions of Cd and Ni in concentrations of 5, 50, and 100 mg L^{-1}, against the control (tap water). At the end of second year of the experiment, the soil total Cd and Ni contents were 973.8 mg kg^{-1} Cd and 2,543.3 mg kg^{-1} Ni under 100 mg L^{-1} treatments. The observed morphological features such as the number of branches; the plant height and the fresh and dry mass of stems, leaves, branches, root system; aboveground part; and total plant did not differ among treated and control plants, indicating the high tolerance ability of giant reed to the examined concentrations of Cd and Ni. Moreover, giant reed showed great potential for growing in nutrient solution supplemented by increasing concentrations of chromium (Cr) up to 900 μg L^{-1} for three weeks under greenhouse conditions (Shabana et al. 2012); generally, results showed that the accumulation pattern of chromium in various plant organs was as follows: root > stem > leaf. However, it shows potential utility of giant reed to decontaminate Cr-contaminated streams.

1.4.1 Antioxidant Capacity of Giant Reed

Study of plant mechanisms for accumulating metals and countering their toxicity stress helps improve our comprehension of the processes of metal nutrition and detoxification. Enhanced tissue tolerance, metal sequestration, and antioxidative response collectively determine the ability of plants to counter the destructive effects of trace metals (Jin et al. 2008).

In plants, high concentrations of trace metals such Cd, As, Ni, and Pb inhibit photosynthesis and diminish water and nutrient uptake (Shamsi et al. 2008; Meng et al. 2009; Dalla Vecchia et al. 2005; Das et al. 1997; Parida et al. 2003) resulting in chlorosis, growth retardation, browning of root tips, ultrastructural damage, and ultimately to death (Daud et al. 2009; Zhou and Qiu 2005; Baryla et al. 2001; Yang et al. 1996). The higher concentration of trace metals causes oxidative stress to the plants by accelerating the production of reactive oxygen species (ROS) and lipid peroxidation (Jin et al. 2008). In order to combat oxidative damage, plants have evolved an antioxidative enzyme defense system consisting of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) (Kanazawa et al. 2000). These enzymes catalyze the dismutation of highly reactive O_2^- into nontoxic forms like O_2 and H_2O. However, in spite of irrigating giant reed plants with aqueous solution containing 100 mg L^{-1} of Cd and Ni for two years, such symptoms have not been observed, all plants were very healthy indicating that giant reed is a good candidate

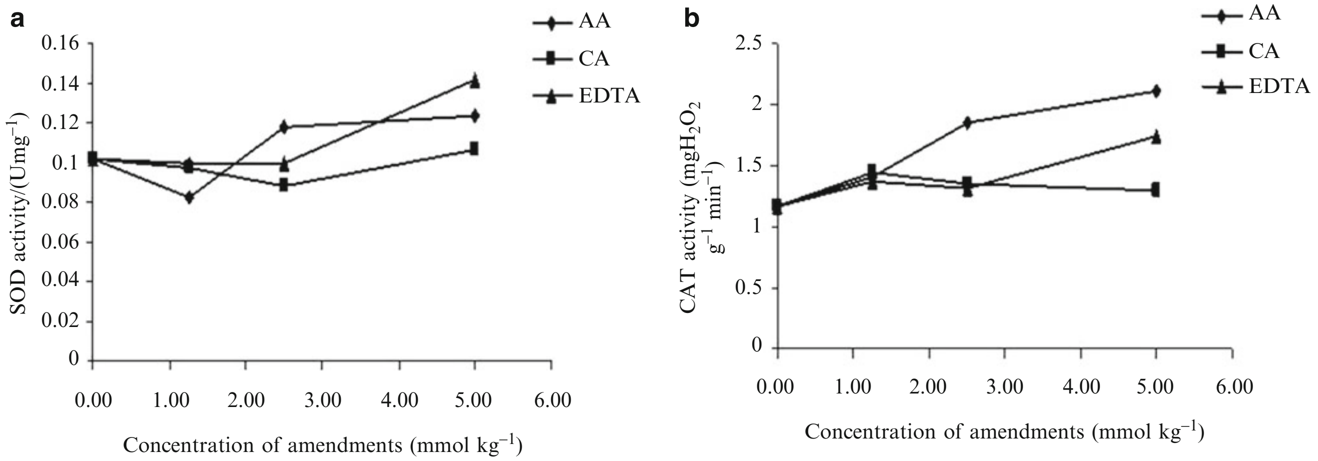

Fig. 1.3 Effect of acetic acid (AA), citric acid (CA), and EDTA on (**a**) SOD and (**b**) CAT activities in giant reed plants grown on soil contaminated by AS, Cd, and Pb (modified after Miao et al. 2012)

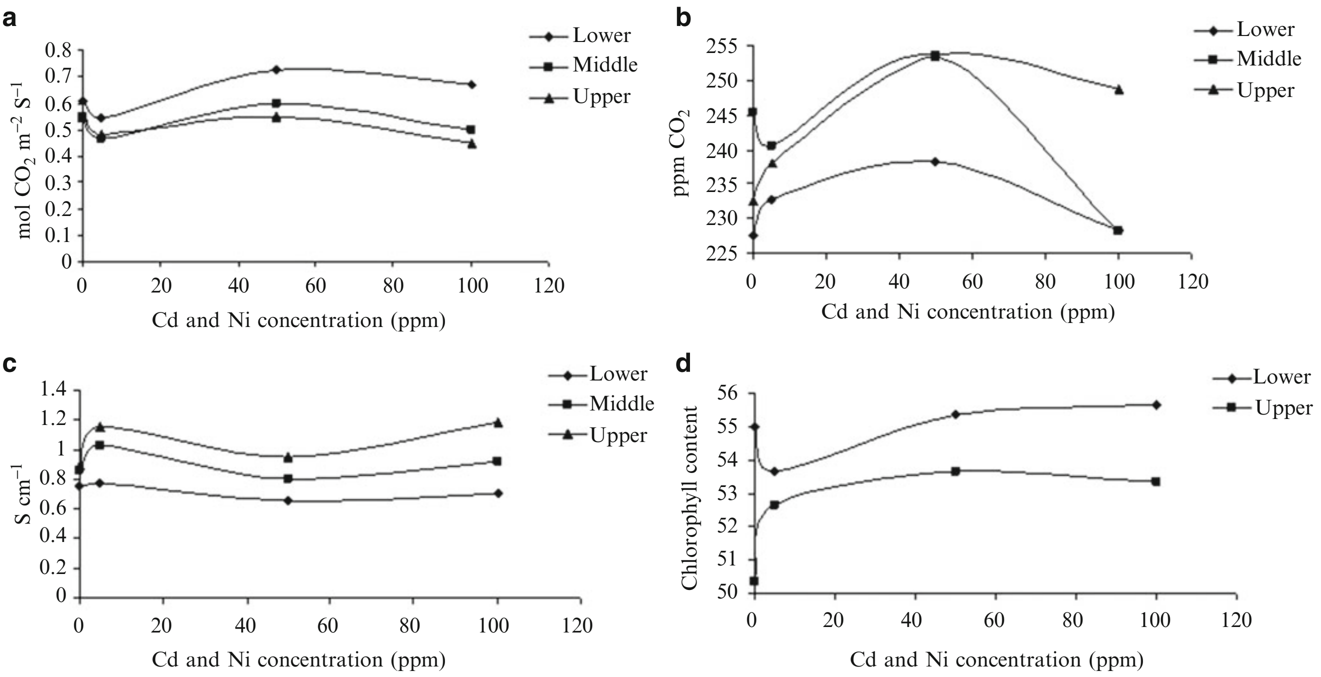

Fig. 1.4 The effect of different concentrations of Cd and Ni on (**a**) stomatal conductance, (**b**) intercellular CO$_2$ concentration, (**c**) stomatal resistance, and (**d**) chlorophyll content of lower, middle, and upper leaves of giant reed (modified after Papazoglou et al. 2007)

for trace metal phytoremediation applications (Papazoglou et al. 2007). The activities of SOD, CAT, and increased resistance to the stress of multi-metals; especially CAT (Fig. 1.3), played a role in countering As-, Cd-, and Pb-induced oxidative stress (Miao et al. 2012).

Physiological processes, such as photosynthesis and water status, are sensitive to trace metals (Monni et al. 2001) in several plant species. Trace metals have been found to inhibit electron transport in photosynthetic systems (Becerril et al. 1989) and the regenerative phase of the Calvin cycle (Weigel 1985). Papazoglou et al. (2005) reported that photosynthetic rates (Pn) of giant reed were unaffected by irrigation with trace metal aqueous solution containing 5, 50, and 100 mg L^{-1} of Cd and Ni, indicating that the photosynthetic system was not harmed and showed a strong tolerance of this plant to the increased heavy metal concentrations in the soil. The values of giant reed Pn rates (Fig. 1.4) found in this study were higher than those usual for C$_3$ plants (18–20 µmol CO$_2$ m^{-2} s^{-1}; Mohr and Schopfer 1995). In a comparative study on photosynthesis of five C$_3$ and three C$_4$ grasses, Rossa et al. (1998) found that giant reed had high Pn rates, higher than the other grasses (37.0 µmol CO$_2$ m^{-2} s^{-1}) under similar environmental conditions.

The same authors suggest that the high Pn rates of giant reed are attributed to the higher level of irradiance for saturation of electron transport through PSII and less photo-inhibition compared to other grasses. The high photosynthetic activity of giant reed indicates the ability of this plant not only to produce high biomass yield compared to other similar grasses with lower photosynthetic but also shows that giant reed is able to grow in trace metal contaminated soil.

Stomata play a crucial role in controlling the balance between water loss and carbon gain, therefore biomass production. Thus, measurements of stomatal conductance and resistance and intercellular CO_2 concentration provide useful tools to qualify the effects of stress factors on stomatal opening (Bolhár-Nordenkampf and Öquist 1993). Decreased stomatal conductance (Pankovic et al. 2000) and increased stomatal resistance (Sheoran et al. 1990) in Cd-treated plants have been reported. The inhibition of stomatal openings in plants exposed to heavy metals may depend on metal concentration, on exposure time, and also on the degree of toxicity suffered by the plants. Prasad (1995) has reported that concentrations above 50 mM for more than 5 weeks cause turgor loss and hydropassive stomatal closure on the exposed plants. Leaves showed toxicity symptoms and senescence with concomitant metabolic breakdown and loss of stomatal control. However, measured values of stomatal conductance, intercellular CO_2 concentration, and stomatal resistance in giant reed plants remain unaffected after treating with 100 mg L^{-1} Cd and Ni separately for two years (Fig. 1.4). These findings probably indicate that giant reed possesses a very effective antioxidant system that protects chloroplast and stomatal function. Organelles with an intense rate of electron flow, such as chloroplasts, are a major source of reactive oxygen species (ROS) production in plant cells. Especially in chloroplasts, the primary sources of ROS production are the Mehler reaction and the antenna pigments. Production of ROS by these sources is enhanced in plants by conditions limiting CO_2 fixation under stress factors such as high concentration of trace metals (Mittler et al. 2004). Indeed, trace metals are effective inhibitors of photosynthesis and cause alterations in PSII activity to various degrees (Pankovic et al. 2000; Zhou and Qiu 2005; Chugh and Sawhney 1999). PSII, including the water-oxidizing step, appears to be particularly sensitive to a number of stress factors (Papazoglou et al. 2007).

Chlorophyll fluorescence is used as a tool in quantifying the stress response under both laboratory and field conditions. The Fv/Fm ratio is a very useful measure of photo-inhibition, which can be observed as a secondary stress response if the photosynthetic process, as a whole, is restricted by a primary stress factor (Bolhár-Nordenkampf and Öquist 1993). Chlorophyll content declines in leaves of plants growing in trace metal contaminated soils or even on irrigating with wastewater (Das et al. 1997; Parida et al. 2003; Monni et al. 2001; Chugh and Sawhney 1999) and therefore the plants would diminish their efficiency to absorb light. By interfering with the activities of PSI and PSII, they would slow down the operation of photochemical reactions even in pigment-sufficient photosynthetic units. However, the maximum chlorophyll A content in giant reed leaves grown on Cr-amended nutrient solution was observed to be 1.893 mg g^{-1} at 200 μg L^{-1} supplied Cr. The chlorophyll B content was the maximum (1.187 mg g^{-1}) at 200 μg L^{-1} supplied Cr. As far as carotene content was concerned, its amount was the maximum (1.56 mg g^{-1}) at 100 μg L^{-1}. Further increase in the supplied Cr caused a significant ($p < 0.05$) decrease in carotene content (Shabana et al. 2012). Furthermore, measured chlorophyll content (Fig. 1.4) in giant reed plants was unaffected by high concentration of Cd and Ni and no significant differences were observed compared to control plants (Papazoglou et al. 2007). Miao et al. (2012) reported that chlorophyll content in leaves of giant reed growing on As-, Cd-, and Pb-contaminated soil was increased by 72.2 % amended with 5 mmol kg^{-1} citric acid in comparison to the control, which was in agreement with the change of biomass, suggesting that citric acid application did not deteriorate photosynthetic parameters of plant (Jean et al. 2008). These results indicate that trace metals such Cd, Ni, As, Cr, and Pb are most likely sequestered in a very effective approach within giant reed plant, thus providing a potent protection of the photosynthetic machine. Such sequestration is usually caused by trace metal-binding peptides, and as giant reed plants were able to grow under high Cd, Ni, As, and Pb concentrations with no effects on the measured physiological parameters, it seems that this sequestration mechanism is also strongly supported by an effective protection by a highly dynamic ROS-scavenging system (Mittler et al. 2004).

1.5 Bauxite Residue (Red Mud)

Bauxite ore contains aluminum hydroxides in large percentage and is therefore largely used for the production of alumina (Al_2O_3). Red sludge, or more euphemistically red mud, is the solid fine-grained residue left after alumina has been extracted from bauxite by the Bayer process, the principal industrial means of refining bauxite in order to provide alumina by digestion with sodium hydroxide (caustic soda) under heat and pressure. Alumina is the raw material for production of aluminum metal and also has important uses in the ceramics and pharmaceutical industries (Christoph (2006); Satish (1996); Babel and Kurniawan 2003).

Bauxite residue material is often described as red mud due to the color of the original bauxite ore and the iron oxide it contains (Table 1.2), which can make up to 60 % of the mass of the red mud (Christoph (2006); Satish (1996); Babel and Kurniawan 2003). In addition to iron, the other dominant particles include

Table 1.2 The main chemical constituents of red mud originated from different countries

Chemical constituents (%)	Weipa (Australia)	Trombetas (Brazil)	South Manchester (Jamaica)	Darling range (Australia)	Iszka (Hungary)	Parnasse (Greece)	Guizhou (China)	London (UK)
Al_2O_3	17.2	13.0	10.7	14.9	14.4	13.0	18.94	16.63
SiO_2	15.0	12.9	3.0	42.6	12.5	12.0	8.52	7.60
Fe_2O_3	36.0	52.1	61.9	28.0	38.0	41.0	26.41	42.58
TiO_2	12.0	4.2	8.1	2.0	5.5	6.2	7.40	5.00
LOI	7.3	6.4	8.4	6.5	9.6	7.1	9.71	12.20
Na_2O	9.0	9.0	2.3	1.2	7.5	7.5	4.75	3.49
CaO	–	1.4	2.8	2.4	7.6	10.9	21.84	11.36
Others	3.5	1.0	2.8	2.4	4.9	2.3	1.19	0.63

Table 1.3 Chemical and biological characterizations of red mud collected from Ajka (Hungary, 2010)

Characters	Red mud
pH	9.80
EC, dS m^{-1}	3.44
Soil organic carbon, g kg^{-1}	39.9
Avail. N, NH_4^+ mg kg^{-1}	0.761
Avail. P, P_2O_5 g kg^{-1}	21.44
Avail. K, K_2O mg kg^{-1}	900
$CaCO_3$ (%)	7.16
Dehydrogenase, μg TPF g^{-1} soil	59.6
Phosphatase, mg P_2O_5/100 g/2 h	0.22
Urease, NH_4^+ mg/100 g	417
Catalase, O_2 ml/2 min	4.3
Total bacterial count, $\times 10^7$ CFU	1.47
Actinomycetes, $\times 10^6$ CFU	4.11
Total fungi count, $\times 10^5$ CFU	0.29
Azospirillum number, $\times 10^4$	2.46
Azotobacter number, $\times 10^4$	1.04

After Alshaal et al. (2011)

silica, unleached residual aluminum, and titanium oxide. Red mud has a large acid-neutralizing capacity (ANC) between pH 12 and 7, and this is primarily attributed to the dissolution of tricalcium aluminate ($Ca_3Al_2O_6$) and Na, Al-silicate ($NaAlSiO_4$) and to the neutralization of $Al(OH)^{4-}$, NaOH, and $NaCO^{3-}$ in the pore water (Babel and Kurniawan 2003).

The red mud is separated during the refining process when caustic soda and alumina solution passes into thickener tanks in the clarification section. At this time, solid impurities sink to the bottom as a fine, red mud. The red mud is washed several times with water to recover caustic soda. Red mud from the washing stage is alkaline at pH 14 (Table 1.3). Seawater, at pH 8, is added to the mud to neutralize any residual caustic, reducing the pH of the mud to 8. The mud is then thickened and deposited in the dam as a slurry from multiple points around the dam perimeter to form drying beaches. The water that drains from the red mud is of similar pH to sea water and is discharged continuously from the residual disposal area into the estuary (http://www.qal.com.au/Press_Release/red%20mud%20fact%20sheet.pdf).

Red mud is composed of a mixture of solid and metallic oxide-bearing impurities and presents one of the aluminum industry's most important disposal problems. Red mud cannot be disposed of easily. Red mud is generally deposited in lagoons or dumped in the sea (Christoph (2006); Satish (1996); Babel and Kurniawan 2003). Approximately 0.5–2.5 t of red mud is produced per 1 t of alumina produced (Fig. 1.5). The amount of red mud produced depends on the quality of the bauxite and the processing conditions. By the year 2000, the alumina industry had produced circa two billion tons (Bt) of bauxite residue and is estimated to reach the 4 Bt mark at its current production rate by 2015 (Power et al. 2009).

It is well known that the continued production of alumina is restricted by a reliable and long-term disposal system for bauxite residues (red mud). Consequently, in the last 25 years considerable efforts have been undertaken to find suitable exploitation for red mud, rather than storage. In particular, utilization as a soil amendment or conversion into soil is high priority as they would enable a high-tonnage end-use outside of existing bauxite residue disposal areas (Klauber and Gräfe 2009). However, the physical and chemical properties of red mud make revegetation challenging and established floras are difficult to sustain without some special treatments before adding red mud into soil. Neutralization of red mud is considered one of the most important treatments, where it is intended to reduce the environmental impact of this highly alkaline material. Also, it can potentially reduce the need for ongoing management of disposal impoundments post-closure, as well as open opportunities for reuse of the residue material. Several methods are used to lower the alkaline pH to an acceptable level to decrease the impact on the environment such as sea water, mineral acids, soluble calcium or magnesium, and carbon dioxide. However, pH 10 is reduced to less than 1 % of its initial value by using mineral acid or a soluble calcium or magnesium sources. On the other hand, using carbon dioxide the solution alkalinity is reduced by about 40 %. Research is being performed to find a suitable way to use the mud for other applications (Christoph 2006; Satish 1996; Babel and Kurniawan 2003). Likewise, drying red mud is considered an effective way to decrease the pH and make red mud somehow safer than fresh

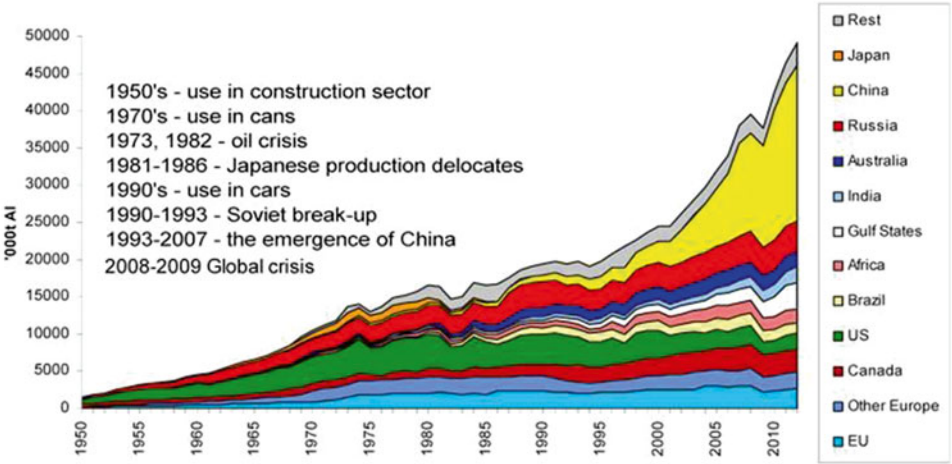

Fig. 1.5 History of primary aluminum production from 1950 to 2012. Source: EAA, AA, JAA, ABAL, RTA, Metallstatistik

Table 1.4 The concentrations of trace elements in red mud (ppm)

Red mud samples	As	Pb	Hg	Cd	Cr	Ba	Zn	Cu	Mn	Ni	Co
Guizhou (China)	267.3	56.6	67.3	27.1	537.8	212.0	103.2	78.2	187.5	984.9	na
Ajka (Hungary)	140.0	210.0	1.7	5.2	<0.1	100.0	125.0	52	187.0	64.0	45.2
London (UK)	na	na	na	na	2,390	na	na	214.0	463	1,423	244

na not available data

red mud, but drying the mud requires much energy (latent heat for water evaporation) and can represent high costs if fossil fuels have to be used in the drying process. In the event of improper disposal or willful discharge of red mud in rivers or lakes, there are fatal environmental hazards. The rainwater runoff from the red mud ponds increases river and stream water alkalinity which will convert lands into alkali soils when used for irrigation purposes. In some cases, the red mud is partly neutralized, but in all cases its chemical and physical characteristics inhibit the establishment of vegetation and pose a barrier for many possibilities for reuses.

1.6 Trace Elements Content in Red Mud

As there is a great deal of industrial alkali, fluoride, trace elements, and other potential pollutants in red mud (Table 1.4), long-term stockpiling would not only occupy scarce land resources but also easily lead to serious pollution of the surrounding soil, air, and groundwater (Agrawal et al. 2004; Ruyters et al. 2010; Mayes et al. 2011). In addition, the continuous increasing of stockpiling yard height may lead to potential geological disasters (Qiao 2004). Likewise, marine dumping of red mud may destroy the ecological balance of the ocean. The dike breach at the red mud stockpiling yard at the Ajkai Timfoldgyar Zrt alumina plant in Hungary on October 4, 2010 released between 600,000 and 700,000 m^3 of caustic red mud suspension (Fig. 1.6). This incident is unprecedented, given the scale of the release and the type of material involved (Alshaal et al. 2013). Furthermore, it is warning us to pay adequate attention to the comprehensive treatment of the red mud.

For many bauxite residues, trace elements can be of concern and may exceed regulatory soil limits in certain circumstances, and this has created public concern (Goldstein and Reimers 1999; Anon 2000; Kutle et al. 2004) (Table 1.4). The concentrations of trace elements (e.g., As, Cr, Cd, Ni, Pb, Zn) depend on the composition of the bauxite ore. Ruyters et al. (2010) reported that aqua regia soluble trace element concentrations in red mud are above the soil limits for Co, Cr, Ni, and Pb. Red mud application to soil increased the $CaCl_2$ extractable Na 70-fold compared to the control, but did not affect any $CaCl_2$ extractable trace element except Fe. Wang and Liu (2012) reported that the total concentrations of several hazardous trace elements including As, Pb, Hg, Cd, and Cr in Bayer red mud were larger than that of Sintering red mud.

1.7 Radionuclide Activities in Red Mud

Some bauxite residues may emit ionizing radiation above natural background rates due to the presence of naturally occurring radioactive materials (NORMs): ^{238}U and/or $^{228,\ 230,\ 232}$Th and members of their decay chains (McPharlin

Fig. 1.6 Aerial photographs of the Ajka alumina waste pond, Veszprém County, Hungary (photographs 1 and 2) (source of photograph 1: http://mysterytricycle.com/wp-content/uploads/2010/10/Sludge.jpg) (source of photograph 2: http://forums.atomicmpc.com.au/lofiversion/index.php?t36557.html)

Table 1.5 ^{40}K, ^{226}Ra, ^{232}Th, ^{238}U, and ^{137}Cs activity concentration of the samples

	Radioactivity (Bq kg^{-1})					
Red mud samples	^{40}k	^{226}Ra	^{232}Th	^{238}U	^{137}Cs	References
Guizhou (China)	113	302	404	na	na	Wang and liu (2012)
Ajka (Hungary, 2009)	48	360	292	na	na	Jobbágy et al. (2009)
Ajka (Hungary, 2010)	250	na	640	550	5.5	Ruyters et al. (2010)
Western Australia	350	310	1350	400	na	Cooper et al. (1995)

na not available data

et al. 1994; Cooper et al. 1995; Somlai et al. 2008). Little is known about the speciation of various metals and NORMs in bauxite residues, particularly with regard to pH neutralization and the accompanying changes in the mineral and solution phases (Goldstein and Reimers 1999; Kutle et al. 2004). Potentially toxic metals and NORMs arise via the original bauxite (Table 1.5); therefore, bauxite residues from different bauxite ores are likely to vary in type and concentration of respective metal species. Thus, any single study is limited in its scope to the origin of the bauxite and the processing conditions at a particular alumina refinery. Application of red mud to vegetable and crops has been proposed as a solution to increase the phosphorous and water retention and thereby reduce the leaching of nutrients. If the thorium (Th) and radium-226 (Ra) concentrations in the red mud are in excess of 1,000 Bq kg^{-1} and 300 Bq kg^{-1}, respectively, the use of this red mud on agricultural land could result in increased levels of radionuclides in food crops grown in amended soils (Cooper et al. 1995). However, data demonstrated that there is no significant increase in radionuclide levels in vegetable crops such as lettuce, cauliflower, cabbage, and potato grown on these soils (Table 1.6). The ^{40}K and ^{137}Cs activity concentrations in the red mud are about four-times lower than in soil, roughly in proportion to the total elemental K. The ^{238}U and ^{232}Th activities, here measured as ^{214}Bi and ^{228}Ac gamma activities, are five- to tenfold above the soil background values and correspond to activities detected in several Hungarian red mud samples (200–600 Bq kg^{-1} for each isotope) (Ruyters et al. 2010).

1.8 Effects of Red Mud on Soil and Plant

Red mud presents a problem as it takes up land area and can neither be built on nor farmed, even when dry. Red mud has a fine particle size (mostly <150 µm in diameter), contains large amounts of iron oxides, and thus has a very high sorption capacity for phosphorus. Due to residual caustic soda (used in the Bayer extraction process), the red mud is strongly alkaline and has a very high pH (9–12) and has a high salt content and EC (electric conductivity) dominated by sodium (Na^{+}); thus disposal is problematic (Alshaal et al. 2013; Snars et al. 2004). The high sodium (Na) content and fine structure of the red mud deteriorate soil structure. In addition, the salinity and alkalinity of the red mud can affect plant growth on the short term. These factors primarily determine the adverse effect of red mud on soil quality. The presence of high-soluble Al concentrations in the alkaline red mud additionally affects plant growth as confirmed in independent solution culture studies at alkaline pH (Fuller and Richardson 1986). Field

Table 1.6 Radionuclide concentrations in vegetable crops grown on red mud amended soil

			Activity concentration (Bq kg^{-1} dry weight)		
Red mud application (t ha^{-1})	Radionuclide	Soil concentration (Bq kg^{-1})	Lettuce	Cauliflower	Potato
0	^{238}U	8	<2	<2	<2
	^{232}Th	9	1.9	<1	<1
	^{226}Ra	7	1.7	<1	<1
	^{40}k	184	1,350	1,180	730
	^{137}Cs	1.5	3.6	0.6	1.1
60	^{238}U	13	<2	<2	<2
	^{232}Th	21	1.8	<1	<1
	^{226}Ra	11	1.4	<1	<1
	^{40}k	170	1,580	1,320	660
	^{137}Cs	1.2	2.4	0.4	0.7
120	^{238}U	21	< 2	<2	<2
	^{232}Th	47	0.8	<1	<1
	^{226}Ra	22	1.0	<1	<1
	^{40}k	212	1,410	1,240	660
	^{137}Cs	2.1	4.6	0.8	0.7
240	^{238}U	38	<2	nd	nd
	^{232}Th	89	2.5	nd	nd
	^{226}Ra	37	2.0	nd	nd
	^{40}k	206	1,290	nd	nd
	^{137}Cs	2.1	2.7	nd	nd
480	^{238}U	71	< 2	nd	nd
	^{232}Th	188	3.3	nd	nd
	^{226}Ra	78	2.1	nd	nd
	^{40}k	225	1,170	nd	nd
	^{137}Cs	1.3	2.5	nd	nd

nd not detected
Modified after Cooper et al. (1995)

trials have shown that bauxite residues can be vegetated by a combination of amendments with sand, compost, and gypsum; leaching and equilibration; and the use of salt- or Al-tolerant plants (Courtney and Timpson 2004; Courtney and Mullen 2009). Gypsum applications improve the red mud structure and gradually reduce the red mud pH to about pH = 8, likely due to the formation of calcite (Ippolito et al. 2005; Jones et al. 2010; Courtney et al. 2009). Revegetation of red mud accelerates the pH neutralization, probably because of enhanced carbonation in rooted zones.

The feasible detrimental effects of agricultural use of red mud on human health as a result of its inherent radioactivity and trace element content (Tables 1.4 and 1.5) have also been investigated. Red mud contains measurably high levels of thorium and uranium and may pose a risk if these elements leach into aquifers or if there is direct uptake by plants and/or animals (Summers et al. 1993; McPharlin et al. 1994; Cooper et al. 1995). However, application of red mud into soil by low rates up to 20 t ha^{-1} recorded negligible contents of radioactivity and trace elements in soil (Cooper et al. 1995; Ho et al. 1989; Summers et al. 1996a; Summers and Pech 1997).

Ruyters et al. (2010) conducted pot experiment in which they investigated the effect of different rates of red mud (0.1, 0.5, 1.4, 4.9, and 16.5 % based on wet mass) adding to soil on growth of spring barley (*Hordeum vulgare* L. cv Mauritia). Data showed that low red mud doses (<1 %) stimulated the barley root elongation and barley plant yield for unknown reasons. At the 4.9 % and 16.9 % red mud doses, barley plant yield decreased significantly and effects did not disappear with leaching. Unexpectedly, barley root elongation was almost completely inhibited at the largest red mud dose after leaching.

Many researches focused on the effects of red mud on soil and plant in three broad agricultural contexts: (1) increasing P retention of very sandy soils including the use of red mud to prevent P runoff to rivers and estuaries (Snars et al. 2003; Kullman et al. 1982; Scheffer et al. 1986; Scheffer 2000; Ward and Summers 1993; McPharlin et al. 1994; Summers et al. 1996a; Summers and Pech 1997); (2) increasing yields of horticultural crops and pastures in Western Australia with and without extra additions of P, K, etc. (Summers et al. 1996b; Robertson et al. 1999; Summers et al. 2001); and (3) liming acidic soils (Snars et al. 2004; Simons 1984; Summers et al. 1996b).

1.9 Effect of Red Mud on Phosphorous Availability

Addition of red mud simultaneously increased soil pH and decreased P availability. However, this reduction in the availability of extractable-P is not a consequence of increasing pH by adding red mud, where using NaOH to raise the

Table 1.7 Available concentrations using DTPA extraction of trace metals (mg kg^{-1}) in soil and red mud after giant reed plantation

Samples	Cd	Ni	Pb	Co	Fe
Control soil	(0.22) 0.04	(1.20) 0.45	(1.46) 0.56	(0.64) nd	(52.0) 9.8
Red mud-polluted soil	(0.18) nd	(0.84) 0.28	(1.06) 0.36	(0.22) nd	(36.0) 8.3
Red mud	(0.28) 0.06	(0.50) 0.32	(3.44) 0.51	(nd) nd	(74.0) 14.1

Values in parentheses are data before the experiment, whereas the rest represents data after experimentation
nd not detected

soil pH resulted in increase extractable-P. Accordingly, the decrease in extractable P with addition of red mud is not primarily a function of the increase in pH, where red mud contains large amounts of Fe oxides and other microcrystalline minerals that have a high capacity to adsorb P and it is likely that they play a major role in decreasing extractable P (Snars et al. 2003). An additional mechanism of P retention may be the precipitation of P compounds, particularly at the high soil pH values (up to 8.5) resulting from the high rate of red mud addition. However, there was no corresponding decrease in extractable P when NaOH was used to increase soil pH, so the existence of this mechanism remains uncertain. Snars et al. (2003) reported that drying and adding of microbial suppressant both could use to mitigate the effect of the red mud in decreasing P availability. Furthermore, drying probably has the same effect as the microbial suppressant as it kills and/or suppresses the microbes in the soil, as well as drying decreases red mud pH (Alshaal et al. 2011, 2013; Bottner 1985). Microbes can immobilize P (Mengel and Kirkby 2001), thereby decreasing available P. With drying the microbes are killed and some P may be released so that the effect of red mud addition on reducing available P is smaller. With suppression of microbial growth, the associated biological P retention does not occur, but there was still a decrease in the amount of extractable P, indicating that some of the decrease is due to chemical reactions with the red mud. Neutralization of soil acidity by applying red mud may increase the agricultural production (Summers et al. 2001), and this could partly compensate for the cost of replacement P fertilizer as will the decrease in leaching losses of fertilizer P achieved by addition of red mud. The increase in electrical conductivity (EC) due to the addition of red mud is not sufficient to adversely affect crop growth.

1.10 Phytoremediation of Red Mud by Giant Reed

Many authors have discussed the possibility of application red mud into soil as an effective and safe way for disposal red mud (Alshaal et al. 2013; Courtney and Timpson 2004; Ippolito et al. 2005). The major constraints in rehabilitation are the high alkalinity, salinity, and sodicity of red mud. Reclamation of red mud requires pretreatments to decrease the high pH and Na content; so many amendments have been suggested such as gypsum, acidic gypsum, sulphuric acid (Ippolito et al. 2005; Wong and Ho 1993), drying (Alshaal et al. 2013), calcium chloride, and leaching (Ruyters et al. 2010).

Establishing vegetation on fine fraction red mud is limited initially by elevated pH, Na content, and trace metal content (Alshaal et al. 2011; Ruyters et al. 2010). So, giant reed (*Arundo donax L.*) is a plant which has the ability to be tolerant of these abiotic stresses and can decontaminate the polluted soil and is not an edible plant. However, few studies have discussed rehabilitation of red mud by plants. Ruyters et al. (2010) have reported that the red mud reduced the shoot yield of barley seedlings by 25 % when red mud was applied to normal soil at 5 %. The biotechnologically propagated giant reed plantlets grown on pure red mud and red mud-contaminated soil did not show any symptoms of toxicity. After 3 months of pot experiment in greenhouse, the vegetative parameters of giant reed such as wet mass, plant length, and number of new buds recorded high values in pure red mud samples and red mud-polluted soils compared to control soil (Alshaal et al. 2013). On the other hand, giant reed has potential effects on the chemical properties of red mud, red mud-polluted soil. However, most of the chemical properties of red mud were influenced positively (Table 1.7). The electrical conductivity (EC) of red mud was decreased in comparison with initial values before plantation by 24.9 % for red mud, 18.1 % for control soil, and 5.9 % for red mud-polluted soil. Furthermore, pH of red mud was 9.80 before experiment and decreased to 9.69 by the end of the experiment. The most encouraging result for using giant reed as phytoremediation plant for soil health point of view was the organic carbon content (OC %) in soils after giant reed harvesting. Giant reed increased the OC content of all soils as a result of the robust growth of root system and huge plant's residues (Alshaal et al. 2013).

Many articles have reported that giant reed has the ability to uptake and accumulate huge amounts of different trace metals with different concentrations in its root and shoot tissues (Balogh et al. 2010; Papazoglou et al. 2007; Tzanakakis et al. 2009; Mirza et al. 2011). It can grow in contaminated environments without symptoms of toxicity (Table 1.8). Uptake rate depends on certain concentration of such pollutants. The trace metals' tolerance capacity for giant reed has been tested with red mud and different soils through the

Table 1.8 Vegetative parameters and contents of Fe and Ni in giant reed growing in red mud and red mud-polluted soil

				Fe (mg kg^{-1})		Ni (mg kg^{-1})	
Samples	Wet weight (g plant^{-1})	Plant length (cm)	Number of new buds per plant	Shoot	Root	Shoot	Root
Control soil	1.63	41.0	4.0	182	5,022	49	76
Red mud-polluted soil	1.87	30.3	4.8	779	11,937	46	99
Red mud	2.97	34.0	6.0	899	36,422	67	97

analysis of Cd, Co, Pb, Ni, and Fe. The first three metals were not detected in both giant reed root and shoot samples. Many reasons may explain why Cd, Co, and Pb were undetected: (1) low available concentrations in soil samples; (2) high pH of red mud, red mud-polluted soil; and (3) high phosphate content in red mud. The pH of the soil is usually the most important factor that controls metal uptake, with low pH favoring Cd accumulation, and phosphate and zinc decrease Cd uptake (Kirkham 2006). pH of red mud was 9.80 and decreased to 9.66 inducing Fe and Ni uptake by giant reed roots. The highest Fe and Ni contents in both root and shoot of giant reed were measured in red mud sample. Irrigated giant reed with trace metal aqueous solutions did not show any differences among treated and control plants, indicating the high tolerance ability of that species to Cd and Ni (Papazoglou 2007). Giant reed rapidly grows with big biomass of shoots in Cd-, As-, and Pb-contaminated soil, possessing strong metal tolerance with limited metal translocation from roots to shoots (Guo and Miao 2010). Papazoglou et al. (2007) reported that stem height and diameter, number of nodes, fresh and dry weight of leaves, and net photosynthesis were not affected, indicating that giant reed tolerates the high concentrations of Cd and Ni. Giant reed has high potential for cadmium removal in hydroponics culture; the removal rate was estimated as 0.12–0.15 mg Cd/day/plant after contact with cadmium solution of 1 mg Cd L^{-1} for 7 days (Sagehashi et al. 2011).

Three months after giant reed plantation, the available concentration of tested trace metals was decreased in soils; however, there were significant differences between treatments and soils (Table 1.7). The reduction percentage of these metals in soils and red mud was found in the following order:

Control soil: Co (100) > Fe (80.4) > Cd (78.8) > Ni (57.8) > Pb (55.3).
Mud-contaminated soil: Co (100) > Cd (83.3) > Fe (74.8) > Ni (61.9) > Pb (60.4).
Red mud: Pb (85.7) > Fe (79.1) > Cd (73.8) > Ni (25.3).

Translocation factor (TF) or mobilization ratio was calculated to determine relative translocation of metals from soil to other parts (root and shoot) of the plant species as follows: TF = (concentration of trace metals in shoots/concentration of trace metals in roots) × 100 (Gupta et al. 2008). The TF indicates the ability of the plant to translocate the metal species from roots to shoots. Ghosh and Singh (2005) reported TF = 54.3 for *Phragmites karka* under 10 mg Cd kg^{-1} soil condition for a 90-day experiment. The results suggest that Fe in giant reed under such circumstances is less mobile, whereas Ni is mobile. In the case of Fe, the highest value was 8.51 % in red mud-polluted soil, whereas it was 114.12 % for Ni. It indicates that Ni has higher mobility from root to shoot than Fe. Mirza et al. (2010a, b) reported that the highest TF value of As was 4.93 by giant reed in hydroponics cultures and all values were above the reference value (TF = 1). It could be concluded that giant reed can act as good hyper accumulator plant for trace metals. Finally, giant reed is considered one of the most promising red mud phytoremediation plants that have huge capacity to take up not only one type of metals but also different types of metals from polluted soils with significant rates.

1.11 Restoring Microbial Activity by Giant Reed

Microorganisms play a crucial role in the transformation of nutrients and energy flow. Microbial communities rapidly respond to environmental stress or ecosystem disturbance, affecting the availability of energetic compounds that support microbial population (Marinari et al., 2007). A pot experiment was conducted out in order to investigate the effect and possibility of using giant reed in microbial-depleted soil. Data showed that soil autoclaving negatively affected the microbial communities (e.g. bacterial, fungal and actinomycetes counts) growth under giant reed. However, autoclaved treatment recorded higher numbers than non-autoclaved treatment, perhaps because autoclaving allowed a large number of bacteria in autoclaved soil to grow within a less diverse community. These findings possibly demonstrate that giant reed maybe pose an especial type of exudates that can quickly improve and help a few numbers of microbes to recover within a short time. On the other hand, there is a shortage in available information about the microbial community that is supported by the root system of giant reed plants. Therefore, further studies about microbial communities that associate with giant reed root system are needed (Alshaal et al. 2013b). As mentioned above, red mud has high pH and salinity, mainly dominated by Na ion, for this reason it was expected that microbial community in red mud treatment should be lower compared to control soil but three months of giant reed cultivation in pot experiment under greenhouse conditions illustrated that giant reed increased the microbial counts in red mud sample. So, it could conclude that giant reed is a promising candidate for reclamation the marginal lands that suffer from low productivity especially within initiated seasons (Alshaal et al. 2013a).

1.12 Summary

The present chapter on *Arundo donax* L. (giant reed) is considered a pioneer report highlighting its phytoremediation potential when grown in red mud has high pH, EC, and trace metals content. The plant was capable of transferring the metals absorbed into the shoot to give higher translocation factors especially with Ni. The ability to accumulate metals in the stems and leaves above the root concentration is a positive indicator of its potential capacity to serve as a phytoremediation. The plant being not consumed by animals might serve as an effective phytoremediation plant. Giant reed has improved some soil quality indicators such pH, EC, OC, microbial counts, and soil enzyme activities. Here, we show that giant reed is one of the most promising red mud phytoremediation plants that have huge capacity to uptake not only one type of metals but also different types of metals from polluted soils with significant rates. In summary, giant reed appears to be a good candidate species to restore ecosystems in wetland and marginal soils, while at the same time generating feedstocks for the production of bioenergy.

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The Method of Dynamic Factors in Bioindication and Phytoremediation

2

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2.1 Introduction

Complicated mechanisms of the accumulation of chemical elements in plants are depicted in scientific literature for at least 60 years, whereas their practical usage in exploration geochemistry is applied for over 70 years. The chemical composition of plants (as well as of all other organisms) depends upon three main factors: their systemic position, environment (the composition of soil, rock, etc.), and individual peculiarities of the plant (first of all, of their evolutionary stage). Furthermore, separate organs of the plants (roots, trunks or stems, leaves, seeds or fruit, etc.) are distinct by the qualities of their composition with regard to chemical elements (Lietuvninkas 2012; Marozas et al. 2013).

The main factors of chemical element concentration in plants, with regard to plants, are normally subdivided into internal (physiological) and external (ecological). Among the external (ecological) factors, the concentration of chemical elements in the nutrient medium (in soil in the case of plants) and the availability of a chemical element to plants have the greatest influence. It depends upon a large number of interrelated factors: the chemical and geochemical characteristics of a chemical element, the parameters of its environment (pH, Eh, temperature), the presence or absence of geochemical barriers, climate, the geochemical characteristics of the landscape, etc. The level of bioaccumulation is governed by lighting conditions for plants. Internal (physiological) factors encompass specific biochemical barriers that are characteristic of some plants, as well as the plants' classificatory position, species, phases of vegetation, and organs (Kovalevsky 1987; Dobrovolsky 2008).

In the latest publications, plant tolerance with regard to chemical elements is associated with two directions of their perception in plants: immunity (resistance) when they are eliminated by plants or immunity (resistance) when they are assimilated and later immobilized in the cells together with the immobile forms (Prasad 2006; Shaw et al. 2006). Actually, the issue is far more complex because separate organs of plants (roots, trunks and stems, leaves, reproductive organs) under the same conditions accumulate chemical elements differently—the acro- and basipetal types of chemical element accumulation (Kovalevsky 1987; Pulford and Dickinson 2006). These aspects are not going to be discussed in this paper. We will confine to the ability of plants to accumulate a larger or smaller amount of chemical elements under certain conditions with regard to the background. This may be used both to evaluate their environmental condition (bioindication) and to rehabilitate a contaminated soil (phytoremediation).

Bioindication and phytoremediation are applied aspects of the accumulation of chemical elements in plants. Bioindication is a means of contamination indication that is most widely investigated in Lithuania and the whole world. Plants most commonly used for heavy metal bioindication are the following: Scots pine (*Pinus sylvestris* L.), silver birch (*Betula pendula*), European ash (*Fraxinus excelsior*), European mountain ash (*Sorbus aucuparia*), small-leaved lime (*Tilia cordata*), and domestic apple (*Malus domestica*) (Kupčinskienė 2011; Stravinskienė 2010, 2011; Butkus and Baltrėnaitė 2007a). Some of the most important directions of fields in Lithuania are discussed in Sect. 2.2.

Phytoremediation, when using trees to rehabilitate contaminated with heavy metals has several relevant advantages: a large biomass of trees, genetic variety, and successful forestry, converting biomass into fuel by means of anaerobic decomposition, fermentation, or thermochemical processes; positive attitude of the society towards afforestated territories; and stability of those territories when dealing with erosion caused by wind and water (Pulford and Dickinson 2006).

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To summarize, it may be stated that plant-based methods of environmental bioindication and environmental quality improvement would receive more approval from the interested parties if they assumed a clear quantitative expression of bioindication.

The aim and content of this paper is to present investigations on the application possibilities of chemical composition qualities of higher plants that have been carried out in Lithuania, as well as to introduce a new evaluation method for chemical element uptake from soil to plants and within plants, applying **the dynamic factors of bioaccumulation, biophilicity, phytoremediation, and translocation**.

2.2 Aspects of Applying the Scots Pine (*Pinus sylvestris* L.) and Other Trees for Bioindication and Phytoremediation of Metals in Lithuania

Trees are qualified as sensitive indicators of the environment condition (Cook and Kairiūkštis 1999; Stravinskienė 2010; Kupčinskienė 2011). The Scots pines (*Pinus sylvestris* L.) are most widely spread compared to their congeners. The whole territory of Lithuania accesses the habitat of the Scots pines (Navasaitis et al. 2003; Navasaitis 2008). Due to the wide distribution and usually non-barrier type of absorption of variety of contaminants, *Pinus sylvestris* L. is suitable for monitoring changes in the environment. As the academician L. Kairiūkštis (Cook and Kairiūkštis 1999) states, the dendrochronological and dendroindicational methods of environment analysis are the theoretical basis for searching the evaluative criteria for the anthropogenic effect on the forest ecosystems and growth of trees.

With increase of environmental pollution, this problem is examined more widely, and the issues of the influence of xenobiotics on organisms, the significance of contamination uptake through the food chain, and the usage of plants for engineering solutions in the environmental security (phytotechnology) are discussed; therefore such contaminants as heavy metals (i.e., remaining in the nature for a long period of time, a large part of which, according to the biochemical role, are toxic) have gained great significance. Also, a lot of attention to metals has been paid in the following international scientific programs and conferences: *COST* 859 *Phytotechnologies to Promote Sustainable Land Use and Improve Food Safety*, *COST FA* 0905 *Mineral Improved Crop Production for Healthy Food and Feed*, *SETAC*, *TRACEL*, and *ICOBTE*.

In the analysis of the uptake of heavy metals from soil into the Scots pine wood, carried out in 2001 in Lithuania, a number of more important pathways of metal (Ni, Cu, and Zn) uptake by the Scots pine have been evaluated in the Rukla–Gaižiūnai (Jonava district) and Kairiai (Klaipėda district) military grounds; they are air–pine bark; air–soil–pine wood, and soil–pine wood. Zn concentration about four times above the average has been found in wood samples from the Scots pines that grew near the shooting range. This could be explained by the fact that Zn concentration in the shooting range soil is three times higher than the average concentration in that territory. About five times higher concentration of Ni and about three times higher concentration of Cu have been found in wood samples from the Scots pine near a water body that was used for military purposes. Ni and Cu might have got into the atmosphere and precipitate on the bark of the trees as constituents of fuel; they also might have got onto the soil together with rainfall and through roots—into the wood (Butkus and Baltrėnaitė 2007a). The local increase of metal concentration in the environment is also confirmed by the fact that concentration of Cu and Ni found in the water body exceeds the highest permissible concentration for drinking water 19 and 22 times, respectively (Baltrėnas et al. 2005). In the bark samples of the Scots pine that grew in this area, concentration of Cu has been found about 1.3 times higher than the average concentration of other pines that have been analyzed (1.75 mg/kg); the concentration of Ni detected was two times higher (4.56 mg/kg) (Butkus and Baltrėnaitė 2007a). This justifies the application of the Scots pine's property to accumulate more metals in its bark in various countries, such as Portugal, Jordan, Germany, Finland, Czech Republic, and the United Kingdom (Machado et al. 2006; Schulz et al. 1999; Harju et al. 2002). This phenomenon is also investigated in Lithuania (Butkus and Baltrėnaitė 2007a; Pundytė and Baltrėnaitė 2011; Baltrėnaitė et al. 2014).

In 2005 investigations were carried out in the territory of a former forest; they took place 7 years after sewage sludge, contaminated with heavy metals, was spread on the soil and 6 years after planting the trees (*Pinus sylvestris* L.; *Betula pendula*; *Alnus glutinosa*). Air contamination with metals in that area was not detected. The main uptake of metals was most probable in the system *soil–tree*. Concentration of Cu and Pb was approximately six times higher and of Ni four times higher in the Scots pine than in the black alder. Concentration of Cu was 2.5 times, of Pb 4 times, and of Ni 3 times higher in the Scots pine than in the silver birch. Compared to control samples, the concentration of these metals in the Scots pine was about five times higher. These investigations allowed assuming that the spread of metal-contaminated sewage sludge in the soil stimulates the accumulation of metals in the seedlings during the first 6 years after planting. Concentration of Ni, Pb, and Cu in the Scots pine can moderately reach up to five times higher values compared to control trees and from two to six times compared to the silver birch and the black alder (Butkus and Baltrėnaitė 2007b; Baltrėnaitė and Butkus 2007).

Such results encourage continuing to regard trees as a potential option when phytoremediation is preferred and when seeking to eliminate or stabilize contamination. On the other hand, it has been observed that sewage sludge, contaminated with heavy metals, does not stimulate rapid increase of the wood biomass. After having evaluated the increase of the biomass in the same territory after 10 years time, the difference between the control trees and trees that grew in the soil with applied sewage sludge was not statistically significant. It has been found that the increase of the Scots pine biomass was by 87 % larger than that of the silver birch, and the accumulated Cu, Cd, and Pb concentration in the Scots pine biomass was higher than that in the silver birch by approximately 60 %. This analysis allowed inspecting two tree functional trait groups of the Scots pine and the silver birch: better nutritional qualities of the soil can be better identified by the increase of the specific root length (SRL), the reduction of the root–shoot ratio and wider root branching, whereas the effect of potential heavy metals can be better identified by a lower height of the trees, the trunk diameter, and the dry biomass (Vaitkutė et al. 2010).

Analyses of element concentration (Pb, Cu, Zn, K, and Mg) in the wood of *Pinus sylvestris* L. that have been carried out in a formerly intensive industrial territory (Panevėžys, Lietuva) have confirmed that Scots pine has the qualities to biomonitor aerogenic metals. Compared to the control site, concentration found in the wood of the Scots pine was higher: Cd ($p < 0.05$) about two times higher, Zn ($p < 0.001$) and Cu ($p < 0.05$) about 1.5 times higher, K ($p < 0.05$) 1.3 times higher, and Mg ($p < 0.05$) 1.9 times higher (Pundytė and Baltrėnaitė 2011; Pundytė et al. 2011).

Trees are affected not only by anthropogenic, but also by natural (abiotic and biotic) factors, which often become stressors. Tree diseases, as a biotic factor/stressor, as well as the interaction of the tree and the pathogen, gain relevance in the understanding of natural resistance and the biochemical aspects of this concept. *H. annosum* is known as a dangerous rot pathogen of the main roots of a tree, which further damages the conifer wood. In 1999 it had damaged approximately 1.2 thousand hectares of pine forest (Navasaitis et al. 2003). During the analysis of a damaged pine wood composition, in the tree ring of 1959–1960, the concentration of Ni and Cr was about five times higher than in other tree rings. In this tree ring, according to the micromorphological symptoms of the mycelium, the root rot pathogen's mycelium has been detected. The increase of Ni and Cr concentration in the damaged wood can be explained by the tree's resistance to the disease, when the concentration of metals increased to fight the disease pathogens (Poschenrieder et al. 2008). When the pathogen gets into the wood, it destroys its constituent part—lignin—in order to reach cellulose which is in the cell walls and which is a very important source of energy. Stress, caused by these processes, stimulates the tree's protective functions. It is probable that metals also take part in the protective reactions. It is known that Ni improves metabolism because while accumulated on the cell walls, it enhances their permeability, and Cr is necessary for the production of glucose, from which glucoside and lignin are formed. Although these data are primary, they allow suggesting that the changes in metal concentration can identify biotic and abiotic factors/stressors that affect trees (Baltrėnaitė and Butkus 2006; Vaitkutė and Baltrėnas 2011).

The effect of other contaminants on pines and other trees in Lithuania has been discussed in the following works: Stravinskienė and Dičiūnaitė (1999), Juknys et al. (2003), Juknys et al. (2006), Kupčinskienė (2011), Ozolinčius et al. (2005), Augustaitis et al. (2007a, b), Augustaitis and Bytnerowicz (2008), Butkus et al. (2008), Stravinskienė and Šimatonytė (2008), Stravinskienė and Erlickytė-Marčiukaitienė (2009), Baltrėnaitė et al. (2010), Pliopaitė Bataitienė and Butkus (2010), Stravinskienė (2010), Stravinskienė (2011), Baltrėnas and Vaitkutė (2011), Pundytė et al. (2011a, b), and Markert et al. (2012).

This paper will further concentrate on metals and their uptake and translocation in trees, although the presented evaluation method based on dynamic factors can be applied in the cases of all other chemical elements and trees.

2.3 Dynamic Factors in Evaluations of Bioindication and Phytoremediation

2.3.1 The Concept of Dynamic Factors

The fields of bioindication and phytoremediation require evaluating the processes of the absorption of chemical elements by the plants, in order to compare the plants by their capacity to absorb chemical elements and to compare the chemical elements by their possibilities to get into the plants. The changes in uptake of metals by plants, which depend upon the plant species and the genotype, are elaborately described by Prasad (1997), Brooks (1998), and Prasad and Hagemeyer (1999). However, besides the biochemical viewpoint, the biogeochemical attitude is also of great importance. The latter is concentrated on the link between plant and its environment, firstly the soil.

The interface of the soil and a plant is the main criterion when evaluating the peculiarities of the concentrative function of the plant. The basic parameters of the soil, which control metal uptake by plants, are different subject to the native rock, landscape, type of ecosystem, and soil modification, and altogether they determine the processes of metal mobility and uptake by plants. These parameters are as follows: pH; Eh; CEC; the content of clay particles and the organic material in the soil; the concentration of Fe, Mn, and Al oxides and hydroxides; the variety and abundance of microorganisms; and finally, the proportion of the mobile forms of metals which are being evaluated. The interface of the soil and a plant, or in other words, the concentration of metals in a plant with respect to the soil, is the basis for a widely used Biological Absorption Coefficient (BAC), also known as Index of Bioaccumulation (IBA), Transfer Factor (TF) (Kabata-Pendias 2010), Transfer Coefficient (TC) (Antoniadis et al. 2006), Bioaccumulation Factor (Prasad 2006), Mobility Ratio, (MR) (Mingorance et al. 2007; Baker 1981; Chamberlain 1983), Bioconcentration Factor (BCF) (Pulford and Dickinson 2006; Gál et al. 2008; Yoon et al. 2006), or Plant–Soil Coefficient[1] (Kovalevsky 1987), which is expressed by the proportion of metal concentration in a plant and soil. This ratio indicates qualities of metal absorption by plants (when the value of the ratio is more than one, plants are termed as accumulators; when the value is close to or equal to one—as indicators—when it is less than one—as excluders), the degree of metal hazard as well as risk assessment for biota. In Russia, bioaccumulation sequences of metals and other chemical elements have been created by B. Polynov and later on improved by A. Perelman (1989). Chemical elements were divided into four groups according to their uptake: *elements of vigorous uptake (P, S, Cl, Br, I), of strong uptake (Ca, K, Mg, Zn, Se, etc.), of medium uptake (Mn, Ni, Cu, Co, Pb, As, Hg, etc.) and of weak/very weak uptake (V, Cr, Sb, Cd, etc.).* Mingorance et al. (2007) used the Enrichment Factor (EF) to evaluate the accumulation of metals and other chemical elements in the analyzed soil and plant, comparing it to the control objects.

Although the abovementioned factors/coefficients express the uptake of metals into the plant in relation to the soil, they do have some disadvantages. From the biogeochemical point of view, they provide a comparison of metal concentration in different media (plant and soil), but only in a particular place (with its typical environmental conditions) or at a particular time (e.g., 10 years after the sewage sludge had spread on the soil). ***Firstly***, it would be inaccurate to compare different plants according to the abovementioned factors/coefficients in the biogeochemical respect, because they may have grown under different circumstances, in different soil and in different elementary landscape, and these facts determine the diverse qualities of metals (and other chemical elements) mobility and uptake. ***Secondly***, the necessity arises to compare not only metal concentration in the plants to metal concentration in the soil or in the control plant but also to compare the changes in the metal uptake processes and the intensity, compared to the control case. Moreover, in order to evaluate the process (in this case, the uptake of chemical elements), it is also necessary to compare the processes, not the concentration. ***Thirdly***, there is a lack of comparative proportion between the metal uptake by the plant and the control case, expressed in numbers, which would facilitate the evaluation of metal uptake. ***Fourthly***, the integration of the effect of natural processes, which have influence on the metal uptake, is very important. These goals can be achieved by using second-level factors[2]. They are calculated by comparing the value of the uptake factor in the territory of investigation to the corresponding factor value in the control territory.

We proposed to use the second-level factors to describe four types of the metal behavior in plants after soil modification. The factors are called the *dynamic factors*[3] because they are sensitive to changes of variables that are involved in calculation.

The dynamic factor of metal bioaccumulation ($\mathrm{BA_{dyn}}$) reflects the physiological sensitivity of plants to the general soil contamination degree [Eq. (2.1)]:

$$\mathrm{BA_{dyn}} = \frac{C^i_{\mathrm{T\_t}} \times C^i_{\mathrm{S\_c}}}{C^i_{\mathrm{S\_t}} \times C^i_{\mathrm{T\_c}}}, \tag{2.1}$$

where $C_{\mathrm{T\_t}}{}^{\mathrm{i}}$ concentration of metal *i* in tree wood ash on the treated site, mg/kg; $C_{\mathrm{S\_t}}{}^{\mathrm{i}}$ concentration of metal *i* in the treated soil, mg/kg DW; $C_{\mathrm{S\_c}}{}^{\mathrm{i}}$ concentration of metal *i* in the control soil, mg/kg DW; and $C_{\mathrm{T\_c}}{}^{\mathrm{i}}$ concentration of metal *i* in the control tree wood ash, mg/kg.

The dynamic factor of metal translocation reflects the changes in metal translocation from plant roots to vegetative organs [Eq. (2.2)]:

$$\mathrm{TR_{dyn}} = \frac{C^i_{\mathrm{v\_t}} \times C^i_{\mathrm{r\_c}}}{C^i_{\mathrm{r\_t}} \times C^i_{\mathrm{v\_c}}}, \tag{2.2}$$

where $C_{\mathrm{v\_t}}{}^{\mathrm{i}}$ concentration of metal *i* in tree vegetative organs on the treated site, mg/kg DW; $C_{\mathrm{r\_t}}{}^{\mathrm{i}}$ concentration of metal *i* in tree roots on the treated site, mg/kg DW; $C_{\mathrm{r\_c}}{}^{\mathrm{i}}$ concentration of metal *i* in tree roots on the control site, mg/kg DW; and $C_{\mathrm{v\_c}}{}^{\mathrm{i}}$ concentration of metal *i* in tree vegetative organs on the control site, mg/kg DW.

The dynamic factor of metal biophilicity ($\mathrm{BF_{dyn}}$) reflects changes in metal participation in plant metabolism [Eq. (2.3)]:

$$\mathrm{BF_{dyn}} = \frac{C^i_{\mathrm{T\_t}}}{C^i_{\mathrm{T\_c}}}. \tag{2.3}$$

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[1] The Plant–Soil Coefficient defines proportion of metal concentration in the plant's ashes and the soil. In the works of other researchers, the concentration of metal in the plant has been recalculated on the basis of dry biomass.

[2] There can be several levels of comparison of metal concentration in plants: (1) direct comparison of their concentration in biomass (incorrect because the concentration of respective metals in the soil that nourishes plants is not included); (2) bioconcentration (bioaccumulation) coefficients (the effect of higher or lower metal concentration in the soil on their uptake and accumulation in plants is not evaluated); (3) dynamic factors of bioaccumulation, which include the effect of soil metal concentration in control (background) and contaminated territories on their uptake and accumulation in plants.

[3] The concepts and calculations of dynamic factors correspond to Baltrėnaitė et al. (2012) of the reference list.

Generally, metal biophilicity is the ratio of metal accumulation in living biomass and metal concentration in the Earth's crust, and thus it indicates metal involvement in the metabolism on the global vegetation basis.

The dynamic factor of phytoremediation reflects changes in phytoremediation effect and phytoremediation capacities of plants [Eq. (2.4)]:

$$\mathrm{FR}_{\mathrm{dyn}} = \frac{C^{i}_{\mathrm{T\_t}} \times B_{\mathrm{t}} \times C^{i}_{\mathrm{S\_c}} \times \rho_{\mathrm{c}}}{C^{i}_{\mathrm{T\_c}} \times B_{\mathrm{c}} \times C^{i}_{\mathrm{S\_t}} \times \rho_{\mathrm{t}}}, \tag{2.4}$$

where B_t and B_c annual tree increment on treated and control sites, respectively, kg/ha; ρ_c and ρ_t soil density on treated and control sites, respectively, g/cm^3. Usually $\rho_c = \rho_t$.

If we rearranged Eqs. (2.1) and (2.4) and presumed that the soil density before and after the modification did not alter much ($\rho_c = \rho_t$), we would have an expression of the interface between the dynamic bioaccumulation factor and the dynamic bioremediation factor [Eq. (2.5)]:

$$\mathrm{FR}_{\mathrm{dyn}} = \mathrm{BA}_{\mathrm{dyn}} \times \frac{B_{\mathrm{t}}}{B_{\mathrm{c}}}. \tag{2.5}$$

Besides the biogeochemical meaning, the dynamic factors have practical advantages:

(a) They integrate four types of information—metal content between two mediums (or plant organs) and between reference and treated sites—into one value and thus make metal transfer evaluation less complicated.
(b) They are dimensionless and thus provide easy comparison.
(c) They eliminate systematic errors of analysis and thus improve the precision and quality of evaluation.

2.3.2 Dynamic Factors: Practice

Experimental analyses designed to establish the factors have been carried out in a former forest territory (Gitėnai forest Taruškos forestry, Panevėžys region, the western part of Lithuania, at E024°34′38.8″ latitude and N55°43′31.6″ longitude) where 14 years ago a part of the territory (2 ha) was modified with industrial sewage sludge. A site more than 200 m away from the modified site has been selected as the control site. A year after modification, the site was planted with Scots pines (*Pinus sylvestris* L.) and silver birches (*Betula pendula*). In natural conditions, black alders (*Alnus glutinosa*) sprout in the site. Samples of wood and soil were taken from the modified and the control sites. Six samples of each tree species were sampled (*Pinus sylvestris* L., *Betula pendula*, *Alnus glutinosa*); meanwhile in the tree growth place complex, soil samples were taken at a depth of 0–40 cm. The physical and chemical preparation of wood and soil samples has been accomplished according to the methodology depicted by Baltrėnaitė et al. (2012).

The statistical analysis of the data has been performed using *Microsoft Office Excel 2007*. The array of primary data has been evaluated according to the 3D criteria. To calculate the values of dynamic factors, the mean values of measurements were used. The dynamic factors, expressing metal uptake and translocation processes in the cases of the Scots pine, the silver birch and the black alder, are provided in Figs. 2.1, 2.2, 2.3, and 2.4. Columns reflect the values of dynamic factors that are larger than one, i.e., express increase with regard to the control site.

As it can be seen in Fig. 2.1, the $\mathrm{BA_{dyn}}$ values of the majority of metals were higher than one. Values below one were only found in the cases of Mn and Pb and only in the cases of the black alder and the silver birch (they cannot be seen in Fig. 2.1 because the *Y*-axis starts with 1). The highest value of $\mathrm{BA_{dyn}}$ was found in the Scots pine, and the highest value among metals was common for Ni in the case of the Scots pine ($\mathrm{BA_{dyn}} = 120.8$).

In the cases of almost all the metals (except Mn in the black alder), the dynamic factors of biophilicity ($\mathrm{BF_{dyn}}$) were higher than one (Fig. 2.2), i.e., the contamination of the soil with heavy metals increased their biophilicity and metal concentration in plants, compared to metal concentration in the soil. The Scots pine also was distinct for its highest values of $\mathrm{BF_{dyn}}$ for all analyzed metals.

Opposite tendencies emerged regarding the dynamic translocation factor ($\mathrm{TR_{dyn}}$): only the values of $\mathrm{TR_{dyn}}$ for Mn and Ni and only with regard to the black alder were higher than one (Fig. 2.3). In the cases of other metals, this factor indicated a decreased metal translocation in the contaminated site, compared to the control site.

The individual qualities of analyzed trees in respect of phytoremediation are reflected by the dynamic phytoremediation factor ($\mathrm{FR_{dyn}}$): it was the highest in the case of the Scots pine and especially pronounced with regard to Pb (Fig. 2.4).

2.3.3 Advantages of Dynamic Factors

2.3.3.1 Dynamic Factors Allow Comparing the Changes in the Process of Metal Uptake by Different Plants (Trees), by Evaluating the Geochemical Features of the Analyzed Area

By using dynamic factors, not only plants can be compared on the level of their capacity to uptake metals, but also the changes in the process of metal uptake can be evaluated after soil modification, comparing the results to the control site, which must be identical in other geochemical respects, except the fact of modification.

In our investigation, after soil modification with sewage sludge, the Scots pine was distinct from the three tree species of investigation. Compared to the control site, the metal uptake became more intense in the Scots pine than in the silver birch or in the black alder. This was indicated by higher values of

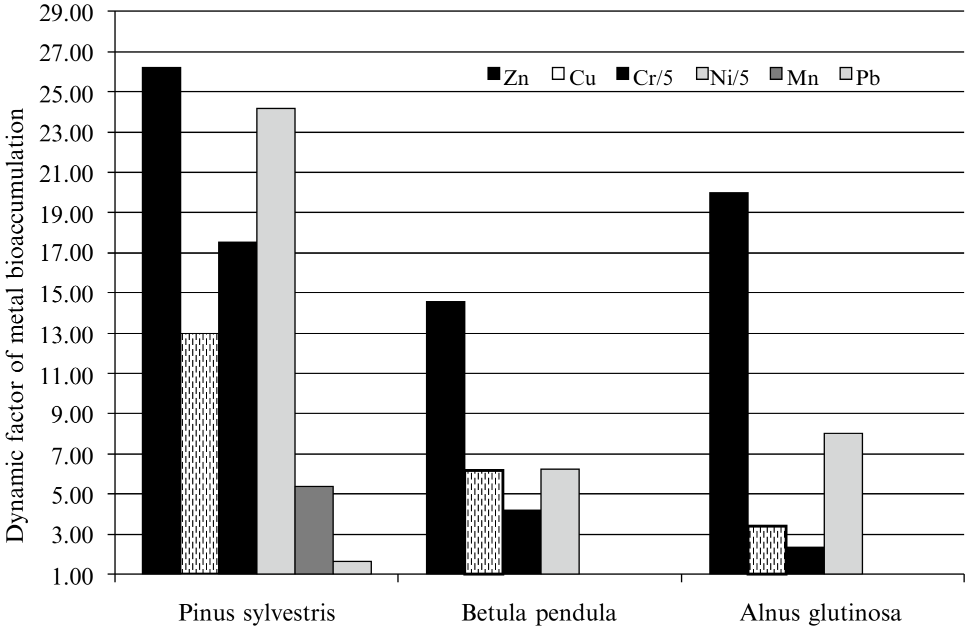

Fig. 2.1 Dynamic factors of metal bioaccumulation (BA_{dyn}). In the case of Cr and Ni, the factor values were reduced 5 times so that they could be compared to other metals

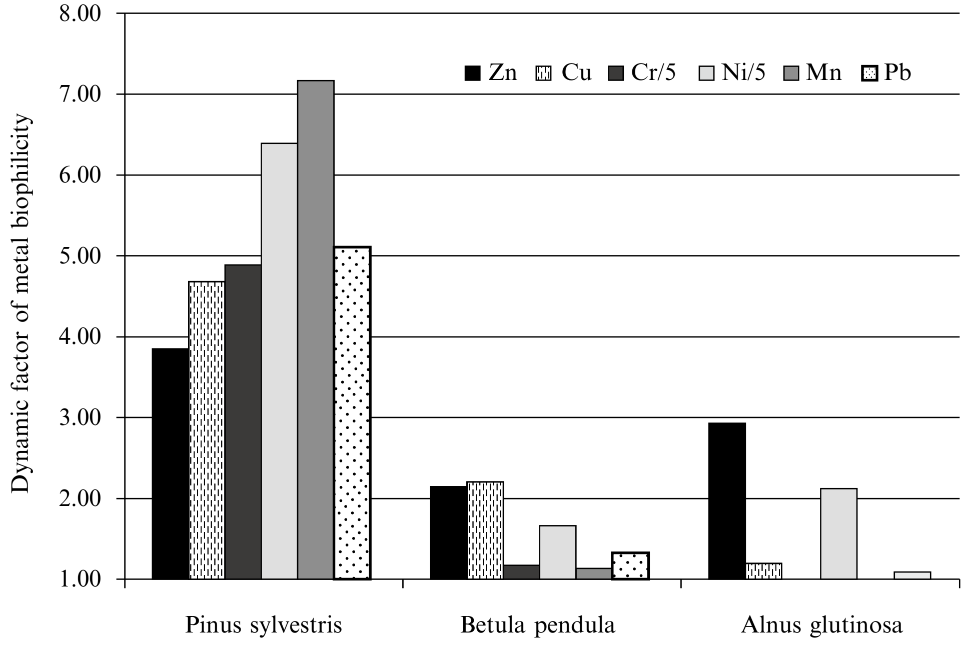

Fig. 2.2 Dynamic factors of metal biophilicity (BF_{dyn}). In the case of Cr and Ni, the factor values were reduced 5 times in order to make them comparable to other metals

BA_{dyn}, BF_{dyn}, and FR_{dyn}, which allow suggesting that on the one hand, the Scots pine accumulated higher amounts of metals and the accumulation was more intense when the soil contamination with metals increased. The higher accumulation common for the Scots pine was confirmed by Kovalevsky (1987) who studied the mechanisms of metal uptake by trees in non-contaminated areas. He stated that in respect of Pb and Cu, a non-barrier type of accumulation mechanism is common for the Scots pine. On the other hand, a more intense metal accumulation in the Scots pine can indicate its weakened protective functions and weaker immunity from environment's pollution, compared to the silver birch and the black alder. The weakened photosynthesis intensity of the Scots pine and its decreased immunity from the effect of contaminated air of its

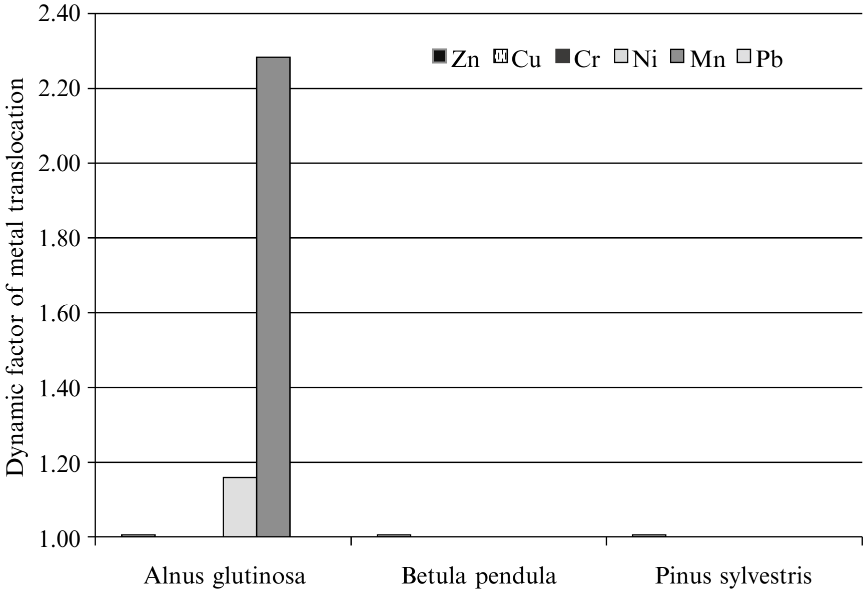

Fig. 2.3 Dynamic factors of metal translocation (TR_{dyn})

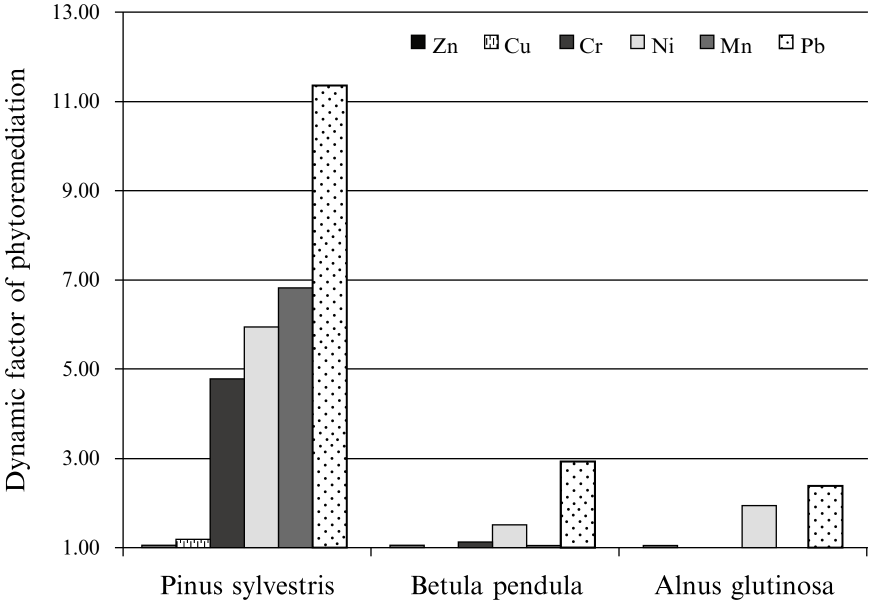

Fig. 2.4 Dynamic factors of phytoremediation (FR_{dyn})

environment, compared to the silver birch, has been established by Neverova and Jagodkina (2010). Thus, coniferous trees (as the example of the Scots pine showed) could be more affected by metal load than deciduous trees.

The TR_{dyn} value found in the black alder was higher (except for Pb with regard to the silver birch and Cr) than in other trees, which suggest that in a contaminated site the uptake of metals from the roots into the vegetative organs of the black alder was more intense. This does not oppose the fact that the black alder has a higher intensity of transpiration flow (2.59) than the silver birch (2.41) and the Scots pine (0.6) (where that of larch is equal to one) (according to L. Ivanov) (Milburn 1979; Zimmermann and Milburn 1982; Navasaitis 2008), which determines a faster transpiration flow uptake with metals dissolved in it, from the roots towards the vegetative organs.

2.3.3.2 Dynamic Factors Provide the Possibility to Compare the Influence of Soil Modification on the Participation of Chemical Elements (Metals as Well) in the Metabolism of Plants (Trees as Well)

Anthropogenic activity is also a stress to the vegetation, and heavy metals are classified as a stress factor (Kupčinskienė 2011); during the activity of which, the plants' metabolism alters, and other phenomena appear that are not common for the normal state of the plant. Soil modification with sewage sludge is also considered a stressor, and the participation of analyzed metals in the tree's metabolism is reflected in the dynamic factors of biophilicity and bioaccumulation, as well as in the altered sequence of metal biophilicity. Soil modification increased Ni and Cr biophilicity in the analyzed trees because compared to the tree's biophilicity sequence in the world's vegetation biomass—$\mathbf{Zn_{19.6}}$ $Cu_{9.1}$ $Mn_{6.9}$ $Pb_{3.7}$ $Ni_{1.5}$ $\mathbf{Cr_{1.0}}$ (Dobrovolsky 2008)—in our case Ni and Cr "moved" from the end to the beginning of the sequence.

Pinus sylvestris L.—$\mathbf{Ni_{31.94}}$ $\mathbf{Cr_{24.41}}$ $\mathbf{Mn_{7.16}}$ $\mathbf{Pb_{5.11}}$ $\mathbf{Cu_{4.67}}$ $\mathbf{Zn_{3.85}}$
Betula pendula—$\mathbf{Ni_{8.32}}$ $\mathbf{Cr_{5.88}}$ $\mathbf{Cu_{2.20}}$ $\mathbf{Zn_{2.14}}$ $\mathbf{Pb_{1.33}}$ $\mathbf{Mn_{1.13}}$
Alnus glutinosa—$\mathbf{Ni_{10.61}}$ $\mathbf{Cr_{3.27}}$ $\mathbf{Zn_{2.93}}$ $\mathbf{Cu_{1.19}}$ $\mathbf{Pb_{1.09}}$ $Mn_{0.84}$

Furthermore, in all the cases of analyzed trees, the BA_{dyn} and BF_{dyn} values of Ni and Cr were higher than one. These metals are known for their active participation in processes when a tree is affected by stressors. Jhee et al. (2005) found that Ni stimulates metabolism and enhances the permeability of cell walls, meanwhile Cr is an important stimulator of the lignification process that has been affected by stressors. Enlarged concentration of Ni and Cr was found in the Scots pine wood that has been affected by biotic factors (Baltrėnaitė and Butkus 2006).

2.3.3.3 Dynamic Factor of Phytoremediation Concretizes the Evaluation of Phytoremediation Efficiency

FR_{dyn} helps to decide which plant is more appropriate for phytoremediation. The results of our research revealed that the Scots pine was distinct for its higher metal accumulation and its elimination from the modified soil in the growth area: in 1 year it eliminated from 0.07–0.15 % (Cu, Pb, Ni, Zn) to 0.23–0.29 % (Cr, Mn) of the metals accumulated in a 40 cm soil surface layer. Meanwhile the silver birch rehabilitated the modified soil by 0.04–0.07 % (Cr, Cu, Pb and Ni) and 0.2–0.3 % (Zn, Mn), and the black alder by 0.01–0.04 % (Cr, Mn, Cu, Pb and Ni) and 0.1 % (Zn)%, compared to the control trees. Compared to the control site, the Scots pine accumulated more intensely and thus eliminated ($FR_{dyn} > 1$) Ni, Mn, Cu, Cr, and Pb from the modified soil; the silver birch Pb, Ni, and Cr; and the black alder Pb and Ni.

2.4 Conclusions

1. Bioindication and phytoremediation are applied aspects of the uptake of chemical elements from soil to plants. For bioindication and phytoremediation purposes, more and more graminaceous and ligneous plants are being evaluated, and more different media (soil–plant, plant–air) or the interfaces of different organs of the same organism (roots–vegetative organs) are analyzed. If the plant-based methods of improving environment observation gained a quantitative expression, they would receive more approval from the parties interested.
2. Dynamic indicators of bioaccumulation, biophilicity, translocation, and phytoremediation allow comparing changes in the processes of metal uptake in different plants (trees), by evaluating the geochemical features of the area of interest; they help to evaluate the influence of soil modification on the participation of chemical elements (metals as well) in the metabolism of plants (trees as well); they allow a quantitative evaluation of phytoremediation efficiency during a specific period of time. Dynamic factors integrate the internal (physiological) and external (ecological) factors.
3. In the case with trees, dynamic factors revealed several relevant tendencies: (1) *the dynamic factor of bioaccumulation* showed that after soil modification with sewage sludge, the metal uptake by trees became more intense in the following sequence: black alder < silver birch < Scots pine, while the Scots pine had the highest metal accumulation; (2) *the dynamic factor of biophilicity* highlighted the tendency that soil contamination may result in the metal biophilicity shift, e.g., the biophilicity of Ni and Cr increased significantly in respect of other metals within the sequence of generic biophilicity of the world vegetation; (3) by means of *the dynamic factor of translocation*, it was found that in the black alder the metal uptake from the roots to the vegetative organs increased more than in the silver birch or the Scots pine, due to the soil modification; (4) *the dynamic factor of phytoremediation* quantitatively estimated that the Scots pine, in respect of analyzed metals, is a better soil phytoremediator than the silver birch or the black alder.

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3 The Bioavailability Processes as a Key to Evaluate Phytoremediation Efficiency

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3.1 Introduction

Heavy metal soil pollution has been increasing since the beginning of the Industrial Revolution. As metals are not biodegradable, they tend to persist and accumulate in soils; however, the risks to man and the environment strictly depend on their bioavailability. Bioavailability can be defined as the fraction of the total amount of a contaminant in the soil that is available or can become available for uptake by organisms in a given time span (Peijnenburg and Jager 2003; van Gestel 2008). Bioavailable contaminants have a negative effect in an organism only if absorbed, being the pathways of exposure and physiological characteristics differ depending on the organism.

Bioavailability concept is essential in the relationship between soil and plants since plants are able to uptake the substances only if present in available forms in the soil environment. This, in turn, highlights the importance of bioavailability for the phytoremediation strategy used to clean up sites contaminated by heavy metals. The initial idea of phytoremediation originated from studies on hyperaccumulator plants (Brooks et al. 1977), which can uptake and tolerate extremely high levels of heavy metals. As later stated by Brooks in 1998 "a small perennial shrub in Tuscany, Italy, was destined to lead the way to a whole range of new technologies and discoveries …". Much time has passed since the appearance of the article (Minguzzi and Vergnano 1948), which described a plant that could accumulate very high amounts of nickel. This research gave rise to the remediation technology that has since evolved by exploiting the multiple properties of plants. Today the term phytoremediation broadly defines a series of plant-based technologies to remediate or contain contaminants in soil, water and sediments (ITRC 2009). In the case of inorganic contaminants, phytoremediation uses two main strategies: phytoextraction and phytostabilization. Phytoextraction involves firstly the cultivation of plant species that are suited to contaminated soil and are able to uptake the metals present. Secondly, the vegetable biomass, enriched in metals, has to be removed. Finally, the resulting biomass has to be disposed of or reused. Phytostabilization regards the ability of roots to immobilize the contaminants in the root zone while stabilizing the soil, thus reducing metal leaching and aerial dispersion of contaminated soil particles.

Heavy metal phytoextraction is very attractive since it enables the use of a biological technique to remove non-biodegradable contaminants from a contaminated site.

Phytoextraction has essentially followed two strategies: continuous or natural phytoextraction, in which hyperaccumulator species are involved (Ghaderian et al. 2007), and assisted phytoextraction, in which additives are used to mobilize the metals in soil solution, thus increasing the bioavailable amounts of metals to plants.

Both strategies depend on biomass production and the amount of metal uptaken by plants. These variables are in turn determined by the plant's ability to grow in contaminated soils as well as bioavailability of heavy metal. The efficiency of phytoextraction thus strictly depends on the soil's properties. These properties determine the growing conditions and regulate the distribution of contaminants in the soil liquid phase, since plants uptake metals only if they are present in the soil solution.

Soil characteristics are often not fully considered in the technology evaluation; however, the ability of the same plants to uptake metals is quite different in soils with different properties that determine metal bioavailability. The total heavy metal concentration in soil is a parameter of a relatively low importance in the phytoextraction mechanism. Contaminants present in soil solution are generally the only readily bioavailable, and these must be primarily considered to evaluate the feasibility of phytoextraction. When the available metal pool is exhausted, for example, for absorption by plants, only a certain amount is

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replenished from those soil surfaces where the metals are associated with the weakest bonds, and the efficiency of the technology is progressively reduced (Shelmerdine et al. 2009).

3.2 Soil Properties and Metals Bioavailability

In environmental studies, bioavailability should take into account very different processes ranging from the release of a contaminant from the solid phase of an environmental matrix to the absorption by a living organism. These aspects are interrelated, and the complexity of interactions between an organism and the environment defines the processes of bioavailability (NRC, National Research Council 2002). The environmental behaviour and toxicity of a contaminant can only be evaluated in terms of its molecular form. Bioavailability is the key to understanding the risks from pollution and to defining remediation strategies, since organisms only respond to the fraction that is biologically available (bioavailable). In soil, the bioavailable fractions of contaminants are dependent on soil properties and processes, which vary with time. Due to ageing, the binding of a contaminant to the soil can become stronger and the effects on the environment consequently smaller. On the other hand, contaminants may become more available by natural or anthropogenic change of soil properties (e.g. pH).

In terms of heavy metals, the path from unavailable to available forms is regulated by chemical, physical and biological conditions which determine the transfer of metals from the solid to the solution phase of soils. After a contaminant has reached the soil, it interacts with the surfaces of the solid phase through a series of reactions of adsorption–desorption and precipitation–dissolution which determine the amount of contaminant in the liquid phase.

Once the chemical has been released into the liquid phase of the soil, it is transported with the soil solution and fills the macropores network. During transport, the contaminant may interact again with the soil surfaces with specific and non-specific adsorption reactions. Following ageing, the chemicals that were initially adsorbed on the surface are slowly redistributed within aggregates of soil even in pores of subatomic dimensions.

These reactions play an important role in determining a contaminant's mobility and bioavailability for plants which is strictly related to the chemical characteristics of the contaminants and above all to the characteristics of the soil such as pH (Li et al. 2003; Chaney et al. 2005), organic matter (Wanga et al. 2010), clay content (Abdullah and Sarem 2010), cation exchange capacity and redox potential (Cherlatchka and Cambier 2000).

Table 3.1 reports the effects of the main soil properties on bioavailability.

Other factors influence the mobility of metals in soils and phytoextraction efficiency. Temperature can modify the mobility of organometal complexes and their absorption by plants. Ionic strength can reduce the sorption of heavy metals by soil surfaces due to the increased competition from alkaline metals (Petruzzelli and Pezzarossa 2003).

Heavy metals are essential elements for plant and animal life but become toxic at high concentrations, and living organisms must be able to adjust their concentration at the cellular level. Consequently, living organisms have developed transport systems to adjust the absorption and distribution of the various metals. Plants have a high metabolic capacity, which make them able to selectively uptake the heavy metals from the soil. Plants have also developed a natural genetic diversity to survive in soils with high concentrations of toxic metals and other contaminants. Metals are absorbed by plants mainly through the roots, where the majority of mechanisms that prevent the toxicity of metals are located. The root system provides a very high surface area, able to absorb and accumulate the water and nutrients needed for growth, together with other elements such as heavy metals.

Metals move from the soil towards the roots essentially through the processes of mass flow driven by plant transpiration and diffusion along a concentration gradient determined by the plant's uptake.

The roots of plants are able to change the chemical and physical properties in the adjacent soil, in particular at the interface between the soil and the roots (rhizosphere) where organic and inorganic compounds (root exudates) are released. The composition and amount of exudates vary depending on species, plant physiological status, environmental conditions (soil, climate) and the architecture of the roots. There are many compounds released: inorganic ions (H^+, OH^- etc.), gas (CO_2, O_2), soluble organic acids, phenols, amino acids, sugars and particulate organic matter (mucilage, residues cell) (Dakora and Phillips 2002). The root exudates influence the number and extent of the microorganisms, the aggregation and stability of the particles of soil around the roots and the bioavailability of the elements.

The action of the root exudates can then directly or indirectly increase or decrease the availability of the metal elements in the rhizosphere through mobilization or immobilization processes. These are caused mainly due to changes in pH and in the redox potential, release of a chelating agent that complexes the metals and an increase in microbial activity (Fitz and Wenzel 2002). In the rhizosphere, the root exudates are also an important source of the carbon required for the development of bacteria and fungi (Badri and Vivanco 2009; Kozdroj and van Elsas 2000). The interactions between plants and microorganisms promote the growth of plants and their resistance to biotic and abiotic stresses (Gray and Smith 2005).

In whatever environmental conditions, metals to be uptaken by plants must be present in soil solutions in soluble forms (Panich-Pat et al. 2004). This means that bioavailability is the key to evaluating the feasibility of phytoextraction as a remediation technology. One of the main difficulties in the practical application of bioavailability in remediation stems from the lack of a general consensus on which methodology

Table 3.1 Effect of soil characteristics on bioavailability

Parameter	Effect on bioavailability
pH	– Affects the concentrations of metals in soil solutions by regulating precipitation–dissolution, specific adsorption and complexation processes – Regulates metal hydrolysis: beyond a threshold pH (specific for each metal), these reactions drastically reduce the concentration of most metal ions in the soil pore water – At low pH levels, sorption processes are reduced due to the acid-catalyzed dissolution of oxides and their sorption sites, whereas complexation by organic matter tends to decrease with increasing acidity
Clay content	Ion exchange and specific adsorption are the mechanisms by which clay minerals adsorb metal ions from the soil liquid phase. This is done through the adsorption of hydroxyl ions followed by the attachment of the metal ion to the clay by linking it to the adsorbed hydroxyl ions or directly to sites created by proton removal. Highly selective sorption occurs at the mineral edges. Notable differences exist between clay minerals in terms of their ability to retain heavy metals
Organic matter	– The organic matter of soils has a great influence on metal mobility and bioavailability due to the tendency of metals to bind with humic compounds in both the solid and solution phases in soil – The formation of soluble complexes with organic matter, in particular the fulvic fraction, is responsible for increasing the metal content of soil solutions. However, higher molecular weight humic acids can greatly reduce heavy metal bioavailability due to the strength of the linkages
CEC	– The negative surface charges may be pH dependent or permanent. To maintain electro-neutrality, they are balanced reversibly by equal amounts of cations from the soil solution – Weak electrostatic bonds link cations to soil surfaces, and heavy metals can easily substitute alkaline cations on these surfaces by exchange reactions – Specific adsorption promotes the retention of heavy metals, also by partially covalent bonds. This can drastically reduce the possibility of plants absorbing inorganic contaminants
Redox potential	– Reduction–oxidation reactions in soils are controlled by redox potential (Eh). High levels of Eh are encountered in dry, well-aerated soils, while soils with a high content of organic matter or subject to waterlogging tend to have low Eh values – Plant-induced reductions of the redox potential and low Eh values can promote the solubility of some metals such as arsenic, thus increasing metal phytoextraction. This can be ascribed to the dissolution of Fe–Mn oxyhydroxides under reducing conditions, thus resulting in the release of adsorbed metals. Under anaerobic conditions, the solubility of heavy metals could decrease when sulphides are formed from sulphates thus diminishing plant uptake
Oxides/hydroxides	– Hydrous Fe and Mn oxides are particularly effective in influencing metal solubility in relatively oxidizing conditions. They reduce metal concentrations in soil solutions by both specific adsorption reactions and precipitation – Under reduced conditions the dissolution of Fe and Mn oxides/hydroxides can release adsorbed elements such as arsenic, and phytoextraction is promoted

should be used to measure the bioavailability. To overcome this obstacle rather than searching for a universal method that is valid in all conditions, it is essential to apply more site-specific tests to assess bioavailability. In phytoextraction, the processes that determine the metal bioavailability are the release from the solid phase into the soil solution and the uptake of the element in soluble forms by the root system of the plants. This means that phytoextraction feasibility tests need to consider both a chemically driven process, i.e. the release of the metal from soil surfaces, and a physiologically driven process, i.e. the uptake by plants. Soil characteristics and plant traits determine bioavailability and phytoextraction efficiency. The bioavailability of metals in contaminated soils should be evaluated using chemical extraction and a bioassay test.

Chemically, the amount of metals in the liquid phase of the soil and/or that can be easily released from the solid phase (metals retained with electrostatic bonds) can be determined using both direct sampling of soil pore water or bland extractants, such as water or dilute solutions of alkali metals that do not modify the surfaces of the soil. Extraction with more strong agents, commonly used in soil chemistry, may be useful in "assisted phytoextraction" since their action is much more aggressive than that of plants.

The reagents to evaluate the potential bioavailability should be selected according to the specific site conditions. The same reagent may not produce useful information in different soils with different sources of contamination. However, a chemical extractant cannot provide a good estimate of metal uptake for different plant species.

The chemical test must be accompanied by a biological test, with plant species that are grown in the real polluted soil under controlled conditions. The growth of plants in contaminated soil is essential when deciding which type of technology to use, because it allows to define the actual retention of metals from the surface of the soil, often increased by "ageing". At the end of the growth period, an analysis of the metal content in plants will provide information on the bioavailable fraction.

Chemical and biological tests alone do not define the bioavailability, but they do provide information on the contaminant bioavailable fractions (Petruzzelli and Pedron 2006).

According to Harmsen (2007),

$$\text{bioavailability} = f_1\left(\text{bioavailability}\,\text{bio}\right) \times f_2\left(\text{bioavailability}\,\text{chem}\right)$$

where f_1 and f_2 are mathematical functions.

Chemical and biological tests can be further combined with toxicity tests and measurements of other plant parameters, such as seedling emergence, survival, root length, shoot height and root and shoot mass (Almansoory et al. 2013; Lucas García et al. 2013).

3.3 Bioavailability at Contaminated Sites

Knowledge of the bioavailability processes can be used at various stages in the selection and application of remediation strategies. In recent years, there has been a development of remediation technologies that use what has been learn about the bioavailability of contaminants in the soil. Two different strategies can be used either to reduce or increase bioavailability.

Techniques that reduce bioavailability prevent the movement of pollutants from the soil to living organisms by:

1. Removing the labile phase of the contaminant, i.e. the fraction that is more dangerous for human and environment
2. Converting the labile fraction into a stable fraction (e.g. the precipitation of metals)
3. Increasing resistance to the mass transfer of contaminants

There are also other procedures which have the aim to increase the bioavailability of pollutants that can be used in the frame of technologies that remove or destroy the solubilized contaminants.

These procedures are based on:

1. Sieving to increase the mass transfer from absorbed phases
2. Increasing the temperature
3. Using chemical additives to mobilize the contaminants

In biological techniques such as phytoremediation, an understanding of the bioavailability of the pollutants plays a primary role in assessing the applicability and efficiency of a technology.

Full-scale applications have revealed that phytoextraction is severely limited by the long time required to achieve remediation goals due to the growing cycles of plants. To increase the efficiency of phytoextraction, it is essential to promote metal bioavailability in soils. This can be achieved with amendments, such as chelating agents, which promote the desorption of metals from the solid phase thus increasing their concentration in the soil solution where are uptaken by plants. This procedure is the basis of assisted phytoextraction.

Numerous amendments have been used and several promising results have been obtained due to the increase in metal solubility, particularly in the lab or greenhouse. Organic acids with a low molecular weight, such as ethylenediaminetetraacetic acid (EDTA), hydroxyethyl ethylenediamine triacetic acid (HEDTA) and diethylenetriamine pentaacetic acid (DTPA), were among the first additives to be used. EDTA has been repeatedly used since it can complex many heavy metals (Gupta et al. 2008; Wu et al. 2004; Seth et al. 2011; Pedron et al. 2010). Although these ligands generally increase the transfer of metals from the soil to the roots, their high mobilizing capacity, together with their long persistence in the soil, may increase the concentration of the metal in the soil solution, thus exceeding the bioavailable quantity that the plants are able to assume (Luo et al. 2005; Santos et al. 2006; Cao et al. 2007).

An evaluation of the complexing agent must also take account of the possible side reactions between the ligand and other chemical species in the soil solution.

In fact, the efficiency of the treatment also depends on the kind of soil, since other ions, such as Ca and Fe, interact with the chelating agent and can be co-solubilized. This can reduce the mobilization of metal contaminants; thus, the amount of chelating agent to be used should be more than that theoretically required (Nowack et al. 2006).

For example, although the Ca-EDTA complex has a much lower constant of complexation than that EDTA-Pb, the high solubility of Ca together with its high concentration in soil makes this an important competing cation when phytoextraction is carried out in soils with neutral or basic pH values. Other complexing agents, for example, ethylenediamine-N,*N′*-disuccinic acid (EDDS), have a lower Pb complexing ability than EDTA. However, the low stability of the complex Ca-EDDS does not reduce the amount of Pb mobilized and bioavailable to plants.

The use of chelating agents that are not easily biodegradable has been criticized for the potential residual toxicity (Evangelou et al. 2008). To avoid the effects of toxicity, highly biodegradable organic acids of a low molecular weight have been used such as citric, oxalic and tartaric acids, all of which have a much lower toxicity (Wu et al. 2004; Evangelou et al. 2006; Doumett et al. 2008, 2011).

Fertilizers have also been used as additives (Giansoldati et al. 2012). Very positive results were obtained with the use of phosphate and thiosulphate in soils contaminated by arsenic and mercury, respectively (Tassi et al. 2004; Moreno et al. 2005; Pedron et al. 2011, 2013). Physical characteristics of soils at contaminated sites are often very poor and it is difficult for microorganisms and soil fauna to live in these environments. Moreover root growth is hindered due to a scarce nutrient supply. Bioavailability and the uptake of heavy metals can be modified by the use of plant microbial consortia, which change the rhizosphere environment by modifying pH, redox conditions and the chemical speciation of metals (Vetterlein et al. 2007; Wenzel 2009; Lin et al. 2010). Foliar treatment with phytohormones, such as cytokinin, has also increased the phytoextraction efficiency of crop plants in mercury-contaminated soil, through the increase in plant biomass and evapotranspiration (Barbafieri and Tassi 2010; Cassina et al. 2012).

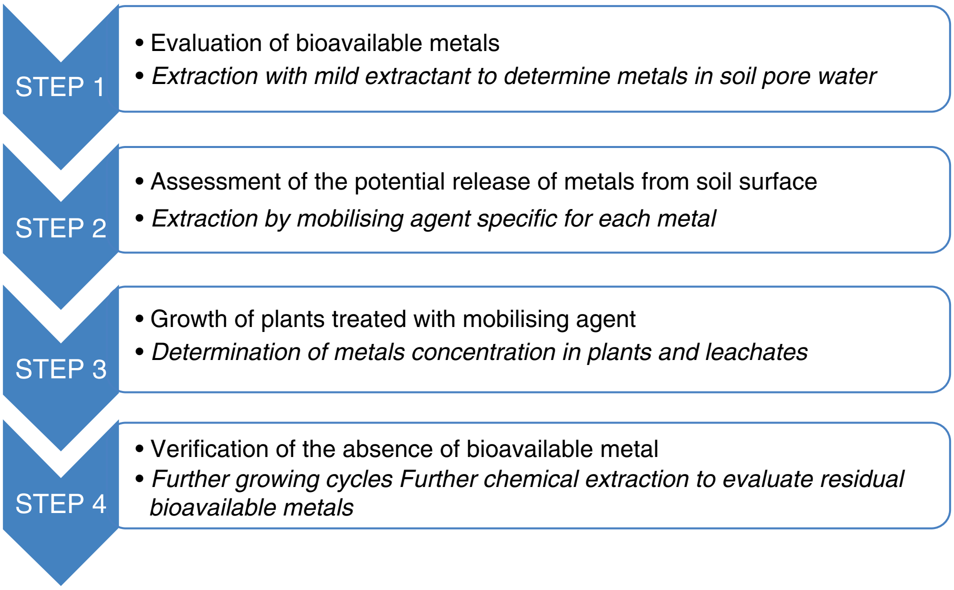


Fig. 3.1 EBCS scheme

Using phytoextraction, the amounts of metals that can be removed from soils is generally no more than 1–2 % of the total concentration in the soil, since plants only act on the contaminants present in the soil solution. The technology has also been constrained by the expectation that site cleanup should be achieved in a comparable time to other remediation technologies (Robinson et al. 2003; Koopmans et al. 2007). Positive results were obtained with the genetic modification of high biomass plant species which increased the accumulation of metals and the resistance to adverse environmental conditions (Bizily et al. 2000; Meagher and Heaton 2005; Hussein et al. 2007). In those countries where genetic engineering is only permitted on a limited scale, the remediation target for phytoextraction should be limited to remove the fraction (bioavailable) that is the most hazardous to the environment and human health (Fitz et al. 2003; Wenzel 2009). This remediation strategy derives from the intrinsic properties of phytoextraction which is strictly related to heavy metal bioavailability. This means that phytoextraction can only be used to decrease bioavailable metals, in this way the clean up time can also be substantially shortened. This approach was defined as "bioavailable contaminant stripping" and was introduced by Hamon and McLaughlin (1999) and a recently revised and implemented as "enhanced bioavailable contaminant stripping" which also takes into account the ability of soil to resupply metals from less available pools (Lehto et al. 2006; Petruzzelli et al. 2011, 2012; Pedron et al. 2013).

The EBCS approach (Fig. 3.1) is a four-step procedure to use in phytoextraction to remove bioavailable metals from soil:

1. Identification of metals in bioavailable forms (soluble or easily solubilizable).
2. Determination of long-term metal releasable from soil surfaces. This step is performed by one or more extractions with a metal-specific mobilizing agents. Since the extractive capacity of the specific agent is much greater than any natural process, the amount extractable can be considered (on a precautionary approach) as the maximum available to plants.
3. Pot experiments with addition of the same mobilizing agent, by growing plant species able to uptake available fractions. Control of the possible presence of metals in the leachates from pot trials.
4. After harvesting, further cycles of plant growth are performed on the same soils, with and without the addition of the specific mobilizing agent, to evaluate any residual metals in bioavailable forms. At the same time further extractions with the specific additive are carried out on soil to verify the absence of extractable metals. When the metal concentration in plants is negligible and no amount of metal can be extracted from the soil by the specific mobilizing agent, the residual metal fractions in soil can be safely considered to be permanently unavailable.

EBCS belongs to "Green Remediation" strategies, a complete new approach to remediation that takes into account environmental impacts of remediation activities, to maximize the environmental benefits of cleanup procedures (USEPA 2008). An essential feature of Green Remediation is the aim to minimize bioavailability of contaminants using wherever possible low invasive technologies such as phytoremediation. This strategy has been encouraged by new legislation which no longer defines pollution on the basis of target concentrations but according to a site-specific risk analysis. The inclusion of the concept of bioavailability in risk analysis would be an important choice for the management of contaminated sites. The acceptance of this option will strictly depend on the capability of the scientific community to clearly communicate the results obtainable to the stakeholders and legislators.

3.4 Case Studies

Two case studies show how it is possible to increase the efficiency of phytoextraction by manipulating the bioavailability. In the first case, the addition of various additives to a very acid soil reduced the toxic effects arising from a too high bioavailability of the metals, thus enabling the plants to grow. In the second case, the addition of a single fertilizer simultaneously increased the bioavailability of arsenic and mercury, thus promoting a greater plant uptake.

3.4.1 Case Study 1: Decreasing Bioavailability in a Very Acidic Soil Contaminated by Different Metals

The site is located in an agricultural area where residues from stainless steel production had been discharged for more than 30 years (Pedron et al. 2009). The soil contamination derived from high levels of Cu (1,140 mg kg^{-1}), Zn (235 mg kg^{-1}) and Ni (141 mg kg^{-1}).

In this soil (Table 3.2), due to the very acidic pH (4.8), an excess of metal bioavailability promoted a very high uptake of heavy metals thus inducing phytotoxicity in plants, leading to a strong reduction in growth and biomass production. In these conditions phytoextraction was not practicable.

To overcome phytotoxicity, agents were used that are able to reduce metal bioavailability.

3.4.1.1 Experimental Procedure

Soil characterization was performed including, in addition to total concentration, an evaluation of heavy metal bioavailability. A sequential extraction procedure (SEP) with H_2O, 1 M KNO_3 and 0.01 M EDTA was performed in original and treated soils in order to assess changes in potential heavy metal bioavailability.

A feasibility test at a microcosm scale was carried out using three different treatments to decrease metal solubility. The materials used were zeolites (10 % w/w with respect to soil weight, Z-Soil); compost (10 % w/w with respect to soil weight, C-Soil); and $Ca(OH)_2$ to increase soil pH value to 6.2 (Ca-Soil).

Table 3.2 Soil characteristics

pH	CEC (cmol kg^{-1})	Sand (%)	Silt (%)	Clay (%)
4.8	26.4	47.4	46.0	6.6

Microcosm experiments were carried out by sowing three plant species—*Brassica juncea*, *Poa annua* and *Helianthus annuus*—in 200 g of soil using approximately 0.20 g and 0.10 g of seeds per microcosm for *B. juncea* and *P. annua* and four seeds for *H. annuus*, respectively. The original soil (O-Soil) without any additions was used as a control.

Five replicates of vegetated microcosms for each kind of soil were prepared and watered daily with tap water. Experiments lasted 45 days. At the end of the growing period, the plants were harvested and the aerial parts were separated from the roots. The dry mass of the plants was gravimetrically determined, and shoots and roots were analyzed for heavy metal content. After an acid–oxidant digestion (HNO_3/$HClO_4$ mixture 2.5:1 ratio), using a microwave system "ETHOS-900" (MILESTONE) with a pulsed-mode emission in Teflon vials, plant samples were analyzed by flame AAS (Varian AA 240FS).

3.5 Results

Figure 3.2 reports the data from the adopted SEP.

The highest water extractability was found in the original contaminated soil.

Treatments reduced the amount of water extractable metals, with the lowest efficiency with the zeolite treatment.

The second step in the sequential extraction (KNO_3) provides an evaluation of the "exchangeable" metal retained by soil surfaces with low-energy electrostatic linkages.

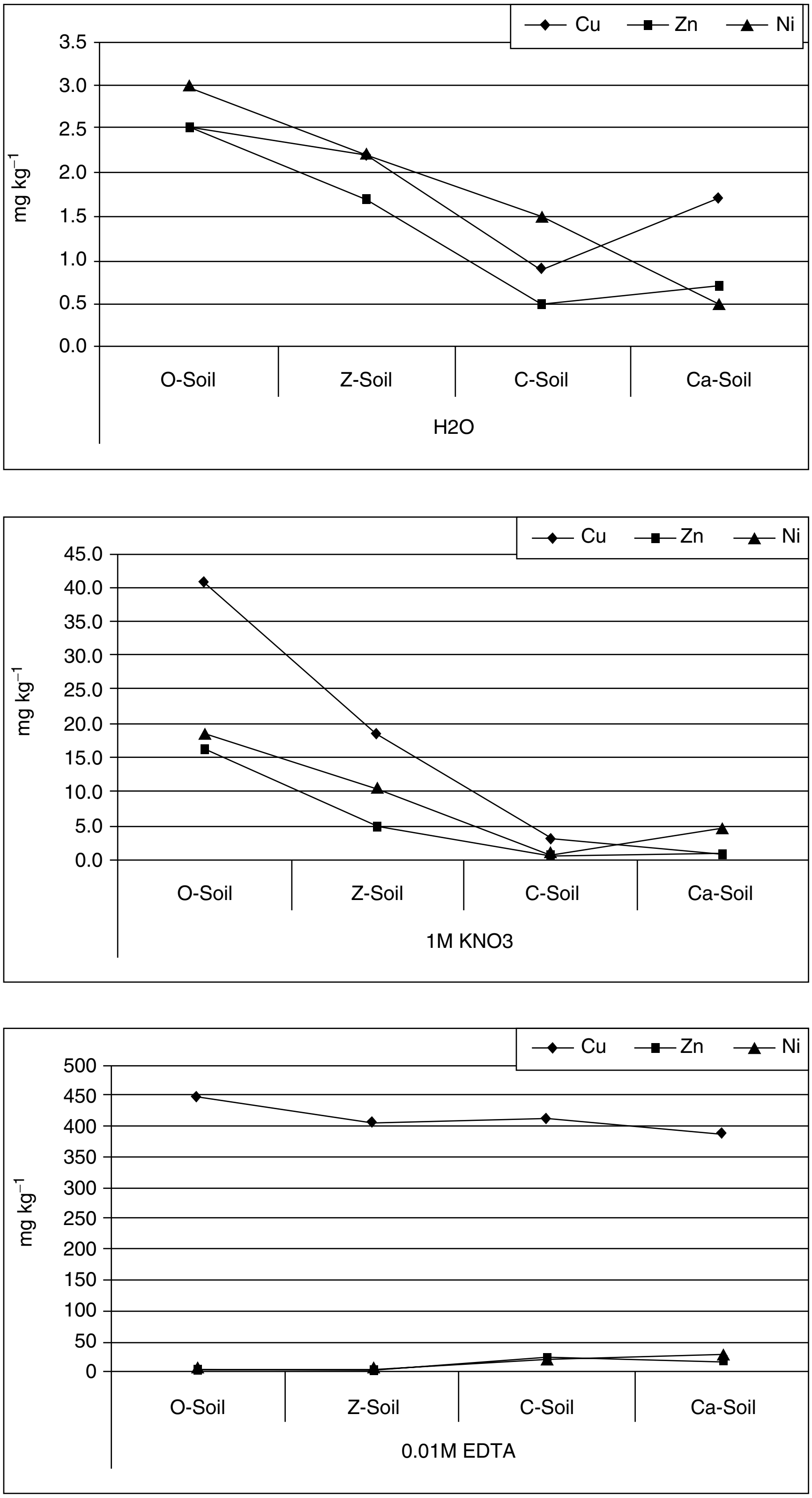
Zeolite addition significantly reduced the extractability of the three metals from 40 to 70 %. However, the greatest reductions (over 90 %) were obtained after the compost and $Ca(OH)_2$ treatments.

The EDTA extraction, which also identifies the metals bound by linkages of a covalent nature, showed a reduction (10 %) of Cu extractability with respect to the original soil, while for Zn and Ni, the addition of compost and $Ca(OH)_2$ drastically reduced their solubility leaving a greater amount available for EDTA extraction.

In the microcosm experiments, the effects of the treatments on biomass production were positive with an increase for all the plant species (Fig. 3.3). The addition of compost produced the most favourable conditions for the growth of plants, probably also due to the increase in fertility of elements such as carbon, nitrogen and extractable phosphorous and potassium in the contaminated soil, thus promoting microbial activity.

The performance data can be evaluated by considering the amounts of metals removed. This amount, which can be expressed as "total accumulation", is the result of metal uptake and biomass production. The results showed that all the treatments increased the metal removal efficiency as expressed by total accumulation (Fig. 3.4).

Fig. 3.2 Soil sequential extraction with H_2O, KNO_3 and EDTA



With *H. annuus* plants, the highest value was detected in the Z-Soil and C-Soil for Zn with an 11-fold increase compared to the total accumulation in the O-Soil. The best results obtained for Ni were in the Z-Soil, where the increase in the total accumulation was 13 times greater than the O-Soil. In the case of Cu in *H. annuus* plants, the highest increase in total uptake was found in the C-Soil, 28 times higher than in the O-Soil.

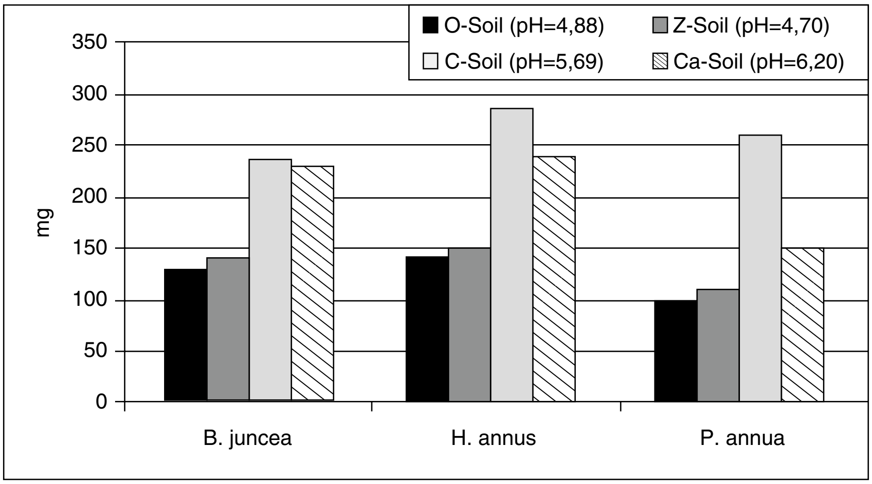


Fig. 3.3 Biomass production following different treatments

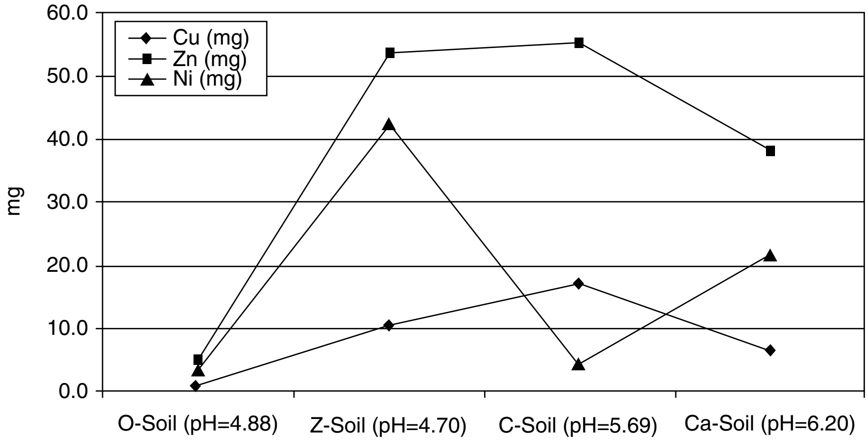


Fig. 3.4 Total accumulation in *H. annuus*

In conclusion, in this soil the high level of metal concentrations could drastically reduce seed germination and the healthy growth of plants, thus creating difficulties in the use of phytoremediation. To limit the negative effects of phytotoxicity, the soil was treated with compost, zeolites and $Ca(OH)_2$. These treatments decreased the metal solubility by increasing metal precipitation as in the case of $Ca(OH)_2$ addition, with a consequent increase in pH, or by increasing adsorption using materials with adsorptive properties for metals, such as compost and zeolites. Humus-like substances in compost are able to complex or adsorb heavy metals, thus reducing their bioavailability. As zeolites are a class of porous aluminosilicates bearing a negative charge, they retain metal cations in their structural sites. A lower solubility of metals after treatment makes them less available to plants promoting an increase in biomass production, leading in the meantime to a significant uptake of metals.

Soil washing, chemical extraction, excavation and landfill disposal were considered as remediation alternatives to phytoremediation, which in the case study would have been difficult to apply due to the acidic nature of the soil. By manipulating heavy metal bioavailability, the final result led to the possibility of using phytoextraction technology also, in this highly contaminated site. This solution led to a cost reduction and improved the soil quality.

3.5.1 Case Study 2: Increasing the Bioavailability in a Soil Contaminated by Mercury and Arsenic

The soil in this case study was from a former industrial site that had been subjected to various chemical activities contaminated by As (41.1 mg kg^{-1}) and Hg (67.0 mg kg^{-1}). The soil was characterized by a basic pH (8.06) and a content of organic matter of around 1.5 %. The soil texture showed a prevalence of the sand fraction (78.9 %), with 13.1 % of silt and 8.0 % of clay. The cation exchange capacity was 15.6 cmol kg^{-1}. All analyses were performed according to soil analysis methods (Sparks 1998).

3.5.1.1 Experimental Procedure

To maintain an elevated soil quality, it was decided not to use complexing agents such as EDTA, and only additives with fertilizing properties were planned. In order to assess soluble and mobilizable fractions of Hg and As, two different extraction procedures were used: ammonium thiosulphate 0.27 M $(NH_4)_2S_2O_3$ for Hg (Moreno et al. 2004; Pedron et al. 2013) and potassium dihydrogen phosphate 0.05 M KH_2PO_4 for As (Tassi et al. 2004). Mercury concentration in soil, plant samples and soil extracts was determined by atomic absorption spectrophotometry with an Automatic Mercury Analyzer (AMA 254, FKV, Bergamo, Italy), according to the SW-846 method 7473 (USEPA 1998). The As concentration was determined using ICP-OES with a method for the generation of hydrides (Sparks 1998). The results obtained are reported in Table 3.3.

From the comparison between the extractants, it was discovered that the effectiveness of the thiosulphate on As was comparable to that of phosphate; while the Hg results confirmed the findings of several studies (Moreno et al. 2004, 2005; Pedron et al. 2011, 2013), highlighting the positive effects of thioligands in increasing Hg bioavailability for plants.

The main objective of this case study was to evaluate whether to use assisted phytoextraction in this soil, which was characterized by high concentrations of arsenic and mercury, by adding a single mobilizing agent for both contaminants, with significant cost savings.

The soil used in the microcosm experiments was prepared by eliminating the coarser materials, homogenized but without sieving to 2 mm, to obtain a more representative sample of the real field situation. Experiments were carried out in a growth chamber in controlled conditions: 14 h of light, with a temperature of 24 °C, and 10 h in the dark at 19 °C. Relative humidity was maintained at 70 % (Pedron et al. 2009).

Using microcosms under controlled conditions of light, temperature and humidity reveals in about 30 days the ability of a plant species both to grow on the matrix under consideration and to absorb and translocate the contaminants to the aerial part of the plant.

The plant species selected for the tests were *Brassica juncea* var. scala, and *Lupinus albus* var. multitalia. Ammonium thiosulphate 0.27 M $(NH_4)_2S_2O_3$ was used as a mobilizing agent.

Table 3.3 Extractability of As and Hg. Data are expressed as mg kg^{-1} dry soil

Extractant	As (mg kg^{-1})	Hg (mg kg^{-1})
0.05 M KH_2PO_4	7.6	n.d.
0.27 M $(NH_4)_2S_2O_3$	7.4	12.9

Each microcosm was filled with 400 g of contaminated soil. After wetting the soil, *B. juncea* (0.5 g of seed per pot) was sown on the surface of each pot, while *L. albus* (three seeds) was pre-germinated and then planted in the microcosms. After about 2 weeks, treatment was started according to an experimental design previously used (Pedron et al. 2013), 2 mL of 0.27 M $(NH_4)_2S_2O_3$ were added daily for 5 days to five replicates for each species, with controls (untreated microcosms) run simultaneously.

The splitting of the total dose was performed over 5 days to minimize possible toxic effects on the plant species. After harvesting, plant aerial parts were separated from the roots and washed with deionized water. The roots were also washed in an ultrasound bath (Branson Sonifier 250 ultrasonic processor; Branson, Danbury, Conn.) for 10 min to eliminate any soil particles that might have remained on root surfaces. Vegetal samples were left in a ventilated oven at a temperature of 40 °C until a constant weight was obtained. The dry mass of shoots and roots was gravimetrically determined.

3.5.1.2 Biomass Production

Assessing the biomass production is of paramount importance in quantifying the removal of metals by plants and enables the removal of the contaminants from the soil to be estimated. In this soil the seed germination of *B. juncea* and *L. albus* was not hindered by the presence of As and Hg. Figure 3.5 shows that the addition of thiosulphate reduced the biomass production of *B. juncea*, probably due to an increase in the concentrations of the two contaminants, in particular mercury, which significantly stressed the metabolism of the plant.

3.5.1.3 Arsenic and Mercury in Plant Tissue

At the end of the growing cycle, plants were harvested; aerial parts and roots were separated and dried in a ventilated oven at 40 °C. The Hg and As concentrations in the plant tissues of *B. juncea*, and *L. albus* are reported in Table 3.4.

The phytoextraction by *B. juncea* and *L. albus* would not have been effective in this contaminated soil without

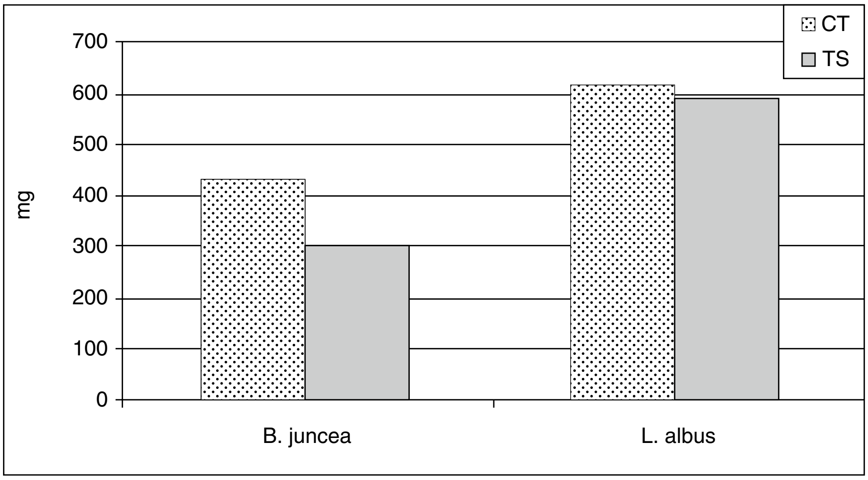


Fig. 3.5 Biomass production of microcosm plants

Table 3.4 As and Hg content in shoots and roots of the selected plants. Data are expressed as mg kg^{-1} dry weight

	Shoots		Roots	
	As	Hg	As	Hg
CT B	n.d.	3.3 ± 0.74	n.d.	16.9 ± 4.9
TS B	14.3 ± 4.4	120 ± 7.5	54.3 ± 4.4	1,451 ± 319
CT L	0.82 ± 0.09	1.1 ± 0.35	1.6 ± 0.60	4.5 ± 0.46
TS L	4.3 ± 1.4	24.1 ± 2.1	14.2 ± 3.2	744 ± 34.6

CT, control; TS, thiosulphate treatment; B, *B. juncea*; L, *L. albus*

modifying the bioavailability of the contaminants by the use of a mobilizing additive. The addition of the mobilizing agent promoted the Hg and As uptake by the plants, both in the case of *B. juncea* and *L. albus*, with respect to the control microcosms.

In the controls, the greatest Hg concentration found in the aerial part was about 3.3 mg kg^{-1} for *B. juncea* plants, while the As concentration was always negligible. In the root portion, the Hg concentration was about 17 mg kg^{-1} in *B. juncea* (As content was negligible) and 4.5 mg kg^{-1} in *L. albus* where As concentration was 1.6 mg kg^{-1}. The addition of the thiosulphate solution to the soil promoted Hg and As uptake in the aerial part of the plants, reaching Hg concentrations of 120 mg kg^{-1} for *B. juncea* and 24.0 mg kg^{-1} for *L. albus*. As concentrations were 14.3 mg kg^{-1} in *B. juncea* and 4.3 mg kg^{-1} in *L. albus*.

Both in the controls and the treated plants, the amounts of As and Hg were higher in the roots than in the shoots. This indicates that within the short time frame of the tests, the plants are able to uptake the metal and partially translocate it to the aerial parts. As is well known, roots retain contaminants to protect plants.

From the results obtained, it appears that Hg is absorbed in greater quantities than As, particularly in the roots both for *B. juncea* and *L. albus*. This is not surprising since the additive used is specific for Hg. However, the effect of thiosulphate on the bioavailability of As is of great interest. The results of plant uptake confirm the results of the extractability tests. This additive is also able to mobilize As in similar quantities to those derived from the use of phosphate.

3.5.1.4 Total Accumulation

The total accumulation, calculated as the product of the concentration of the metal in plant tissues for the respective dry biomass, provides an estimate of the amounts of contaminants removed from the polluted soil and thus the phytoextraction efficiency. The data are reported in Fig. 3.6.

It is clear that the addition of thiosulphate greatly increased the amounts of metals removed by the plants, due to the higher concentrations of As and Hg.

Results showed that increasing the metal's bioavailability with the use of $(NH_4)_2S_2O_3$ promoted more Hg and As uptake by the plants than in the controls. While the effect of thiosulphate on mercury bioavailability is due to the formation of the soluble complex mercury–thiosulphate $Hg(S_2O_3)^{2-}$, which can be absorbed by plants (Moreno et al. 2005), the thiosulphate–arsenic interaction in the soil–plant system has not been studied with a view to phytoextraction.

The increase in arsenic bioavailability promoted by thiosulphate addition could be ascribed to the competition between arsenate and sulphate ions for the same soil surfaces, with the release of arsenic in the liquid phase in potentially bioavailable forms.

3.5.1.5 As Extractable Sulphate

In the soil, thiosulphate decomposes into sulphur and sulphate. Sulphur can give rise to precipitates while sulphate remains in solution. This is why the thiosulphate fertilizer is widely used because the sulphate has an immediate action on the crops, while the sulphur is released over time. In the specific conditions of the soil in this case study, we can assume that the thiosulphate in the soil is transformed into tetrathionate and subsequently to sulphate as shown in the following scheme:

$$S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

The reaction is either abiotic or biotic depending on the microbial community present. The kinetics of the oxidation depend on the characteristics of the soil; in general the first step is very rapid and requires a few hours, after which the reaction is complete in a few days (3–6), but can be speeded up in the microcosms due to the presence of the plants which further stimulate microbial activity. In a previous work (Barbosa-Jefferson et al. 1998) results of thiosulphate incubation in different soils revealed that the final concentration of sulphate deriving from thiosulphate transformation is basically the same as of thiosulphate added.

Thus, with an excess of sulphate ions, deriving from thiosulphate addition, we can suppose that arsenate ions are released in the liquid phase of the soil in a potentially bioavailable form for plant uptake.

To test this hypothesis, arsenic extractions with sulphate were performed in comparison with thiosulphate extraction (Table 3.5).

Data show that in this soil the As extractability was the same with the use of thiosulphate and sulphate. The capability of thiosulphate to promote the release of As from soil surfaces and to improve its phytoavailability was reported also in a different soil (Pezzarossa et al. 2013). Competition between sulphate and arsenate for the same adsorption sites is generally very low; however, in an alkaline environment SO_4^{2-} ions are found to form outer and inner sphere complexes which could substitute arsenate at least in outer sphere complexes (Myneni et al. 1998). Although the issue is highly controversial, the

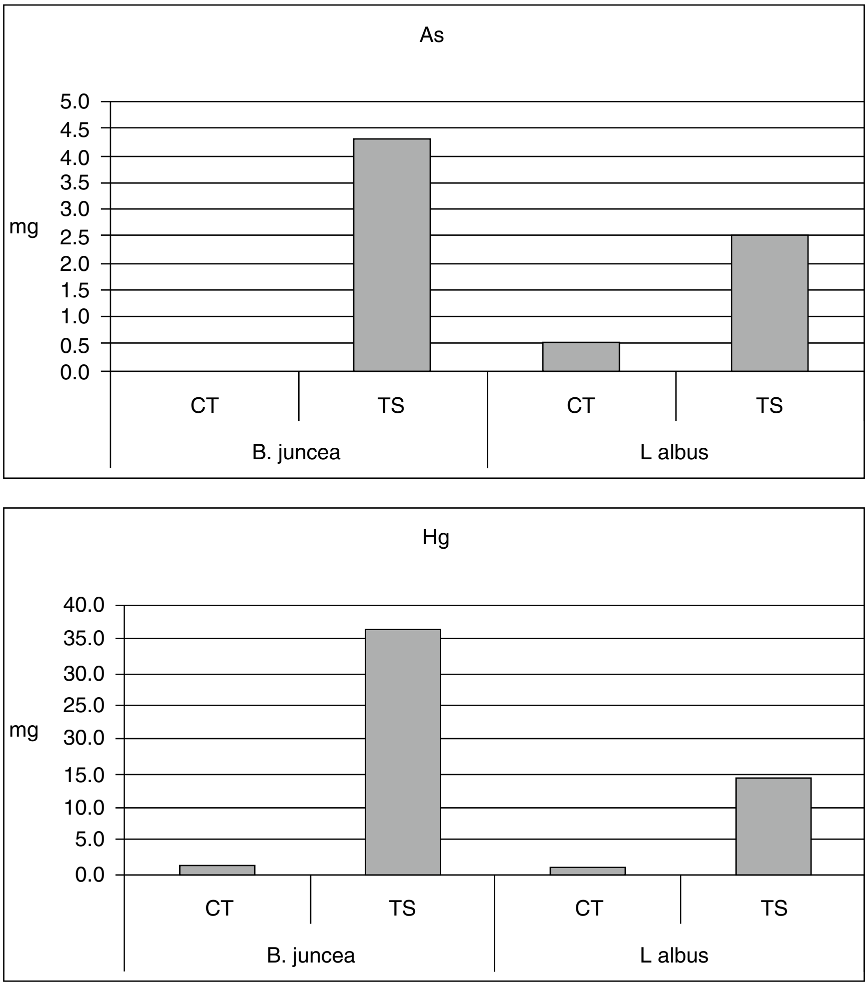


Fig. 3.6 Total accumulation of As and Hg in the selected plants

Table 3.5 Extractability of As by thiosulphate and sulphate. Data are expressed in mg kg^{-1} dry soil

Extractant concentration	$(NH_4)_2S_2O_3$	$(NH_4)_2SO_4$
0.14 M	3.83 ± 0.05	3.87 ± 0.08
0.27 M	6.98 ± 0.08	6.06 ± 0.03

results obtained show a clear correlation between the As amounts extracted by sulphate and those extracted by thiosulphate. This thus confirms that the sulphate–arsenate competition could be the basis for the release of arsenic in solution in bioavailable forms to plants. However, a further aspect needs to be considered: the interactions between sulphur and arsenic affect both the absorption of the contaminant and its transport to the aerial part of the plant. The addition of sulphur to the soil promotes the absorption of arsenic by plants, and sulphur plays anti-stress role in reducing the toxicity of arsenic (Duan et al. 2013). Thiosulphate can act either as nutrient and detoxifying agent, due to the stimulation of plant defensive systems, and influenced either the biomass production and the As accumulation in plant tissues.

3.6 Concluding Remarks

When selecting technologies, contaminant bioavailability has often been neglected, yet in reality it is essential. In fact, there is a noticeable discrepancy between the results based on laboratory tests and those obtained from full-scale cleanup where several difficulties have been encountered that have often been underestimated in theoretical studies (Ernst 2005; McGrath et al. 2006; Robinson et al. 2006; van Nevel et al. 2007).

One of the main reasons for this discrepancy derives from not considering bioavailability. In soil, bioavailability is the result of the complex mechanisms of retention and release which depend on the soil characteristics and on the biology of the organisms involved (Alexander 2000; Ehlers and Luthy 2003). It is thus essential to evaluate the retention ability of soil in order to be able to estimate the possible success of phytoremediation strategies. The level of retention depends on the soil type. In soils of temperate regions, which have a high content of expanding minerals, metal retention is much higher than in soils of tropical regions. This in turn drastically influences the metal bioavailability to plants and as a consequence plant uptake and phytoextraction efficiency. Consequently, any phytoextraction programme that does not consider the specific properties of the contaminated soil may completely fail to predict how efficient it will be in field applications.

Phytoextraction aimed to remove the bioavailable fractions of metals can provide sustainable solution in remediation since at the end of the treatment the soil quality is improved. Moreover the removal of bioavailable fraction can be considered as a fundamental step also to promote prevention of public health (Henry et al. 2012) since in this way most exposure pathways to hazardous substances are eliminated or drastically reduced.

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4 Phytoremediation and Environmental Factors

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4.1 Introduction

Phytoremediation includes a range of plant-based remediation processes such as phytoextraction, phytostabilization, phytoimmobilization, rhizofiltration, and phytovolatilization (Alkorta et al. 2004; Ali et al. 2013; Vithanage et al. 2012). Among all of these techniques, phytoextraction is crucial, because the principle of this technique is metal translocation to shoots and bioconcentration is observed in the above ground parts of the plant species (Peuke and Rennenberg 2005; Vithanage et al. 2012). This is all the more important when the harvest of root biomass is generally not feasible (Zacchini et al. 2009; Tangahu et al. 2011; Ali et al. 2013). However, the efficiency of phytoextraction (as well as other techniques of phytoremediation) depends on numerous coupled environmental factors. Generally, they can be defined as factors influencing the lives of organisms and which are essential to their correct functioning in land and water environments. The most universal division of environmental factors distinguishes two main groups, namely, biotic (living) and abiotic (nonliving) factors (Schulze et al. 2005). The biotic group includes plants, animals, protists, fungi, and bacteria, whereas the abiotic group includes weather (rain, insolation, temperature, cloud, snow), pH, rocks, oceans, and rivers, as well as anthropogenic factors (all the factors that are products of human activity, e.g., organic and/or inorganic pollutants). The characteristic traits of all mentioned factors are in mutual interrelation; therefore, when one factor is changed, the whole system can be altered (Atwell et al. 1999; Beard et al. 2005). However, the phytoextraction potential depends on external environmental factors, but also on internal plant factors. Interest in phytoextraction has grown due to the identification of metal phytoaccumulator plant species (Seth 2012) and their high capacity for adaption to environmental conditions. Plants are able to adapt to disadvantageous environmental conditions (growth in significantly polluted areas, drought, salinity), and two main strategies are used: stress avoidance and tolerance. Selection of plants used in phytoremediation is extremely important and has been presented in numerous scientific works, both in hyperaccumulator and non-hyperaccumulator plants (Hendriks et al. 2003; Mleczek et al. 2010). The mentioned plants produce numerous chemical compounds able to stimulate or inhibit plant growth especially in unfavorable environmental conditions (Davies 1987; Dimkpa et al. 2009). The growth-promoting substances include phytohormones, bioregulators, and biostimulators, where, e.g., auxins (Chalupa 1984; Dimkpa et al. 2008; Liphadzi et al. 2010), cytokinins (Ei-D et al. 1979; Cassina et al. 2011), and gibberellins (Pandey et al. 2007) are stimulators, and abscisic acid is an inhibitor (Park et al. 2009). Also, plants activate a defense mechanism combined with the exudation of specific molecules into the environment and adaptive changes in plant structure and growth to withstand the adverse growth conditions (Drzewiecka et al. 2012). These specific molecules include:

(i) Metal-binding peptides created from glutathione (GSH)—phytochelatins (PCs) (Grill et al. 1987; Rauser 1995)
(ii) Low molecular weight, metal-binding proteins—metallothioneins (MTs) (Cobbett and Goldsbrough 2002; Kägi 1993)
(iii) Amino acids with especially the role of histidine (Leszczyszyn et al. 2007)
(iv) Phytin (Rauser 1999)
(v) Low molecular weight organic acids (LMWOAs) secreted into the rhizosphere (Magdziak et al. 2011; Li et al. 2012a, b)
(vi) LMWOAs produced in plant tissue (Adeniji et al. 2010)
(vii) Flavonoids (Ebrahimzadeh et al. 2008; Fernandez et al. 2002)

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The amount and the kind of molecules occurring in a given plant together with specific traits of certain plant taxa determine the ease of plants' adaptation to new environmental conditions, biomass crop, and at the same time efficiency of trace element accumulation. In this regard, plant selection has fundamental importance in phytoextraction efficiency, but the influence of external factors although variable (interaction between elements in polluted soil) is also permanent (abiotic factors). For this reason, it is not possible to determine the precise efficiency of trace element accumulation. The range of tolerance together with maximum, optimum, and minimum of particular factor values decides about the plant tolerance as well as plant ability to adapt to these factors (Chapin et al. 1987; Atwell et al. 1999). Due to the size constraints on this chapter, the characterization of all environmental factors is impossible. Therefore we present only some of them, which are most frequently analyzed or commented on in scientific environmental studies.

4.2 Environmental Factors

Proper plant growth is not possible without adequate amounts of water fulfilling the primary role in normal plant functioning. Due to the difference in water potential Ψ in the soil, plant, and atmosphere, the natural flow of water from the soil to the atmosphere occurs. The presence of conditions for the occurrence of the phenomenon determines the transport of numerous substances and compounds playing an important role in the life processes of plants (Tien et al. 1979; Chen and Aviad 1990). Therefore, this seemingly small polar molecule with a high thermal capacity allows the elongation growth of cells and tissues of plants. The amount of available water affects the physiological and biochemical processes of plants (Hanson and Hitz 1982; Al-Karaki and Al-Raddad 1997; Maggio et al. 2000; Asrar and Elhindi 2011). For the proper functioning of the individual cell structures, it is important to maintain a sustainable water balance, because changes in water availability result in altering growth, yield, and water relations (Abdel-Fattah et al. 2002; Wu and Xia 2006; Ibrahim et al. 2011) and metabolic pathways (Subramanian and Charest 1995; Asrar and Elhindi 2011; Asrar et al. 2012). However, the root system plays the essential role in providing water, nutrients, and physical support to the plants. It is the major plant organ, which receives most stress factors and as a response to environmental factors starts the modulation of organs and then tissue in response to changing conditions of the external environment (Kummerová et al. 2013). In the event of water deficiency, the plant growth is significantly reduced due to osmotic stress resulting from decreasing soil water potential and uptake of nutrients (Auge 2001; Lee et al. 2007; Seagraves et al. 2011) and increased levels of nitrogenous compounds in plants due to increased protein hydrolysis, and the accumulation of osmoprotectants is observed (Seagraves et al. 2011). Moreover, the changes in root physiology and morphology depend on the level of groundwater (Zencich et al. 2002), providing an additional and important water source, and with it the minerals, which next to rainfall, are considered to be the main source of water for plants (Ehleringer and Dawson 1992). In the case of water stress in the upper soil layer, it leads to increased root length and reduced diameter, which reduces the resistance of roots to water uptake, so crop roots actively absorb water from the subsoil (Costa et al. 2000; Hendrick and Pregitzer 1996; Li et al. 2012a, b), while in the case of water excess, formation of anaerobic conditions in the root zone area is observed. Associated with this is the reduction of selective permeability of plant roots, which then is connected with accumulation of both essential and toxic elements. This situation is especially seen in the case of plants with a small annual growth increase in flooded areas (even more than half the height of plants) and where an increase in water level occurs for a long time, resulting in wilting of most of them (Dinicola 2006). Metals such as Zn, Ni, and Cd in water-logged soils are less available, because of decreased solubility resulting in low redox potential and formation of sparingly soluble sulfides (Rieuwerts et al. 1998; Hammer and Keller 2002). The most important hyperaccumulators of different metals (Ni, Co, Zn) originated from areas with dry, hot summers and/or grow on skeletal soil with very low water holding capacity (Reeves and Brooks 1983). For hyperaccumulator plants metal bioavailability is an important factor affecting uptake. High soil moisture usually does not negatively affect growth and metal accumulation by hyperaccumulators. Angle et al. (2003) confirmed that high soil moisture stimulated growth and metal uptake by hyperaccumulators of Ni such as *Alyssum murale* and *Berkheya coddii* and of Zn such as *Thlaspi caerulescens*, which generally grow on low moisture soil. On the other hand, low moisture enhanced Se accumulation and reduced biomass production of *Festuca arundinacea* (Tennant and Wu 2000).

But, with access to water, it involves not only the structure of the root system and further plant development but also the availability of plant nutrients (essential and toxic elements). Different water content and different chemical and biological properties of soil/roots from the rhizosphere zone and from bulk soil can affect nutrient bioavailability to the plant. It is evident that bioavailability of metals could be artificially increased under optimum or a little above water resources. Water is the main source of macronutrients (K, N, P, S, Ca, and Mg) but also micronutrients (Fe, Zn, Cu, Ni, and Mo) and toxic elements (Pb, Cd, and Hg). Their presence is an important contribution to the life processes of plants and their growth. Their amount in water, as an important medium, is not decisive, because plants developed a selective mechanism to acquire some element ions in spite of others, and selective absorption of elements depends on the membrane transporter properties (Salt et al. 1995; Seth 2012). By these specific transporters are able to recognize, bind, and then mediate the transmembrane transport of ions. However, under metal-polluted soil, plants take up not only essential nutrients but also significant amounts of toxic elements, such as Cd, Hg, and Pb. Essential nutrients and toxic trace elements are absorbed by the same processes, so under increased concentrations of nonessential elements, plants are not able to distinguish between the two ions. Therefore, at high levels of toxic metals, there follows competition between ions, and the plant starts to retrieve the component that dominates in the environment. Moreover, in the case of plants, accumulation of all contents begins, as mentioned above, at the roots, which are a crucial element affecting the efficiency of phytoextraction. The most important is the mobility of elements in the soil and their availability to the cells of the roots, which may result in further transport of metal-containing sap to aboveground parts of the plant. It should be noted that the transfer process is controlled by two factors: the root pressure and transpiration of leaves, which are contingent on water resources in the soil environment (Seth 2012). Stress associated with water availability leads to disruption of water potential gradients, loss of turgor, disruption of membrane integrity, and denaturation of proteins.

Soil is the most important environmental factor in the growth and development of plant life. However, it must be mentioned that soil has indeed a diverse composition, with no regular and predictable structure, as a result of which part of soil may have significantly different physical, chemical, and biological properties from another part located in close proximity. It all depends on many factors, including composition of mineral and organic matter, soil water, aeration, climate, the presence of soil bacteria and fungi, and animals inhibiting the ground. All of these parameters have essential effects on plant life, but also are an important issue in effectiveness of the phytoremediation process. Dynamic changes that occur in the soil environment not only determine development or inhibition of plant growth but also have a significant impact on the remediation process. However, among the numerous abovementioned parameters which determine the process of phytoremediation in soil, the most significant impact is the type of pollution (Wang 1994; Gonzales et al. 2013), because soil may be polluted by natural aromatic and hydrocarbon compounds and man-made chemicals (pesticides, herbicides, fungicides, and antibiotics) and metals. In the case of organic pollutants, their metabolism generally results in degradation into nontoxic substances that can be used by the plants and their microbial partners as a source of carbon, phosphorous, nitrogen, sulfur, and, in the some cases, trace elements (Saier and Trevors 2010). In the case of metals, the situation is much more complicated. Metals are not readily removed or degraded by chemical or microbial processes and in consequence are accumulated in soils and aquatic sediment (Ojegba and Fasidi 2007). This problem can be solved through the use of plants with increased tolerance to metals and the phytoremediation process. During the phytoremediation process, plants may transport trace elements in their cell walls, chelate them in the soil in inactive forms using secreted organic compounds, or complex them in their tissue after transporting them into specialized cells and cell compartments. Metals may be stored in vacuoles safe from the sensitive cytoplasm where most metabolic processes occur. Plants also make chelating peptides such as cysteine and small proteins such us phytochelatins and metallothioneins that are stored safely in vacuoles (Saier and Trevors 2010). However, the effectiveness of phytoremediation depends mainly on the bioavailability of trace elements in soil, and their bioavailability strictly depends on soil solution properties (Chen et al. 2006). Chemical properties of soil—such as pH, Eh, and nutrient content—influenced the metal forms present in the soil and their accumulation (de la Fuente et al. 2008). However, it is important that a large proportion of metals in soil is immobilized, because they are bound with selected matrix components (Sheoran et al. 2011; Ali et al. 2013; de la Fuente et al. 2008), and only a fraction of soil metal is bioavailable for uptake by plants (Lasat 2002). For this reason the cleanup of soils contaminated with heavy metals (HMs) is one of the most difficult tasks for environmental engineering (Li et al. 2013). However, before plants are able to absorb and then accumulate metals, there must be a process involving activation of metals in the environment; in other words, there must be a process in which metals will be bioavailable to the plants.

Transport of heavy metals in the soil depends to a large extent on the chemical form of the metal. In the initial phase of the metal–soil contact, metal reactions are fast (minutes, hours). For this reason, natural consequences are various chemical forms of the metals and their different bioavailability, mobility, and toxicity (Shiowatana et al. 2001; Buekers 2007). The distribution of the metals in the soil is conditioned by factors such as precipitation or dissolution reactions, ion exchange, adsorption and desorption, immobilization of the biological activation, and plant species (Levy et al. 1992). Nevertheless, having an influence on all these parameters is the pH value, which is one of the parameters determining the metal speciation in soil. The trace elements that commonly occur as impurities in soil and for which the pH value is an important factor in the form of their occurrence include lead (Pb), chrome (Cr), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni).

How does the pH affect the metals' occurrence in soil?

Pb

The lead phosphates, carbonates, (hydr)oxides, and sulfide are the dominant Pb compounds presented in the soil environment (Raskin and Ensley 2000). Their properties change depending on pH value, and insoluble forms occur when the pH is above 6 (Raskin and Ensley 2000). In conclusion, the mobility of Pb increases with decreasing pH value.

Cr

Chromium commonly found in contaminated areas can occur in the Cr^{3+} or Cr^{6+} oxidation state, depending on the pH and redox conditions of the soil. Cr^{6+} is the dominant form where aerobic conditions exist, whereas in the presence of organic matter, S^{2-} or Fe^{2+} ions under anaerobic conditions can be reduced to Cr^{3+}. It is also important to note that Cr^{6+} is the more toxic form and moreover more mobile with increasing pH. Chromium mobility decreases with the decrease in the oxidation state degree due to the increasing adsorption reaction to clays and oxide minerals below pH 5, and low solubility above pH 5, due to the hydroxide Cr^{3+} (III) formed in the solid form (Chrostowski et al. 1991). On the other hand, the Cr mobility is relevant to physicochemical properties of the soil, which determine the sorption properties due to the content of clay, iron oxide, and also the amount of organic matter.

As

The toxicity of As is determined by the form in which it occurs (depending on whether it is in the As^{3+} or As^{5+} form or as organic). The As speciation highly determines its toxicity and bioavailability as well as determining the establishment of an adequate transport mechanism (Larios et al. 2012; Larsen et al. 1998). It is known that inorganic forms are more toxic and mobile than organoarsenic forms. Among inorganic forms of As, it was found that arsenite (As^{3+}) is 10 times more toxic and mobile than arsenate (As^{5+}) (Demirbas 2001; Lasota et al. 1968, 1980) and 70 times more than monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) (Demirbas 2001; Le et al. 1994; Londesborough et al. 1999; Niedzielski et al. 2013). However, the bioavailability of As is determined by the pH, and As mobility increases with increasing pH value (Smith et al. 1995).

Cd

Cadmium may be present in the soil as a result of application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge). The disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree (Weggler et al. 2004). But it should be underlined that Cd mobility strictly depends on pH value, and the acidification of soils increases the geochemical mobility of Cd (Campbell 2006). In the case of Cd, the bioavailability to plants (as well as other organisms such as animals or humans) is important because once absorbed by an organism, it remains resident for many years (Wuana and Okieimen 2011).

Cu

Copper is an element that forms stable complex compounds with organic material; therefore, only a small part of Cu ions or in more bioavailable organic forms are present in soil solution. The pH value is strictly associated with Cu solubility in the environment (solution), which significantly increases at pH 5.5 (Martínez and Motto 2000), which is rather close to the ideal farmland pH of 6.0–6.5 (Eriksson et al. 1997).

Hg

In the soil environment mercury may exist in mercuric (Hg^{2+}), mercurous (Hg_2^{2+}), elemental (Hg_o), or alkylated form (methyl/ethyl mercury), but pH is a factor which determines the form in which Hg will be present. Moreover, sorption to soils, sediments, and humic materials is an important mechanism for the removal of Hg from solution. Sorption is a pH-dependent process and increases as pH increases. In consequence, the amount of bioavailable forms of mercury increases with the pH decrease of the environmental (Wuana and Okieimen 2011).

Ni

In the case of Ni speciation under neutral pH or slightly alkaline soil environment, the hydroxide of Ni, which is a stable compound, is precipitated. The precipitate is readily soluble in acid solutions, consequently giving compounds of Ni^{3+}, while under strongly alkaline conditions, a soluble form of nickelite ion (NO_2^-) is formed. However, it should be noted that in the case of the soil, the greater part of all the Ni compounds that are released into the environment are adsorbed on soil particles, which become immobile as a result. In the case of Ni, the mobility significantly increases in acidic soils, making it more bioavailable, and often leaches down to the adjacent groundwater (Wuana and Okieimen 2011).

In the soil solution, siderophores have a significant impact; they are small molecule chelating compounds with high affinity to Fe^{3+} ions and having the ability to bind trace elements (Al, Cd, Cu, Ga, In, Pb, Zn) and are produced by plants, bacteria, and fungi (Kidd et al. 2009; Rabęda et al. 2011). In the case of plants, the metal-mobilizing compounds secreted by roots into the rhizosphere are called phytosiderophores (Lone et al. 2008), which have certain mechanisms for solubilizing heavy metals in soil. Generally, only a fraction of soil metals are bioavailable for uptake by plants (Lasat 2002). In addition, plants growing in areas contaminated by high amounts of trace elements are characterized by a low content of iron in plant tissue. This generally results in chlorosis associated with iron deficiency, which is the consequence of the inhibition of chloroplast development and chlorophyll biosynthesis (Imsande 1998). The presence of bacteria in the rhizosphere, with the simultaneous presence of iron–siderophore complexes, is an additional source of iron for plants in polluted conditions (Bar-Ness et al. 1991; Reid et al. 1986; Wang et al. 1993). Strong binding of heavy metals to soil particles or precipitation makes a significant fraction of soil heavy metals insoluble and therefore mainly unavailable for uptake by plants (Sheoran et al. 2011). Due to secretion of H^+ ions by roots, the rhizosphere is acidified, and in consequence metal dissolution increases. The presence of H^+ ions allows displacement of heavy metal cations adsorbed to soil particles (Alford et al. 2010) and, in most cases, lower soil pH, promoting desorption of metals and increase of their concentration in solution (Thangavel and Subbhuraam 2004).

Furthermore, the rhizospheric microorganisms (mainly bacteria and mycorrhizal fungi) may significantly increase the bioavailability of heavy metals in soil (Vamerali et al. 2010; Sheoran et al. 2011). Interactions of microbial siderophores can increase labile metal pools and uptake by roots (Mench and Bes 2009; Ali et al. 2013). Bacteria are an important factor of soil, because they have the ability to mineralize organic matter, improving bioavailability and effectiveness of nutrient uptake (Domínguez-Crespo et al. 2012). Bacteria present in soil have a significant impact on phytoremediation, especially those bacteria which belong to the plant growth-promoting rhizobacteria/bacteria (PGPR/PGPB) group. Also, the presence of PGPR bacteria is very important in areas contaminated with heavy metals, as it is conducive to the process of plant growth, inhibits the development of chlorosis, and increases the resistance of plants to increased metal content in the soil environment (inter alia abovementioned Ni, Pb, or Cu). Bacteria in the rhizosphere are involved in the accumulation of trace elements potentially toxic to plant organs (Jing et al. 2007). What is more, at the same time, the presence of bacteria affects the reduction of phytotoxicity of contaminated soil. This is due to the fact that the plant–bacterium together forms a specific arrangement in which the plant provides the bacteria the organic carbon (e.g., by molecules/compounds secreted by the cells of the root), which in turn induces the bacteria to reduce the phytotoxicity of the soil. In addition, bacteria and plants are in a symbiotic relationship by which microbial activity results in degradation of contaminations. Moreover, the components secreted into the root zone by the plant roots increase metal bioavailability and consequently increase the possibility of further phytoremediation abilities of bacteria. This mechanism increases the ability of plants to adapt to unfavorable conditions in the presence of metals, as well as increasing the phytoremediation potential by the presence of plant–bacterium symbiosis (Jing et al. 2007). Moreover, bacteria in the soil are usually present in large amounts, have a high surface area and metabolic activity, affect the bioavailability and toxicity of heavy metals by adsorption and dissolution, and carry out oxidation–reduction reactions or association of bacteria with organic and inorganic colloids (Shen and Yang 2008). The presence of bacteria in the root zone may have a beneficial effect because they are able to produce a series of compounds, which may be used by plants, and their presence leads to increased plant ability to absorb nutrients contained in the medium and also changed morphology of the roots (increased spreading), thus increasing the collection surface area (Rabęda et al. 2011). These mechanisms occurring in the soil in the presence of bacteria, presented, for example, in *Brassica juncea* growing on soil contaminated by Cd, Cu, P, or bound Zn with simultaneous presence of *Azotobacter chroococcum* or *Bacillus megaterium*, leading to lower toxicity, and even neutralization of toxic elements with respect to the plants, as a consequence result in increased efficiency of the phytoremediation process. This is due to the fact that on the surface of the cell walls of bacteria are present sulfhydryls and carboxyl groups and also a number of specialized proteins embedded in the cell wall and cell membrane, which limit the toxicity of the metal. Furthermore, microbes will produce metal-resistant genes composed of structural genes and regulatory genes to reduce the toxicity of heavy metals through many approaches. However, it should be noted that the siderophores produced by gram-positive and gram-negative bacteria are the most effective agents in metal complexing in the soil solution. In conclusion, the ability of activating metal absorbed onto the soil fraction, followed by complexation and their accumulation in the phytoremediation process, significantly depends on bacteria present in the soil environment.

An important factor in trace element availability in the soil environment, next to bacteria, is the effect of fungi, due to the fact that they are closely involved in the carbon cycle in nature. Through the ability to decompose organic matter, they take a significant role in the distribution of generated energy, and also conversion of proteins from plant residues on the nitrogen dissolved in the acidic form or NH_4^+ ions, which are more easily available forms for plants. In addition, mushrooms are involved in the circuit of inorganic components (Domínguez-Crespo et al. 2012). Also through the presence of plant–fungus symbiosis, the plant becomes much more resistant to environmental stress (Khan 2005; Gamalero et al. 2009; Rabęda et al. 2011). The presence of mycorrhizal fungi on the one hand can significantly increase the absorbent surface area of the root system and consequently allows better nutrition of the plants and on the other hand results in significantly increased accumulation of metals in the cells (Liang et al. 2009; Rabęda et al. 2011).

Moreover, the mycorrhizal fungi—as outlined above for bacteria—may produce siderophores, but also may secrete other components whose presence has a significant impact on the physical and chemical properties of the soil environment, which in turn contributes to the activation or inhibition of the processes taking place in soil. One of them is glomalin (Gonzalez-Chavez et al. 2004), whose presence in the soil is strongly associated with trace metals present in soil. Also, like plants, fungi secrete into the rhizosphere other molecules, such as organic acids, enabling the activation of the substrate metal, complexion, detoxification, and increased efficiency of accumulation (Magdziak et al. 2012; Vaněk et al. 2012).

It is worth emphasizing the presence of salts in the soil solution, which in addition to water is another important factor significantly influencing the growth of plants, as well as modifying the efficiency of accumulation of trace elements from the environment (Atkinson et al. 2007). The increasing salinity of soil occurring all over the world reduces growth of plants mainly by water deficit in the root zone, causing a number of changes in the homeostasis of cells at the molecular, physiological, and biochemical level. Salinity is a stress factor influencing plants, which is connected mainly with the negative effect of Na^+ toxicity for cell metabolism and oxidative damage of plants related to formation of reactive oxygen species (ROS) (Manousaki and Kalogerakis 2009). It also affects the metabolism of soil organisms, leading to a major reduction of soil fertility. Salinity has toxic and/or osmotic effects affecting the use of hyperaccumulators to decontaminate polluted soil. Because salinity can change the bioavailability of metals in soil, salinity is a key factor in translocation of the metals from roots to aerial parts of the plant (Otte 1990; Fitzgerald et al. 2003; Manousaki et al. 2008). The authors suggested that metal accumulation in saline conditions can be very useful in enhancing phytoremediation processes. Manousaki and Kalogerakis (2009) revealed that cadmium uptake by cadmium and lead-tolerant *Atriplex halimus* L. increased with increasing salinity, because of higher bioavailability of the metal in soil. They suggested that it was achieved by displacement of cadmium from binding sites in soil matrix by Na^+, solubilization of organic matter bound with the metals, or formation of soluble chloro-complexes of Cd which tend to shift Cd from the solid phase (Norvell et al. 2000; Weggler et al. 2004; Wahla and Kirkham 2008). Salinity of storm water could change metal uptake through the toxic effect of Na^+ and Cl^- (Fritioff et al. 2005). Because Na^+ can release Cd from the sediment to the water, it causes an increase of cadmium concentration in water (Greger et al. 1995). Increasing salinity reduced metal accumulation (Cu, Zn, Cd, Pb) in submersed plants *Elodea canadensis* and *Potamogeton natans* L. (Fritioff et al. 2005). The Ni accumulator *Alyssum murale* was documented to be highly salt resistant in terms of seedling emergence and survival of emerged seedlings, while the Zn accumulator *Thlaspi caerulescens* was salt sensitive in low concentrations of Ni and Zn. High concentrations of the metals did not have a clear effect on salt tolerance (Comino et al. 2005). The experiment was carried out with NaCl concentration of 0.25, 50, and 100 mM. Another experiment indicated that in some plant non-hyperaccumulators of any metal, such as *Tamarix ramosissima*, the Cd uptake significantly increased with elevating salinity. Additionally, the salinity affected the translocation of Cd from roots to the aerial parts of plants, and no visible signs of metal toxicity were observed (Manousaki et al. 2008). Because of Cd tolerance and high production of biomass, the authors suggested the use of *T. ramosissima* for phytoremediation of cadmium-contaminated soil.

It should also be noted that the growth of plants significantly depends on the air temperature, implying the widely discussed nowadays global warming. Global warming is associated with increased concentration of carbon dioxide (CO_2) and other greenhouse gases in the atmosphere by natural and anthropogenic activities (Cox et al. 2000; Qaderi and Reid 2009; Qaderi et al. 2012). Industrialization dramatically changes the biological, chemical, and physical properties of the environment via the increase of atmospheric CO_2 concentration despite reduction of CO_2 emissions. A positive effect of elevated atmospheric CO_2 concentration on heavy metal phytoextraction in polluted soil was documented, e.g., *Pinus densiflora* and *Pteridium revolutum*, via increasing production of biomass or uptake of heavy metals from soil (Jia et al. 2010; Kim and Kang 2011; Zheng et al. 2008). What is more, higher CO_2 concentration will result in an overall increase of the surface air temperature in the coming decade, extrapolating from the existing records (Smith et al. 2007; Qaderi et al. 2012), which also may lead to enhanced plant metabolism and increased development (Larcher 2003; Qaderi and Reid 2009). The responses to higher temperatures depend on plant developmental stage and the character of temperature increase. Observed higher temperature, under global warming, stimulates plant growth, photosynthetic capacity, and decomposition of soil organic matter, which affects mobilization of metals. The bioavailability of elements such as Ag, Cu, Zn, Fe, Sb, and Cu increased, which consequently resulted in an increase of phytoextraction efficiency (Baghour et al. 2001; Li et al. 2012a, b; van Gestel 2008). Temperature also had a positive effect on Cu, Zn, and Cd uptake by submersed plants *Elodea canadensis* and *Potamogeton natans* L. (and also for Pb), and high temperature together with low salinity led to nearly twofold elevated metal concentration in comparison to low temperature or high salinity (Fritioff et al. 2005). However, apart from the positive effects of higher temperatures in phytoextraction, one should pay attention to the negative side of warming. It has been predicted that global warming will cause more frequent and prolonged drought periods. Limited water supply can affect tree species and lead to dieback of sensitive species. Drought led to stomata closure and restriction of CO_2 input, which causes imbalances between excitation energy driving electron transport and electron consumption in Calvin cycle reactions (Flexas et al. 2004; Tausz et al. 2004). These imbalances cause the formation of harmful reactive oxygen species (ROS) and (photo-)oxidative stress (Tausz et al. 2004). Antioxidative and photoprotective defense systems counteract damage caused by ROS (Smirnoff 1993). Reactive oxygen species also results in photosynthesis decrease, but increased transpiration and stomatal conductance, and in turn, reduced plant biomass (Jones 1992; Nobel 2009; Qaderi and Reid 2009), due to the fact that plants usually produce smaller leaves and extensive root systems to increase water uptake from soil but reduce water loss from leaves (Gliessman 1998).

It is worth underlining that a significant role in soil is also played by animals inhabiting the soil environment. While the composition of organic matter is conditioned by many factors, as described above, a little should be mentioned about the animals inhabiting the soil environment, in particular earthworms. They are an essential part of the soil fauna and are regarded as a useful indicator of soil health and quality. The most important role of earthworms in soil is organic matter decomposition and subsequent cycling of nutrients. Also, data available in the literature indicate that their presence in the soil may have humifying action, because some humic acids are detected only in soil where earthworms are present (Spurgeon et al. 2003; Sizmur and Hodson 2009). For this reason they have a biological impact on soil pollutants, because it is known that organic metal compounds are more bioavailable due to the fact that microorganisms inhabiting the soil while the metals are released in solution readily decompose organic components. Next, metals consequently released into solution may be chelated by compounds produced by earthworms and further absorbed and taken up by the plants (Ruiz et al. 2011). Moreover, earthworms have a direct impact on the cycle and metabolism of nutrients, which has a significant impact on the physical, chemical, and biological properties of the soil. Sizmur and Hodson (2009) presented a conceptual model of how earthworms may impact metal chemistry in soil, and regardless of the type of soil, the results clearly show that the presence of earthworms affects the bioavailability of elements. Physical and chemical properties of the soil have a significant impact on the phytoextraction process, indicated by the fact that numerous studies on remediation of soils in contaminated areas have shown how by modifying the properties of the soil one can increase the accumulation process and at the same time accelerate the remediation process. For this reason, the development of phytoremediation as a useful technique for soil remediation is focused on soil properties, where metals occur in soluble form, easily accumulated by plants (Lasat 2002).

4.3 Conclusions

- Phytoremediation is the complex of environment purity improvement techniques based on plants' abilities and depends on several environmental factors.
- Oxidative stress associated with water availability influences the techniques' efficiency.
- Soil is the most important environmental factor in the effectiveness of the phytoremediation process.
- Availability for plants of trace elements contaminating the environment depends on pH value of the soil and/or water.
- Rhizospheric microorganisms may significantly increase the bioavailability of trace metals.
- Salinity of soil reduces growth of plants and effectiveness of the techniques.
- Earthworms present in soil have a direct impact on phytoremediation.

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Landscape Frameworks for the Revitalization of Urban Neighborhoods in the Context of Phytoremediation

5

Frank Sleegers

5.1 Scope and Introduction

This study focuses on selected case studies of unbuilt scenarios and built projects that explore brownfield remediation and specifically phytoremediation within urban contexts. It investigates overlooked opportunities that lie in the interdisciplinary overlap of environmental sciences, urban systems design, aesthetics, and cultural crossovers. The study begins with a short introduction of the contexts common to urban brownfield and phytoremediation techniques and is followed with an overview on the current research green infrastructure as it represents a spatial, multi-scalar strategy to transform urban areas. The case studies include: (a) one built example of successful urban brownfield transformation—Westergasfabriek in Amsterdam (NL); (b) one example of communicating and staging the transformation of an industrial brownfield in Lowell, MA; and (c) two visionary research-by-design scenarios. The findings are relevant for scientists focusing on incorporating phytoremediation and remediation in urban contexts, as well as decision makers and community stakeholders.

5.2 Background

5.2.1 Context of Urban Brownfields

Contamination is not the only problem of urban brownfields—a majority of these areas are located in deprived urban neighborhoods with limited access to public open space and a lack of resources and opportunities that range from education over to unemployment or food deserts, while contamination is an impediment for future investment and economic development. These areas are a burden for municipalities as they bring in no tax revenue and leave cities and neighborhoods with a negative perception. While these negative factors play a decisive role, these brownfields share common opportunities as many of them are located within reach of urban centers. With the problem of increasing suburbanization of cities and the excessive consumption of undeveloped land in the peripheries, they could be recycled and balance the lack of open space and lack of housing opportunities in dense urban areas.

5.2.2 Phytoremediation

Phytoremediation has the capacity to assist in the remediation of petroleum hydrocarbons, benzene, and heavy metals, which are among the common toxics found in urban brownfields. The simultaneous treatment of these multiple contaminants is claimed to make phytoremediation a cost-effective and attractive option (Raskin and Ensley 2000, ix; Marmiroli and McCutcheon 2003). Plants typically tested in phytoremediation include trees such as hybrid poplars and willows and herbaceous perennials and grasses including yellow iris, reeds, cattails, legumes, and mustards. The roots of plants used in phytoremediation help to rebuild soil structure in the rhizosphere, and they also contribute to the improvement of soil structure through the deposition of organic material from leaves, branches, and root cells. Repeated planting and harvesting may be required to reduce contaminant levels to permissible limits. Phytoremediation technologies can be classified into five categories that are not mutually exclusive and may occur simultaneously: (a) phytostabilization (plant roots limit the mobility of contaminants and thus stabilize, rather than remove them), (b) phytodegradation (plant metabolism transforms, breaks down, stabilizes, or volatilizes organic compounds from the soil and groundwater into harmless by-products), (c) phytoextraction or phytoaccumulation (absorbs contaminants from the soil and translocates them to harvestable shoots and leaves), (d) phyto- or rhizofiltration (plant roots remove or contain toxics in aquatic environments),

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and (e) phytovolatilization (plants take up organic and inorganic contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere at comparatively low concentrations) (Ghosh and Singh 2005).

While conventional remediation techniques such as excavation and disposal off-site or on-site-treatments by capping techniques are prevailing, phytoremediation has become common practice in centralized stormwater and rainwater treatment facilities (Zhang et al. 2007); as a system and process-oriented technology for urban transformation, it is still on a conceptual level. Unrealistic expectations about time management, performance capabilities of phytoremediation techniques, an underrating of the complexity of the cleansing process, and the intricacy of the urban and social context of contaminated sites may be the prevailing issues. The disadvantage of phytoremediation such as the time factor, the limited applicability for a mix of pollutants, or possibly toxic effects on the plants could be balanced by the advantage of using low capital and operating cost and the great potential of capitalizing on the aesthetical and cultural values of this technique.

Concepts to spatially allocate phytoremediation more strategically could help advance and proliferate it as a technology and are among the key concerns of this paper.

5.2.3 Green Infrastructure and Landscape Urbanism

Green infrastructure can shape urban form and is principally structured by a hybrid hydrological drainage network, complementing and linking relict green areas with built infrastructure that provides ecological and social functions (Benedict and McMahon 2006, 2–4, 35). Green infrastructure applies key principles of landscape ecology to urban environments as a multi-scale and multilayered approach. The green infrastructure pattern derives from ecological and social process relationships with an emphasis on connectivity and context. Benedict defines the green infrastructure as “an interconnected network of green space that conserves natural ecosystem values and functions and provides associated benefits to human populations” (Benedict and McMahon 2006). Ahern and Kato (2007, 287) present a definition of green infrastructure as “Integrated networks/systems of built and protected/managed urban ecosystems that provide multiple, complementary functions in support of urban sustainability.” Ahern (2006, 267–269) further grounds his findings on the corridor–patch–matrix theory (Forman and Godron 1984), yet expands the definition with the widely accepted resource model for landscape planning in the abiotic, biotic, and cultural (ABC) resource model (Ndubisi 2002; Ahern 1995). While proposing interdisciplinary and transdisciplinary collaboration, he emphasizes that the urban hydrological and drainage system principally frames the structure of the green infrastructure. An explanatory example is Weller’s master plan for the Wungong Urban Water Landscape Structure Plan in Perth, Australia that focuses on landscape as an infrastructural system. The existing vegetation and the Wungong River system are part of the landscape structure that ensures the protection and creation of landscape systems—habitat, drainage, and public open space. Park-like avenues 26 m in width become multifunctional linear elements as grass drainage swales and recreational corridors. They create the landscape framework that connects to the Wungong River, larger community parks, smaller patches of active and passive open space, schools, and developable land (Weller 2008).

The current research of green infrastructure and landscape urbanism describes multi-scalar and multilayered spatial landscape frameworks for urban environments that are developed through interdisciplinary and transdisciplinary collaboration. Their application may create new opportunities for phytoremediation with the focus on transforming urban brownfields as a process-oriented and systematic connectivity tool.

5.3 Case Studies

5.3.1 Westergasfabriek Amsterdam: Integration of Open Space System, Historic Heritage, and Mainstream Cultural Venues

A built and well-published example is the Westergasfabriek in Amsterdam, Netherlands (Margolis 2007; Spens 2007). This, an inner-city formerly contaminated site, illustrates how a diversity of strategies redefined an urban brownfield. The Westergasfabriek has been a coal gas plant from 1885 to 1967 and left a large and underutilized contaminated area within the inner-city area of Amsterdam. Artists discovered the area in the early 1990s and created a small cultural center. Simultaneously, the city planned to remediate the complete area which resulted in an invited design competition in 2000. The winners of the competition, Gustafson Porter landscape architects and Mecanoo architects, realized the project in 2005. The challenges of contamination were solved in a safe but rather conservative way—a capping method was chosen to keep contaminants on-site while isolating them from further uses. A strategy of short-term uses and long-term planning was beneficial for the project—short-term uses would incorporate cultural activities within the historic buildings and put the area on the map, while long-term planning would incorporate the remediation of the buildings and contaminated areas to build the park. Today the area provides a diverse pool of destinations and activities. The remaining architecture on-site are industrial heritage monuments that were adapted to house contemporary uses—commercial and noncommercial—from mega-events in a former, gigantic gasholder to small conference rooms at an old meter house for a maximum of 40 people, restaurants, clubs, and a day care center. The new 14-hectare public park accommodates a rich diversity of program elements from picnic to open-air concert areas and paddling on a canal and integrates a pedestrian and bicycling system that connects to the municipal open space system (Westergasfabriek 2013).

This case study is an example of rebranding an urban brownfield through creative adaptive reuse of existing architecture on-site, integration through implementation of diverse cultural and social uses, and the creation of multiscalar and multipurpose public open space that connects to a citywide system for pedestrians and bicyclists.

5.3.2 Tanner Street Initiative: Comprehensive Staging and Communication Strategies

The Tanner Street Initiative is a comprehensive, 25-year plus redevelopment study completed by StoSS Landscape Urbanism of Boston, MA, in 2002 to address larger ecological, cultural, recreational, and cultural issues around the 4.5-acre Silresim Chemical Co. Superfund site in Lowell, Massachusetts (StoSS 2002). This study was conducted because the city decided that the cleanup would not be effective without including the entire district. The EPA had been treating the heavily contaminated groundwater on the site since the mid-1990s (EPA 2013). Since 2011, the property undergoes a major cleanup through a thermally enhanced soil vapor extraction technology that was funded through the 2010 President Obama's "American Recovery and Reinvestment Act." StoSS' study contains valuable aspects for communicating and staging a remediation process. It was developed through numerous meetings with local and regional community stakeholders and residents. The result is a timeline of staging the recovery of the contaminated site and the surrounding neighborhood. Carefully sequenced and overlapping program elements are defined and proposed to create a process-oriented strategy within an overarching time-based framework of temporary events, catalysts, incubators, scaffolds, and speculative futures (Table 5.1).

StoSS' strategy is supported through convincing on- and off-line communication and presentation tools that are integrated into the public processes and targeted the transformation of the whole neighborhood as it engages aesthetics, arts, social and cultural values, and ecological performance (Fig. 5.1). Imaginative visual tools can thus drive, energize, and catalyze public perception and participation. It may facilitate access to new funds. While not all of the ideas were implemented, one tangible positive result of the Tanner Street Initiative is the development of a strategic neighborhood master plan that was published in late 2013 as a result of a more than 10-year-long community participation process (Interview 2013).

5.3.3 Hamburg–Wilhelmsburg: Multifunctional Water-Based Green Infrastructure Network

Samimi and Wang's research-by-design study "Rhizotopia" in Hamburg–Wilhelmsburg, Germany (Samimi and Wang 2007), was a product of an interdisciplinary urban design laboratory at the University of Massachusetts Department of Landscape Architecture and Regional Planning with advising from the Environmental Science Program at the university and a collaboration with the International Building Exhibition Hamburg. The study comprises an approximately 1 km^2 large territory in the less active eastern area of the port area of Hamburg, Germany, at the eastern edge of the channelized Reiherstieg branch of the tidal Elbe River. This place is located just 2 miles south from the center of downtown Hamburg, is mostly protected by dikes, and is only 1–2 m above sea level with a high water table. The territory is contaminated with heavy metals, oils, and organics due to the existence of former oil refineries and industrial activities since the beginning of the nineteenth century. While the refineries were extinct during World War II, some of the industrial activities remained until a big flood in 1962 destroyed the already declining industries. They left behind underutilized and secluded areas of contamination that have not been fully assessed and are close to residential areas further east (Fig. 5.2).

"Rhizotopia" combines phytoremediation techniques with the strategies of applying principles of a green infrastructure framework that transforms over time with places for educational interventions (Figs. 5.3 and 5.4). East-western arteries connect the residential neighborhoods of Hamburg–Wilhelmsburg to the underutilized tidal Reiherstieg branch of the Elbe River. These arteries are complemented by other streets in a grid pattern and form an interconnected system as a spatial infrastructure framework. In the beginning, the proposed phasing plan suggests the construction of a gridded system of ditches with grasses and multilane allées of fast-growing, deep-rooting hybrid poplars (*Populus deltoides*) and willows (*Salix*). The grid system allows for flexible cleansing and development options within the created blocks such as highly desired new housing opportunities in the area. These blocks could be remediated instantly with conventional methods to meet regulatory benchmarks—or they could be cleaned up by longer-term

Table 5.1 Staging timeline for proposed activities on the Silresim site and the adjacent Tanner Street neighborhood. The program elements were extracted from web-based information material that was generated by Stoss Landscape Urbanism in 2002 (Stoss 2002)

Silresim site	Tanner street corridor
Events 0–2 years “**Temporary and interim events, designed to raise public awareness of the site, its diverse resources, and the clean-up process, as well as to allow participation in the site’s recovery**.” (Stoss 2002)	
Site tours Holiday light festival Movie nights	Environmental art installation Pothole repair River meadow Brock clean-up/community day East pond clean-up/family picnic day
Catalysts 0–5 years “**Interim installations, demolitions, and small-scale constructions that physically re-make the image of the site, thus attracting investor interest, bolstering funding plans and allocations, and initiating other transformations**.” (Stoss 2002)	
Re-cladding of water treatment plant (opaque to transparent) Movable elevated walkway installations Movable solar farm test plots, interim park eco-tech	Phytoremedial forest demonstration installation Property screen/hedgerow installation Identity vine installation Recreation remediation trail installation Lowell connector gateway construction Property taking/demolition/sedding
Incubators 2–15 years “**Small-scale business ventures geared to providing short-term returns but serving as long-term models or generators of new business expansion and redevelopment**.” (Stoss 2002)	
Green technology demonstration site	Re-training center Short-term redevelopment sites
Scaffolds 5-20 years “**Physical infrastructures and frameworks that require significant public and private investment, remaking the circulatory, infrastructural, and ecological foundations of the district**.” (Stoss 2002)	
Water processing system construction Remedial treatment construction	Tanner street reconstruction New and extended roads construction River Meadow Brook remediation terraces East Pond park construction Ecological infrastructure installation
Speculative futures 10–100 years	
Distributed energy generation site (solar farm) Metal recycling facility	Tourism service district (motels, retail) Lowell national historical park expansion High—rise commercial condos Junkyards, used car lots, processing facilities, energy corridor (co-firing)/renewable generation Commercial/industrial park Green technology corridor
Network	
Sacred heart, community advisory board, city of Lowell DPD, art advisory committee, city-wide advisory committee, coordinating committee	

phytoremediation techniques. The network of allées will be able to remediate medium to lighter concentrations of soil pollutants and stabilize the flow of hazardous substances into the groundwater. In the future, the ditches can accommodate a coherent system for stormwater cleansing and disposal. The grid layout will also allow new recreational connections and delineate the future layout of neighborhood streets that are integrated with the existing, adjacent residential areas to enhance the accessibility of public open space and the overall quality of life. In addition, the remediation infrastructure is a habitat for wildlife, and the plants can be harvested and used for energy-producing processes (Sleegers 2010a, b).

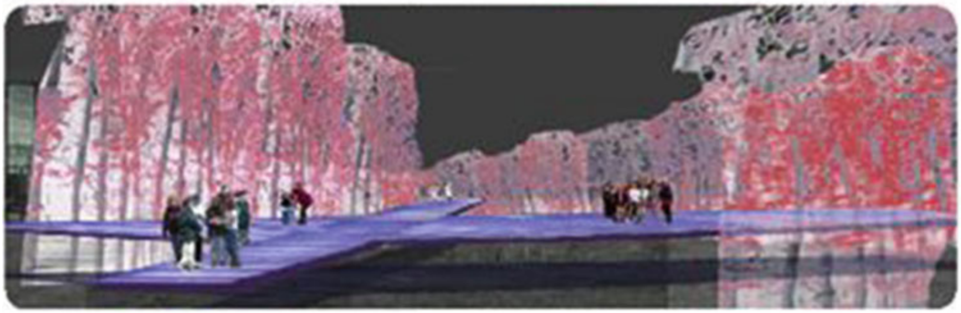
This case study is an example how a spatial network can service phytoremediation and simultaneously hold multiple functions as an interconnected system for recreation and transportation in a once isolated neighborhood. The study utilizes the diverse plant selection to respond to the variety of contaminants while enriching aesthetic values. It also demonstrates that the functions of this phytoremediation network can grow and transform into a system for surface water treatment. Noteworthy is the fact that the research study was developed in an interdisciplinary collaboration including the disciplines of landscape architecture and the environmental sciences.

5.3.4 Amsterdam Buiksloterham: Landscape Framework Through Classification of Pollution Levels and Dynamics of the Landscape

Wilschut et al. (2013) tested the potentials of phytoremediation in Buiksloterham, a former landfill in the harbor area of Amsterdam-North. Like the area in Hamburg, this landfill has been used for industrialization since the beginning of the 1900s and left vacant and contaminated parcels behind that are an impediment for further investment and revitalization.

Speculative review of an art festival along the railroad tracks near East Pond

Speculative review of an environmental cleanup day along River Meadow Brook



Speculative review of a temporary holiday light festival on the superfund site

Fig. 5.1 Examples of proposed temporary and interim events to raise public awareness of the site. This information was obtainable through a specifically created website (StoSS 2002)

Basic information about the contaminants was available through soil quality reports and average pollution concentrations—zinc being the main pollutant, followed by lead and copper, PAKs, and mineral oils. The terrain has a high groundwater table of 0.9–1.5 m. The authors of this study created a spatial framework that was based on three criteria: (a) the levels of contamination with a resulting anticipated cleanup time, (b) allocation of spatial typologies in relation to planned land use dynamics and level of contamination, and (c) a plant selection for the three phytoremediation functions of stabilization, degradation, and accumulation (Fig. 5.5). These criteria were synthesized and resulted in a master plan and a number of illustrative landscape typologies that creatively translate the spatial framework into tangible visual models.

The remediation time needed to reduce the levels of contamination for meeting the regulatory threshold set by municipalities was developed through a regression model (Koopmans et al. 2007) for the zinc-hyper accumulator alpine pennycress (*Thlaspi caerulescens*). Based on average contamination values and the phytoremediation capacity of alpine pennycress, the authors could geographically classify the area into the three groups from clean to highly polluted. These three groups allow for a differentiation into areas where phytoremediation is useful or not and resulting possible land uses. The contamination level also allows for basically two levels of dynamics that can be related to spatial typologies and lead to a framework concept that was originally developed for an integration for nature, conservation, water management, and industrial agriculture in rural areas (Kerkstra and Vrijlandt 1989). Clean parcels allow for all kinds of land uses and allow for quick change over time. Medium polluted areas will take some time to clean up and can thus create a landscape framework including landscape typologies that accommodate phytoremediation such as streets, canals, and parks. Heavily polluted and potentially hazardous areas can also be included in this framework. The authors propose omnipresent barges with displaced soil on the canals as floating phytoremediation gardens. After identifying and strategizing

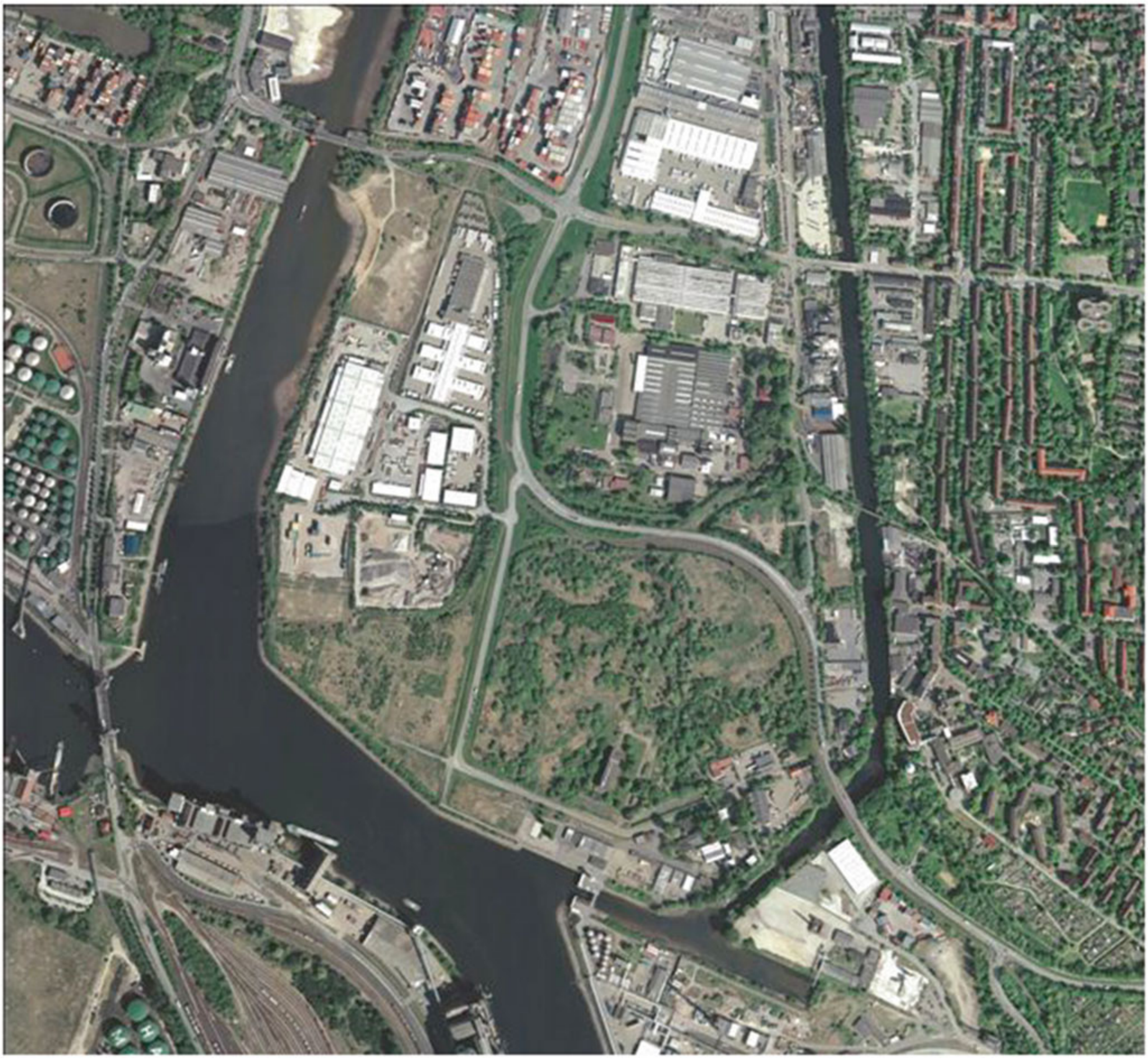


Fig. 5.2 Aerial photograph. The largest concentrations of contaminants are found in the tree-covered areas. This area has been underutilized since the end of World War II (Google earth 2014, © AeroWest 2014)

spatial allocations, it is necessary to select plants according to their phytoremediation capacities and desired aesthetical values. Examplary plants are black willows (*Salix nigra*) and poplars (*Populus deltoides*) that are proposed for the stabilization of organic and inorganic pollutants as these plants create an upward groundwater flow; tufted hair grass (*Deschampsia cespitosa*), broadleaf cattail (*Typha latifolia*), and basket willow (*Salix viminalis*) are used for degradation of pollutants in wet environments; and alpine pennycress (*Thlaspi caerulescens*), Indian mustard (*Brassica juncea*), or hybrid aspens (*Populus tremula x Populus tremuloides*) accumulates pollutants. Like in Hamburg, for Buiksloterham, the authors propose the production of biomass as temporary short-time uses on clean parcels or on medium polluted areas. Overall, the proposed scenario could be a successful framework to spatially allocate different future functions or land uses of urban brownfields in relation to contamination, anticipated economic activity level, and plant selection—the latter one in respect to aesthetics and phytoremediation functions (Figs. 5.6, 5.7, and 5.8).

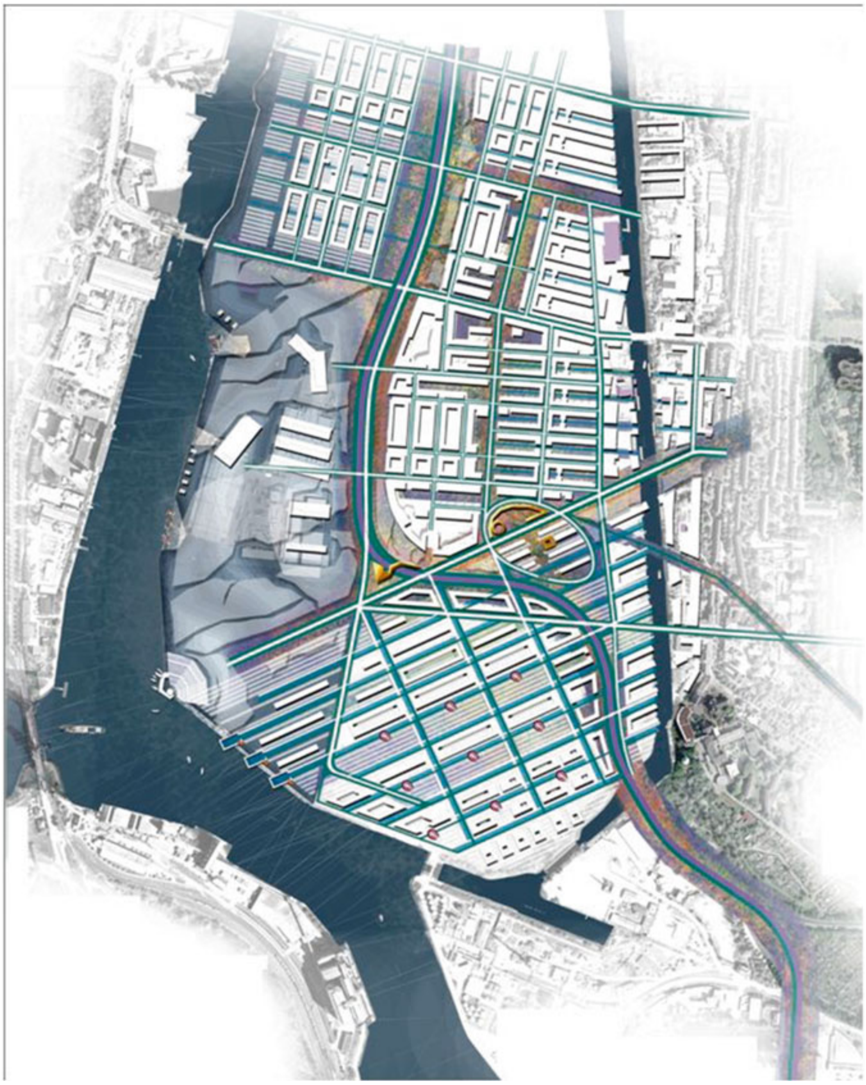
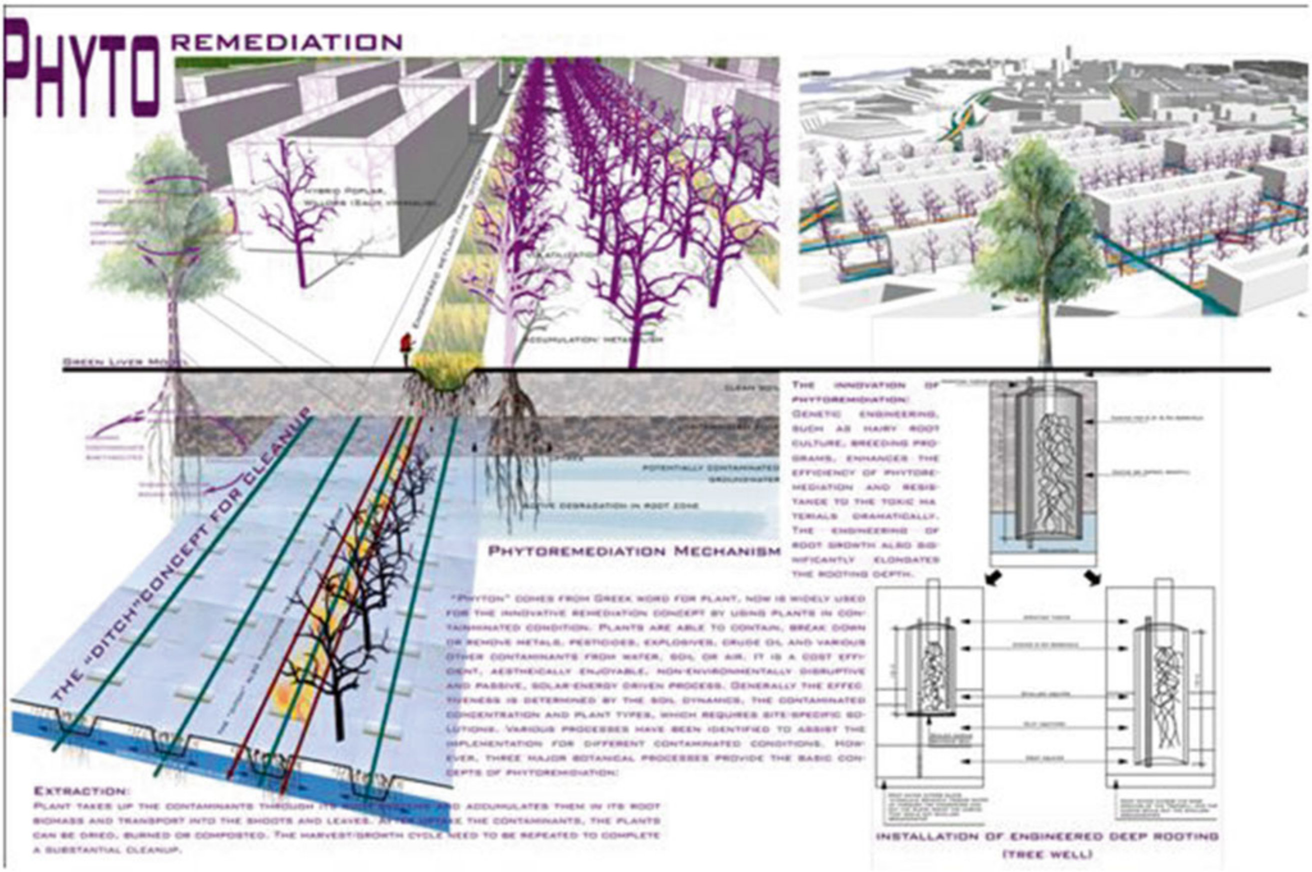


Fig. 5.3 A comprehensive master plan creates new connections from east to west that are part of a green infrastructure network (Samimi and Wang 2007)

5.4 Discussion and Conclusions

The cases introduce comprehensive lessons to learn from more general examples of urban brownfield remediation to specific scenarios of phytoremediation strategies. The findings propose a simultaneous application of multifaceted and overlapping strategies and recommendations:

1. A combination of phytoremediation with a water infrastructure network that is integrated into the open space system as applied green infrastructure and establishes a multi-scalar and multilayered spatial landscape framework for urban environments, transforming urban brownfields through a process-oriented and systematic connectivity tool. This system can grow and transform into a system for surface water treatment.
2. Space–time models include different levels of pollution and anticipate cleanup time at a larger scale. The allocation of different future functions or land uses of urban brownfields in relation to contamination and the anticipated or planned economic activity level is crucial for organizing a landscape framework.
3. The variety of aesthetical values of plants in the phytoremediation has been identified in many visual scenarios. For a success of this strategy, it is important to consider their remediation capability, time aspects, and toxic tolerances.
4. The architectural heritage of remediation sites has possibilities of adaptive reuses that add cultural values.
5. Staged phasing frameworks integrate cultural, social, and educational processes as they secure and extend the physical network.



THE INNOVATION OF PHYTOREMEDIATION: GENETIC ENGINEERING, SUCH AS HAIRY ROOT CULTURE, BREEDING PROGRAMS, ENHANCES THE EFFICIENCY OF PHYTOREMEDIATION AND RESISTANCE TO THE TOXIC MATERIALS DRAMATICALLY. THE ENGINEERING OF ROOT GROWTH ALSO SIGNIFICANTLY ELONGATES THE ROOTING DEPTH.

"PHYTON" COMES FROM GREEK WORD FOR PLANT, NOW IS WIDELY USED FOR THE INNOVATIVE REMEDIATION CONCEPT BY USING PLANTS IN CONTAMINATED CONDITION. PLANTS ARE ABLE TO CONTAIN, BREAK DOWN OR REMOVE METALS, PESTICIDES, EXPLOSIVES, CRUDE OIL AND VARIOUS OTHER CONTAMINANTS FROM WATER, SOIL OR AIR. IT IS A COST EFFICIENT, AESTHEICALLY ENJOYABLE, NON-ENVIRONMENTALLY DISRUPTIVE AND PASSIVE, SOLAR-ENERGY DRIVEN PROCESS. GENERALLY THE EFFECTIVENESS IS DETERMINED BY THE SOIL DYNAMICS, THE CONTAMINATED CONCENTRATION AND PLANT TYPES, WHICH REQUIRES SITE-SPECIFIC SOLUTIONS. VARIOUS PROCESSES HAVE BEEN IDENTIFIED TO ASSIST THE IMPLEMENTATION FOR DIFFERENT CONTAMINATED CONDITIONS. HOWEVER, THREE MAJOR BOTANICAL PROCESSES PROVIDE THE BASIC CONCEPTS OF PHYTOREMEDIATION:

EXTRACTION:
PLANT TAKES UP THE CONTAMINANTS THROUGH ITS [illegible] AND ACCUMULATES THEM IN ITS ROOT BIOMASS AND TRANSPORT INTO THE SHOOTS AND LEAVES. AFTER UPTAKE THE CONTAMINANTS, THE PLANTS CAN BE DRIED, BURNED OR COMPOSTED. THE HARVEST/GROWTH CYCLE NEED TO BE REPEATED TO COMPLETE A SUBSTANTIAL CLEANUP.

Fig. 5.4 Proposed reed and grass planted remediation ditches (*Agrostis* ssp., *Festuca* ssp., *Lolium* ssp., *Panicum* ssp.) and multilane allées of fast-growing, deep-rooting hybrid poplars and willows (*Populus deltoides*, *Salix* ssp.) become part of the street and pedestrian circulation network. This network creates a framework for the future urban form of the project area and connects it to the existing neighborhood (Fig. 5.3). After the area has been remediated, the water remediation network can be transformed into a surface stormwater treatment system, and the multilane allées can become street boulevards with permanent street trees (Samimi and Wang 2007)

6. Interdisciplinary collaborations between scientist and designers secure aesthetic values and integrate the phytoremediation technology with larger urban systems.
7. Illustrative and engaging visual communication techniques for staging, design, and implementation are tools that can well integrate with the public processes and target the transformation of the whole neighborhood as they engage aesthetics, arts, social and cultural values, and ecological performance. Graphic presentation and visualization rebrand neighborhoods and stimulate discussion about phytoremediation, innovative solutions, future research, and new aesthetic paradigms.

Phytoremediation is a complex science that, if realized in complex urban situations, needs multi-scalar and interdisciplinary long-range plans. The spatial concepts of phytoremediation have more opportunities than explored so far. The described frameworks provide lessons to learn for creating more comprehensive and multilayered strategies and thus provide a synthesis that is valuable for scientists, designers, planners, and stakeholders on the regional and municipal level. While many of the described strategies and recommendations are not new, they are currently practiced in isolation. A simultaneous application of this palette of strategies may embrace and synergize visual, spatial, scientific, and cultural elements as multiscalar approach. Executed, they will result in the propagation of phytoremediation as a technique to transform derelict urban landscapes.

I. Degree of Pollution, Remediation Time, and Potential Uses		
Pollution Category	**Remediation Time**	**Potential Uses**
Clean	none	All uses possible - long-term and temporary uses
Medium	< 45 years	Phytoremediation possible
High	> 45 years	No on-site phytoremediation, Displacement nearby possible

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II. Dynamics of the Landscape and Spatial Typologies		
Pollution Category	**Level of Dynamics**	**Spatial Typology**
clean	High dynamic system	parcels
medium	Low dynamic system	streets canals parks
high		

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III. Plant Selection and Phytoremediation Function	
Phytoremediation Function	stabilization
	degradation
	accumulation

Fig. 5.5 Matrix of major elements of a landscape framework

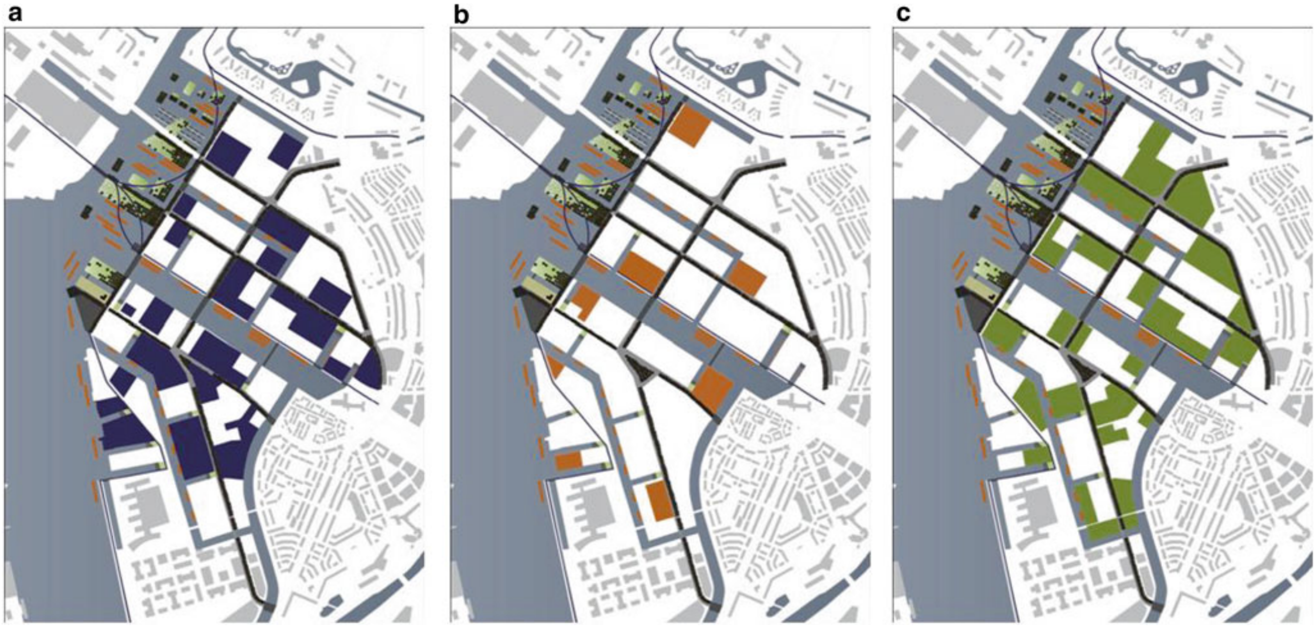

Fig. 5.6 Maps of landscape frameworks and the indication of heavily polluted parcels, medium polluted parcels, and clean parcels—from *left* to *right* (Theuws and Wilschut 2009). From Theuws and Wilschut (2009); published in Theuws P, Wilschut M, Duchhart I (2013) Environ Pollut 183:85–86

Fig. 5.7 Landscape typology "accumulation park." This park is made accessible by a raised path and planted with a mixture of phytoextraction species combined with rows of poplars. From Theuws and Wilschut (2009); published in Theuws P, Wilschut M, Duchhart I (2013) Environ Pollut 183:85

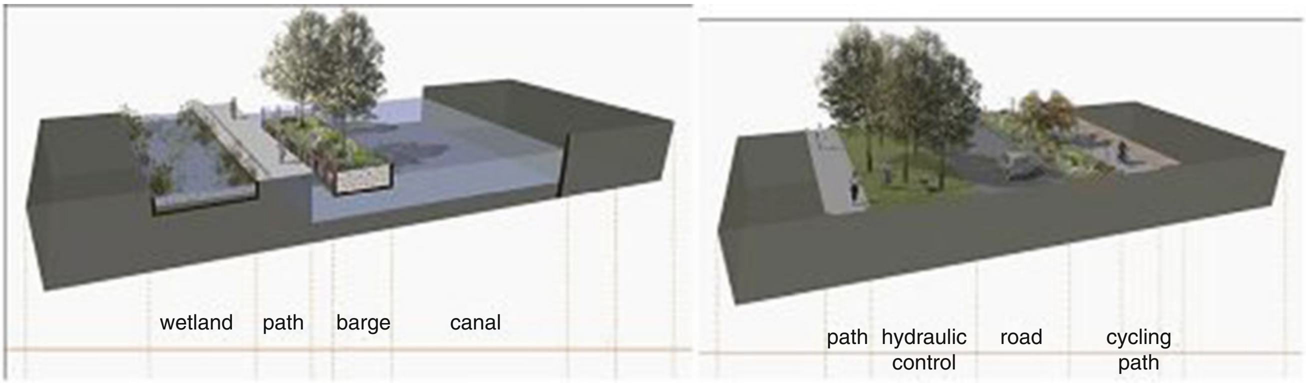

Fig. 5.8 Landscape typologies "canals with barges and wetlands" and "roadsides." From Theuws and Wilschut (2009); published in Theuws P, Wilschut M, Duchhart I (2013) Environ Pollut 183:84

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6

Phytoextraction of Metals: Modeling Root Metal Uptake and Associated Processes

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6.1 Introduction

Phytoextraction is a process which can remove the soluble (bioavailable) metal pool from soil or aqueous solutions using different kinds of terrestrial or aquatic plants. This transport may be divided into three different steps: (1) absorption from the solution (e.g., soil solution), (2) transport to the root xylem, and (3) transport to the shoots (Mench et al. 2009).

A modern approach, how to evaluate the extraction potential of selected plants as well as how to plan and design the most effective metal phytoextraction, is the ability to relevantly model this process. There are many studies aimed at the modeling of metal transport. Nevertheless, there is a limited amount of present studies, which are focused on the modeling of the phytoremediation process. In general, developing an applicable model requires sufficient conceptual model description and a fundamental theoretical understanding of the studied system, as well as its experimental equipment and measurement; these are all crucial aspects required to create a relevant simulation of studied problem. This chapter attempts to describe the problems associated with metal phytoextraction modeling. The term "root metal uptake" is crucial for the simulation of phytoremediation of metals, which is a concrete modification of the general term "root uptake." Root uptake is one of the most important processes considered in numerical models because root surfaces represent the first-phase boundaries in nature for nutrients and toxic elements. In order to efficiently model the phytoextraction processes, it is essential to provide information about (1) the spatiotemporal concentration changes of metals in the root-influenced soil and (2) the cumulative uptake of metals per unit length of root over time (Darrah et al. 2006).

6.2 Root Uptake

Determining water and metal uptake by plant roots and their subsequent translocation into the tissues of the aerial parts is obviously critical for assessing the modeling of phytoremediation options. Because metals are taken up by plants dissolved in water, it is crucial to have information about the transport of water and the hydraulic properties of the roots.

6.2.1 Root Water Uptake

Water is primarily absorbed by the root hairs and other root zones and then transported to the aerial parts in xylem tissues. Water absorption by the plants is mainly a response to the water potential gradient (from low to high energy) from the rhizosphere to the xylem of the roots. Soil water has a lower total solute concentration than plant water, and hence the osmotic gradient tends to drive water from the soil into the root. The rate of water uptake from the soil depends on rooting density, the hydraulic conductivity of the roots, and the difference between average soil–water suction and root suction. The root hydraulic conductivity is a measure of the amount of water transported by the root in an interval of time at a determinate pressure [mg g^{-1} DW h^{-1} MPa^{-1}] (Steudle et al. 1987). It depends on osmotic potential gradients which result from differences in the composition and the concentration of solutes, and also on the plant species and its developmental stage.

In order to reach the central cylinder of the root with the vascular bundles, the movement of water and dissolved ions follows the dimensional network of cells. A portion of the total flow may occur by diffusion through the walls or by passing into the cortical cells and thence to the central cylinder through the plasmodesmata linking these cells. The set of walls and intercellular spaces is called the *apoplast*, and the protoplast interconnected network is called the *symplast* (Fig. 6.1). The proportion of water that follows the apoplastic and symplastic pathways depends on the resistance

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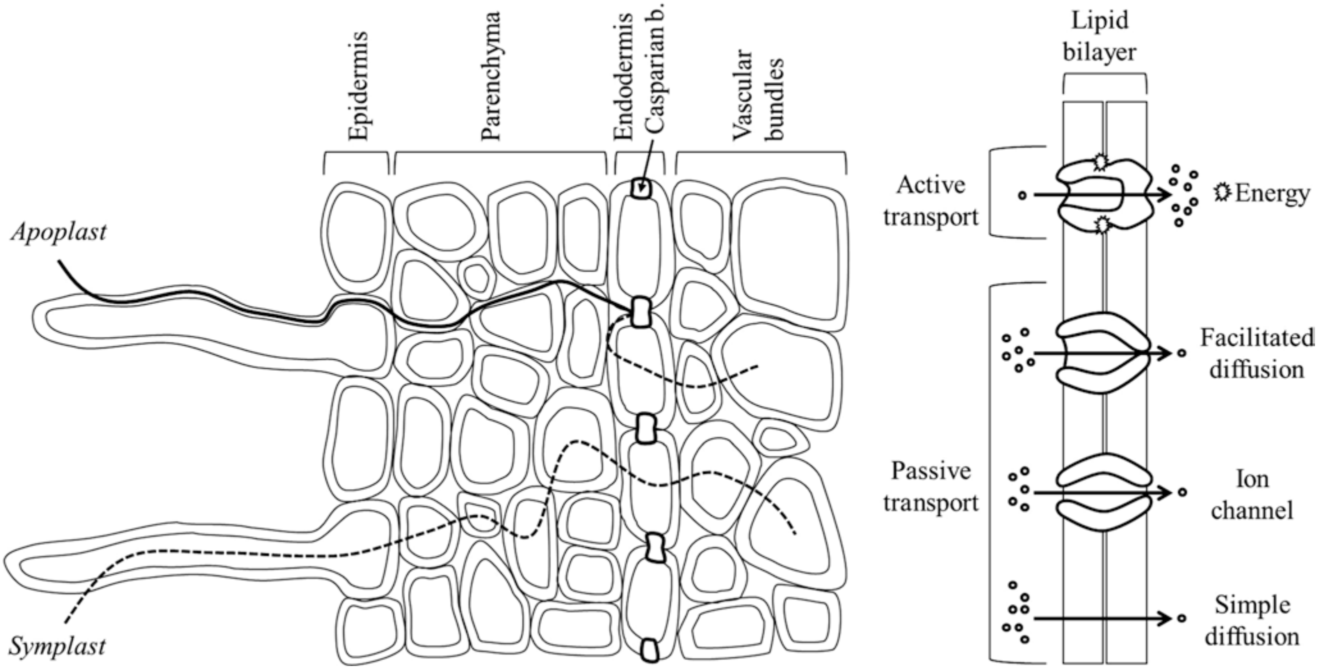

Fig. 6.1 In the area of greatest absorption of the roots, the water with dissolved metals can enter until the parenchyma via the apoplast or go into the cells via the symplast. If water enters the cells, the metals will be forced to go across the plasma membrane using any of the transport systems, whether active or passive. Once in the endodermis, the Casparian band forces all water and metals to follow the intracellular pathway. From the vascular bundles, water and solutes can be metabolized or ascend to the rest of the plant

offered by both paths, but both are controlled by the water potential gradient generated between the two sides of the endodermis. The particular structure of the endodermis confers high resistance to water passage, because of the presence of the Casparian band, a suberized band in the radial and transverse walls of endodermal cells, and blocks the passage of water through the cell walls. Consequently, the water goes across the endodermis by the symplastic route. Due to the nature of the endodermis and its physical properties, it behaves like a semipermeable membrane (Hopmans and Bristow 2002); this can be useful during modeling, since it is possible to assign physical parameters such as diffusivity, permeability, selectivity, etc. Additionally, the effects of environmental factors on the permeability of the roots should be similar to the influence of these factors on the permeability of the membranes, in general.

During daylight hours, the evapotranspiration from the leaves increases the hydrostatic pressure there, resulting in the sub-pressure called tension that pulls water upward from the roots. Therefore, water transport across roots is purely passive, without an input of metabolic energy.

6.2.2 Root Metal Uptake

6.2.2.1 Rhizosphere and Adsorption

In the soil, the metals can be present as free ions, soluble metal compounds, and insoluble compounds such as oxides, carbonates, and hydroxides. Metals can remain in the soil in four different ways: (1) retained in the soil, either dissolved in the aqueous phase of the soil or occupying exchange sites, (2) specifically adsorbed on inorganic soil constituents, (3) associated with soil organic matter, and (4) precipitated as pure or mixed solids.

Plants can adopt different strategies in the presence of metals in the environment, depending on the plant species and the characteristics of the metal content of the soil (Baker 1981; Barceló and Poschenrieder 2003). Roots of plants grown in metal-contaminated sites can develop mechanisms to protect the plant from excessive uptake of a metal (Santa-María and Cogliatti 1988), acting as a “barrier” and basing their metal resistance on an efficient strategy of metal exclusion, restricting transport to the shoot. On the other hand, other species accumulate the metals in the aerial parts in a form nontoxic to the plant. Exclusion is more common for species that are sensitive and tolerant to metals, while accumulation is the preferred strategy for species that always grow in contaminated soil.

There are many physical, biological, and physiological mechanisms that are involved in the uptake of metals by plant roots (e.g., Hopmans and Bristow 2002; Jungk 2002; Darrah et al. 2006). In more detail, metal uptake is influenced by the spatial distribution of the root system, its architecture and morphology, and the presence of more active sites of uptake like root hairs (Somma et al. 1998; Biondini 2001; Javaux et al. 2008).

Table 6.1 Deficiency and toxicity limits (mg kg^{-1} DW) in plants for the main trace elements, their absorption forms, elements with which they compete or interfere, and their main mechanisms of uptake during situations in normal conditions, under the excess limit for the plants (Hilbold 1975; Bergmann 1988; Balsberg Påhlsson 1989; Mengel and Kirkby 1987; Dordas and Brown 2002; Fitz and Wenzel 2002; Meers et al. 2005; Lefèvre et al. 2010; Oliveira 2012)

Element	Limit deficiency	Limit toxicity	Absorption form	Competition	Uptake
As	–	3.50	$(AsO_4)^{3-}$	P as PO_4^{3-}	Active by phosphate transporters
B	5–30	50–200	H_3BO_3	–	Passive
Cd	–	3–10	Cd^{2+}	Ca, Mg	Passive, but also active
Co	Only for legumes	100–1,000	Co^{2+}	Fe, Zn, Cd, Cu, Mn, Cr, Mg, S, Ni	Active
Cr	–	100–300	$Cr^{6+} > Cr^{3+} > CrO_4^{2-}$	Ca, Mg, Fe, Mn, Cu, S, P	Cr^{3+} passive, Cr^{6+} active
Cu	6–10	15–30	Cu^{2+}	Zn, Fe, Mo, Cd, Se, Mn, Ni, Cr, N, P, Ca	Active and passive
Fe	50–100	(500 for rice)	$Fe^{2+} > Fe^{3+}$	Ca, P	Active
Hg	–	1–3	Hg^{2+}	K	Passive and active
Mn	30	100–800	Mn^{2+}	Mg, Ca	Active and passive
Mo	0.1–0.3	10–50	MoO_4^{2-}	–	Active
Ni	–	10–1,000	Ni^{2+}	Mg	Passive
Pb	–	100–200	$Pb^{2+} > Pb(NO_3)_2$	Zn, Cd, Ca	Passive (apoplastic pathway or via Ca^{2+}-permeable channels)
Zn	30–60	100–600	Zn^{2+}	Cd, Cu, As, Fe, N, P, Ca, Mg	Active

Sorption of metals by plant roots is due to binding and electrostatic attraction by a limited number of cell surface cation exchange and binding sites on the negatively charged root surface. The type and number of these sites varies by species due to the composition of root cells and the functional groups they present (Johnson and Singhal 2007). The Langmuir isotherm (see below) has generally been used to represent solute sorption to a finite number of exchange sites in the root and to provide a good fit for metal sorption to plant biomass (Chen et al. 1996; Sun and Shi 1998; Villaescusa et al. 2004; Vijayaraghavan et al. 2006) and to root tissue (Bhainsa and D'Souza 2001). Mobilized metals are able to bind to sites in the root cell walls (Wang et al. 1992) and plasma membranes (Briat and Lebrun 1999).

6.2.2.2 Transport to the Root Xylem

After adsorption, metals can be absorbed into the cytoplasm of the epidermal or cortical cells and continue through the symplast (from cell to cell) or remain in the apoplast. Metal movement through the apoplast may be caused by the flow of the water in which they are dissolved, by diffusion due to differences in the chemical or electrochemical potential of the metal between two points. In the endodermis, the absorbed metals are subject to the selective permeability of the membranes—before reaching the central cylinder—due to the high resistance to water passage of the aforementioned Casparian band, so that the metals circulating in the apoplastic pathway must pass into the symplast. Each time a metal is incorporated into the symplast, it must necessarily go across the plasma membrane of the cell. Pathways that allow metals to cross biological membranes can be passive but also can be based on active transport systems (Russell 1977).

For most metals, plant uptake is passive (non-metabolic), moving into the plant along with the water used for transpiration, by convective mass flow of water by simple diffusion, or through protein channels (Fig. 6.1). It is important to emphasize that when biological and structural properties of root cells are altered, all elements are taken up passively. This is the case when concentrations of elements pass over a threshold value for a physiological barrier (Kabata-Pendias 2011), as happens in contaminated soils with high concentrations of metals (see Limit Toxicity in Table 6.1). Therefore, a longer exposure and a greater amount of water transpired mean that more contaminants will be taken up by the plant.

However, some metals such as Cu, Fe, and Zn are essential to maintain plant metabolism, and for that reason plants have active systems (metabolic), and cell membranes contain proteins known as "proton pumps" that regulate the flow of ions from outside to inside the cell (and vice versa; Fig. 6.1). The existence of active transport mechanisms gives the membranes a selective permeability, which thus acts as a filter and allows the regulation of the inputs and outputs of different metals in the symplast. So, while the passive component represents the mass flow of metals into roots with water, the active component represents a very diverse range of various biological energy-driven processes (Luxmoore et al. 1978; Hopmans and Bristow 2002; Jungk 2002; Neumann and Romheld 2002; Silberbush 2002; Šimůnek and Hopmans 2009). These other mechanisms include, for example, specific ion uptake by electrochemical gradients, ion pumping, and uptake through the ion channels. For example, Ni and Pb are preferably absorbed passively, while compounds that are charged in soil solution (Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}) are likely not to be passively transported across a hydrophobic membrane due to high transport activation energy requirements. Therefore, these compounds must be transported synergistically by proton pumps in which a specifically designed protein, which utilizes the energy supplied from the ATP-ADP reaction, pumps protons across a membrane. Then, mechanisms of uptake differ, depending on the given element (Table 6.1). An additional problem is the interaction of ionic species during uptake of various metals which can be absorbed by specific channels for other elements (Tangahu et al. 2011; see Competition in Table 6.1).

6.2.2.3 Translocation to the Aerial Part

Part of the absorbed metals can be utilized in the root cells, but most of them will be directed centripetally from the root surface to the xylem of the central cylinder. After reaching the root xylem, the metals enter the transpiration stream, in which they are distributed to the rest of the plant, where they will be metabolized. The long-distance transport of metals in plants is partly related to the transpiration intensity. However, numerous other factors such as pH, the oxidation-reduction state, competing cations, hydrolysis, polymerization, and formation of insoluble salts (e.g., phosphate, oxalate, etc.) govern metal mobility within plant tissues.

The complexation of metals prevents their immobilization in the xylem and allows their long-distance transfer to the shoots and metal detoxification. Several plant metal ligands have been identified, including organic acids (e.g., citric, malic, malonic), amino acids (serine, cysteine), and peptides and proteins (phytochelatins and metallothioneins); (Rauser 1999).

In general, easily transported metals, from roots to aboveground parts, are Ag, B, Li, Mo, and Se; moderately mobile are Mn, Ni, Cd, and Zn; and strongly bound in root cells are Co, Cu, Cr, Pb, Hg, and Fe. The transport of trace elements among plant organs also depends on the electrochemical variables of the elements (Kabata-Pendias 2011).

6.3 Numerical Modeling of Root Metal Uptake

The metal uptake process is driven by the movement of water in the unsaturated zone and its uptake rate by plant roots. Hence, for the relevant root metal uptake, the water movement and its uptake by the plant roots should be considered first (Verma et al. 2006). In modeling, the flow of water to the plant roots is described as either microscopic (single root model) or macroscopic (diffuse sink). Microscopic models typically describe the microscale physics of water flow from the soil to and through the plant roots. A single cylindrical root system (consisting of the endodermis, epidermis, and surrounding homogenous soil) is divided into a soil domain and root-tissue domain (Molz 1975; Hillel 1998), which are solved separately. This approach describes the water extraction process by roots but is not applicable for the complex modeling. On the other hand, macroscopic plant water uptake functions are typically an empirical function that describes water uptake in response to soil suction, soil–water content, and time. This approach neglects the effects of the root geometry and flow pathways. The root water uptake is here represented using a single (sink) term, which regulates the final mass balance equation (Šimůnek and Hopmans 2009).

Once the water movement is simulated, the equation for the metal transport is modified to take into account the sorption and desorption processes, respectively (Verma et al. 2006). Next, the root metal uptake is solved, assuming this retardation factor, the temporal and spatial variation of the metal, and the kind of metal transport (passive/active) as affected by mass flow and diffusion.

Generally, two types of process-based models (Table 6.2) are available to describe root metal uptake (Seuntjens et al. 2004). The first types of models are "mechanistic models" (MM) that predict metal transport through the rhizosphere. Multicomponent reactions, such as aqueous and surface complexation or multicomponent transport of metals as well as their complexes, are well defined. In the second type of model, the "transport model" (TM), the metal transport in the air-soil–plant continuum is solved using the convection-dispersion equation (CDE) with the sink terms for the root metal uptake (Šimůnek and Hopmans 2009). Additionally, there are "other models" (O) related with phytoremediation but from a more general point of view, such as (1) models which are able to fit the optimal removal rate (bioaccumulation factor; BCF) or (2) plant growth models related with metal stress (see Table 6.2).

6.3.1 Mechanistic Models

Metallic elements may be present as different species in natural environments, which yields different mobility, availability, and solubility. In order to properly describe and interpret root metal uptake using modeling tools, it is necessary to involve the rhizosphere processes and characteristics. Therefore, the following aspects need to be taken into account: acid-base and redox reactions, solution and surface complexation, ion exchange, adsorption, mineral dissolution, surface speciation, metal speciation, metal diffusion toward the root, root exudation, root uptake, and multicomponent transport of metals, ligands, and their complexes (e.g., Seuntjens et al. 2004; Nowack et al. 2006; etc.). In general, three interrelated parts of the soil–plant system can be distinguished: soil particles, soil solutions, and plant roots. The applicability of a model is highly dependent on values and coefficients that describe the processes in the soil, the plant itself, and the soil–plant interaction.

Table 6.2 Selected studies focused on root metal uptake (RMU) modeling

RMU modeling				
Type of model	Approach	Metal(s)	Plant	Reference
Single root uptake model (Bar-Yosef et al. 1980)	TM + sorption (MM)	Zn	n.a. (artificial roots)	Bar-Tal et al. (1991)
Macroscopic root water and Cd uptake model	TM	Cd	n.a.	Rao and Mathur (1994)
A mechanistic system dynamic model; STELLA II (Brennan 1997)	MM + translocation (TM)	Pb	Maize	Brennan and Shelley (1999)
Predictive model of time and concentration parameters during phytoextraction	O	Ni	*Alyssum bertolonii* Desv.	Gonnelli et al. (2000)
Uptake mechanism (kinetic) of As species model	MM	As	Rice (*Oryza sativa*)	Abedin et al. (2002)
Diffusive gradients in thin films (DGT) model	MM	As	*Pteris vittata* L.	Fitz et al. (2003)
Solute transfer and uptake kinetics models	MM + translocation (TM)	Zn	*Thlaspi caerulescens*	Whiting et al. (2003)
Complex model in the plant root zone which describes root uptake and leaching of metals	MM	Cu	*Oryza sativa* L.	(Seuntjens et al. 2004)
Mechanistic system dynamics modeling approach and the physiology model (MATLAB)	MM + translocation (TM)	Pb	Maize (*Zea mays*)	Chrysafopoulou et al. (2005)
Combined free ion activity model and Solubility-uptake model (WHAM-VI model; Tipping 1994)	MM	Cd and Zn	*Lolium perenne* L.	Hough et al. (2005)
Simple process oriented functional model	TM	Cd	Potato (*Solanum tuberosum* L.), sugar beet (*Beta vulgaris* L.), and winter wheat (*Triticum aestivum* L.)	Ingwersen and Streck (2005)
Coupled model of water flow, heat and solutions transport in the soil–plant–atmosphere continuum (CTSPAC)	TM + translocation (TM)	As	Fern (*Pteris vittata* L.)	Ouyang (2005)
Model to know the time (harvest cycles) and cost for phytoextraction	O	Cr	*Brassica juncea*	Thomas et al. (2005)
Metal uptake and the Free Ion Activity Model; GEOCHEM-PC (Parker et al. 1995)	MM + translocation (TM)	Pb, Cd and Fe	*Brassica juncea*	Schaider et al. (2006)
Model for simulating metal dynamics in the soil–water–plant root system (MATLAB)	TM	As	n.a.	Verma et al. (2006)
Two- and three-parameter isotherm models	MM	Ni	*Sargassum wightii*	Vijayaraghavan et al. (2006)
Diffusive gradients in thin films (DGT) model	MM	Cd, Co, Cu, Ni, Pb and Zn	*Lepidium sativum*	Zhang and Davison (2006)
The Hung and Mackay's model (Hung and Mackay 1997)	MM + translocation (TM)	Cu, Ni, Zn, Mn and Pb	Pine (*Pinus sylvestris*), birch (*Betula pendula*) and black alder (*Alnus glutinosa*)	Baltrënaïtë and Butkus (2007)
Langmuir and Freundlich model	MM	Co, Cu, Ni and Zn	Eurasian water milfoil (*Myriophyllum spicatum* L.)	Lesage et al. (2007)
Free ion activity model based on the biotic ligand model	MM	Cd and Zn	*Thlaspi caerulescens*	Maxted et al. (2007)
Kinetic and equilibrium models	MM + translocation (TM)	Pb	*Salvinia minima*	Sánchez-Galván et al. (2008)
Modeling program REC (Risk reduction, environmental merit and cost)	O	Cu, Cr, Mn, Ni, Pb and Zn	*Lolium perenne*, *Poa pratensis*, *Festuca pratensis*	Jankaite (2009)
Model using sum of the Michaelis-Menten, linear and Freundlich functions which were fitted to the symplastic and apoplastic isotherms (KaleidaGraphTM software)	MM + translocation (TM)	Cd	Maize (*Zea mays*), alpine pennycress (*Noccaea caerulescens*)	Redjala et al. (2009)
Combined free ion activity model and solubility-uptake model (WHAM-VI model; Tipping 1994)	MM	As	*Pteris vittata*	Shelmerdine et al. (2009)
Batch reactor model (Sternberg 2007)	MM + effect of metal uptake on plant growth (O)	Pb	Duckweed (*Lemna minor*)	Uysal and Taner (2009)

(continued)

Table 6.2 (continued)

RMU modeling				
Type of model	Approach	Metal(s)	Plant	Reference
Model proposed by De Leo et al. (1993) (interaction between soil acidity and plants); modified by Guala et al. (2009)	MM + effect of metal uptake on plant growth (O)	Cd, Cu and Zn	Alfalfa, lettuce, radish and *Thlaspi caerulescens*	Guala et al. (2010a)
Model proposed by De Leo et al. (1993) (interaction between soil acidity and plants); modified by Guala et al. (2009)	MM + effect of metal uptake on plant growth (O)	Cd and Ni	Ryegrass (*Lolium perenne*, L.) and oats (*Avena sativa* L.)	Guala et al. (2010b)
Free ion activity model (FIAM); WHAM-VI speciation model (Windermere Humic Acid Model WHAM; Tipping 1994)	MM	Cd	*Zea mays* and *Brassica juncea*	López-Chuken et al. (2010)
Model using sum of the Michaelis-Menten, linear and Freundlich functions which were fitted to the symplastic and apoplastic isotherms (KaleidaGraphTM software)	MM + translocation (TM)	Ni	Leptoplax (*Leptoplax emarginata*) and maize (*Zea mays* L.)	Redjala et al. (2010)
Batch reactor model (Sternberg 2007)	MM	Cd	Duckweed (*Lemna minor*)	Uysal and Taner (2010)
Biotic ligand model (Visual MINTEQ; Gustafsson 2006)	MM + effect of metal uptake on plant growth (O)	Cu	Pea (*Pisum sativum* L.)	Wu and Hendershot (2010a)
Biotic ligand model (Visual MINTEQ; Gustafsson 2006)	MM + effect of metal uptake on plant growth (O)	Cd	Pea (*Pisum sativum* L.)	Wu and Hendershot (2010b)
The ecological model of population growth (r/K-driven model; Verhulst 1838)	O	Cu	*Aldama dentata*	Dasgupta-Schubert et al. (2011)
Model proposed by De Leo et al. (1993) (interaction between soil acidity and plants); modified by Guala et al. (2009)	MM + effect of metal uptake on plant growth (O)	n.a.	n.a.	Guala et al. (2011)
Flux and mass balance model	MM	Ni	*Elodea Canadensis*	Hansen et al. (2011)
Coupling a saturable Michaelis-Menten type accumulation model and an energy-based toxicity model	MM	Cu	Switchgrass (*Panicum virgatum* L.)	Juang et al. (2011)
Stochastic model (yields an analytical, time-dependent probability density function (PDF) for the contaminant mass during the remediation process)	TM	n.a.	n.a.	Manzoni et al. (2011)
Biosorption pseudo-second order kinetic equation model	MM	Cd	Sunflower (*Helianthus annuus*)	Meighan et al. (2011)
Prediction models (linear, exponential, and lognormal) of metals extraction	O	Cd, Cr, and Zn	*Panicum virgatum* L.	Chen et al. (2012)
Model that combined a diffusion equation with a competitive Michaelis-Menten equation	MM	Cd, Zn and Ni	*Spinacia oleracea*; *Lycopersicon esculentum*	Degryse et al (2012)
Speciation model in phloem sap (ECCLES program)	MM + translocation (TM)	Cu, Fe, Mn and Zn	n.a.	Harris et al. (2012)
Model proposed by De Leo et al. (1993) (interaction between soil acidity and plants); modified by Guala et al. (2009)	MM + effect of metal uptake on plant growth (O)	n.a.	n.a.	Guala et al. (2013)
Langmuir model to fit tannin-metal binding (Jain and Snoeyink 1973) (GraphPad Prism)	MM	Al and Fe	*Epilobium angustifolium* (oenothein B and epigallocatechin gallate)	Schmidt et al. (2013)
Model NST 3.0, transport of Cd towards the root, taking sorption processes to the soil matrix into account	MM	Cd	*Zea mays*, *Helianthus annuus* *Linum usitatissimum*, *Spinacia oleracea*	Stritsis et al. (2013)
Radially symmetric single- and dual-porosity transport model (HYDRUS 2D program)	TM	Cd, Cu, Pb and Zn	Willow (*Salix* × *smithiana* Willd.)	Trakal et al. (2013)

Mechanistic models (MM), transport models (TM), and other models (O) approaches, which are connected with phytoremediation modeling, are listed in this table (n.a. = not available)

The distribution between the solution and the solid phase is calculated using equilibrium and kinetic reactions between the individual components of the porous medium, namely, metals, ligands, surfaces, and mineral phases (Seuntjens et al. 2004).

6.3.1.1 Sorption Isotherms

The potential biosorption of metals is often demonstrated using sorption isotherms, which represent the relationship between the concentrations sorbed onto the surface of the biomass and the equilibrium concentration in the solution. Adsorption data can be modeled using two-, three-, or four-parameter isotherms (Khan et al. 1997; Vijayaraghavan et al. 2006); however, Langmuir and Freundlich (two-parameter) models are the most commonly used (e.g., Bar-Tal et al. 1991; Lesage et al. 2007; Sánchez-Galván et al. 2008). The Langmuir model (3.1) provides a description of the equilibrium relationships between a bulk liquid phase and a solid phase and is valid for monolayer sorption on a homogeneous surface with a finite number of identical sorption sites. The Freundlich isotherm (3.2) describes sorption onto heterogeneous surfaces; it is assumed that stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation:

$$s = \frac{s_{\max} K_L}{1 + K_L} \tag{3.1}$$

$$s = K_F c^{\beta} \tag{3.2}$$

where s [M M^{-1}] and c [M L^{-3}] are concentrations in the solid and liquid phase, respectively; $s_{\max}$ [M M^{-1}] is the maximum sorbed concentration; K_F [L$^{3\beta}$ M$^{1-\beta}$ M^{-1}] and K_L [–] are the Freundlich and Langmuir sorption coefficients, respectively; and β [–] is an empirical coefficient of the Freundlich equation.

Vijayaraghavan et al. (2006) demonstrated the applicability of ten different models of sorption isotherm to the biosorption of Ni onto *Sargassum wightii*. The experimental data obtained under different pH conditions were fitted to five two-parameter models (Langmuir, Freundlich, Temkin, Dubinin-Radushkevich, and Flory-Huggins) and five three-parameter models (Redlich-Peterson, Sips, Khan, Radke-Prausnitz, and Toth). Subsequent error analysis showed that the three-parameter models better describe the biosorption of Ni compared to two-parameter models. The closest fit to the Ni biosorption data was observed for the Toth equation.

Sorption models were produced which were well suited to the data obtained from experiments on *Myriophyllum spicatum* L. for the treatment of metal-contaminated solutions. For Co, Ni, and Zn, the sorption process was described excellently by the Langmuir model, whereas the Freundlich model better fitted the Cu data (Lesage et al. 2007). The impact of EDTA on the rate of accumulation of Cd in plants was tested and modeled by Meighan et al. (2011). The uptake data were fitted to pseudo first-order and pseudo second-order kinetic equations commonly used to model the biosorption of metals onto inert materials.

6.3.1.2 Speciation-Solubility Modeling

A number of models are available to define speciation of metals in the solution (e.g., soil pore water, wastewater). They are often sophisticated mathematical models that have been developed to describe complexation, ion exchange, sorption, or dissolution/precipitation reactions. The WHAM-VI speciation model (Windermere Humic Acid Model WHAM) combines the simple inorganic speciation of aqueous solutions with the humic ion-binding model VI (Tipping 1994). This permits the inclusion of the effect of humic substances (humic and fulvic acids) on metal speciation (López-Chuken et al. 2010). Visual MINTEQ (Gustafsson 2006, 2011) is the Windows version of the MINTEQA2 model (Allison et al. 1991), which is a geochemical equilibrium speciation model for aqueous systems. Other examples include GEOCHEM-PC (Parker et al. 1995) and its upgraded version GEOCHEM-EZ (Shaff et al. 2010) and MINEQL (Westall et al. 1972). The PHREEQC speciation-solubility model (Parkhurst and Appelo 1999) provides various options for hydrogeochemical modeling involving a large number of reactions in water and geological environments. The equilibrium compositions of the solutions can be calculated with respect to the possible formation of precipitated compounds. The ORCHESTRA modeling framework (Meeussen 2003) has been developed for modeling equilibrium chemistry. All model definitions are separate from the calculation engine or equation solver, which allows the user to adapt the model structure and equations. It comprises also advanced sorption models that provide calculations for pH-dependent sorption and electrostatic interactions. Moreover, both the PHREEQC and ORCHESTRA models have been extended to combine geochemical processes with transport calculations (Nowack et al. 2006).

A combined solubility-uptake model has been developed to predict the suitability of *Pteris vittata* for the phytoremediation of As-contaminated soils (Shelmerdine et al. 2009). For this purpose, as speciation in the soil solution was calculated using the WHAM-VI model (Tipping 1994) including several complex-formation constants (Ca-arsenate complex formation), which were added to the original database. In phytoextraction strategies it is crucial to increase the solubility of metals in soils, often using the addition of synthetic chelates such as EDTA. The formation of metal-chelate complexes in phytoextraction has been modeled and shown in many studies (Nowack et al. 1996; Schaider et al. 2006; Komárek et al. 2007, 2008, 2009, 2011; López-Chuken et al. 2010; Meighan et al. 2011). The PHREEQC-2 speciation-solubility code (Parkhurst and Appelo 1999) was used to determine the metal speciation in extracts of different chelating agents (EDTA, EDDS, NH_4Cl) studied by Komárek et al. (2007, 2008). In addition, the interactions of Fe/Al oxyhydroxides with complexing agents and thus the extraction efficiency has been addressed (Nowack et al. 1996; Komárek et al. 2009, 2011). Models such as PHREEQC-2 represent a useful tool to study chelate-assisted phytoremediation and the effects of ligand exudation on plant uptake and leachability of metals. This was also documented by Seuntjens et al. (2004), who simulated the influence of EDTA addition and the effect of oxalate exudation by roots on Cu leaching and plant uptake.

Harris et al. (2012) have reported an important aspect related to metal behavior in plants, that is, the speciation of metals (Fe^{3+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}) in the phloem sap. The total concentrations of 20 amino acids and other components, including organic acids, were included in the speciation calculation model developed using the program ECCLES (May et al. 1977). The model confirmed that the metal ions are strongly chelated in the phloem, mainly by nicotianamine, and that very few free metal ions are present (Harris et al. 2012).

6.3.1.3 Metal Bioavailability and Root Uptake

It is important to assess the bioavailability of metals and thus to distinguish between the total and available metal concentration (van Leeuwen 1999; Guala et al. 2010b, 2013). The free metal ion activity in solution may explain the metal uptake and toxicity to a greater degree than the total soil metal concentration (Sauvé et al. 1998). Although the "free ion activity model" (FIAM) has been used for predicting metal uptake in many studies (Hough et al. 2005; Maxted et al. 2007; Shelmerdine et al. 2009; López-Chuken et al. 2010), its general applicability has been a matter of debate. The model suggests that metal ion activity (M^{2+}) is the major determinant of bioavailability in the soil (Sparks 1995). However, chemical species other than M^{2+} are taken up by plants as intact metal complexes, which play a significant role in overall metal uptake and accumulation (Campbell 1995; Parker et al. 2001; Schaider et al. 2006; López-Chuken et al. 2010). Moreover, local depletion of ions around roots leads to changes in the rhizosphere conditions that may not reflect bulk soil conditions (Zhang et al. 2001), and this is not parameterized in FIAM (Hough et al. 2005). The use of the FIAM depends on a number of simplifying assumptions: (1) the rate of contaminant transport from roots to shoots depends on the density of occupied transport sites at the root surface and reaches a theoretical maximum when all sites are occupied; (2) the root-to-shoot ratio is constant throughout the exposure period of the plant; (3) the density of sorption sites on the roots is constant irrespective of physiological age; and (4) the free ion activity near the root surface is constant throughout the exposure period of the plant and is identical to that measured in the bulk solution (Shelmerdine et al. 2009; López-Chuken et al. 2010).

Cadmium uptake by plants as a function of free and Cl-complexed ions in soil pore water was modeled by López-Chuken et al. (2010) using a form of FIAM also called the "biotic ligand model" (BLM; Datta and Young 2005; Hough et al. 2005). This approach assumes the initial sorption of free metal ions (M^{2+}), or defined metal complex species (e.g., MCl^+), from the solution onto hypothetical plant root sorption sites. The model also considers competition between cations and protons for sorption sites (Maxted et al. 2007; López-Chuken et al. 2010).

The bioavailability of metals may be affected by the presence of organic acids, which are exuded by plant roots (e.g., Jones 1998; Seuntjens et al. 2004) as a response to environmental stimuli and stresses (Ryan et al. 2001). Exudation rates vary depending on the roots' structure, the position along the root, and the external impulses (Ryan et al. 2001). For modeling purposes (see Eq. 3.3), the exudation of organic anions is assumed to be at a constant rate (Geelhoed et al. 1999):

$$\frac{dc_L}{dt} = Wu \tag{3.3}$$

where c_L is the concentration of the organic anion [M L^{-3}], W is the root biomass [M of FW], and u is the exudation rate [M M^{-1} of FW T^{-1}]. More complex calculations involving organic acid degradation may be included.

Modeling of root metal uptake involves a combination of equations, which describe the uptake of the root active species at the root surface as well as the diffusion of the free metal and metal complexes in solution (van Leeuwen 1999; Seuntjens et al. 2004). Uptake of metals at the biosurface by the root and root hairs is assumed to follow a nonlinear Michaelis-Menten rate equation (3.4; Barber 1995; Seuntjens et al. 2004; Wilkinson and Buffle 2004; Redjala et al. 2010):

$$J = \frac{J_{\max} c_M^0}{\left(c_M^0 + K_m\right)} \tag{3.4}$$

where J is the actual metal uptake flux [M L^{-3} T^{-1}], $J_{\max}$ is the maximum metal uptake flux [M L^{-3} T^{-1}], K_m is the half saturation constant [M L^{-3}], and $c_M{}^0$ is the free metal concentration at the (root) surface [M L^{-3}]. However, this equation assumes that only free metals are taken up. The diffusion of both metals and metal complexes to the root surface plays an important role for metal uptake:

$$J_m = \bar{k}\left(c_t^* - c_t^0\right) \tag{3.5}$$

where J_m is the diffusion flux of the metal [M L^{-3} T^{-1}], $\bar{k}$ is the mean diffusion rate coefficient [T^{-1}], $c_t{}^*$ is the total metal concentration in the bulk solution [M L^{-3}], and $c_t{}^0$ is the total metal concentration at the root surface [M L^{-3}].

A combination of a Michaelis-Menten equation with other types of models has been performed in many other studies. Coupling a Michaelis-Menten model and an energy-based toxicity model aims to enhance the ability to assess the feasibility of phytoextraction (Juang et al. 2011). The bioaccumulation and phytotoxicity parameters of Cu in switchgrass (*Panicum virgatum* L.) were adapted by fitting the models to the data obtained from hydroponic experiments under various exposure concentrations (Juang et al. 2011). Lehto et al. (2006) presented a "dynamic plant uptake model" which describes plant Zn uptake using Michaelis-Menten kinetics but includes consideration of the labile metal K_D value, advective replenishment of metal at the soil–root interface, and the short-term kinetics of desorption from soil solids. Degryse et al. (2012) aimed to describe the uptake of Cd, Zn, and Ni by plants in a single comprehensive model that combines Michaelis-Menten kinetics with a diffusion equation. A solute transfer model was developed by Whiting et al. (2003) to predict the concentration of Zn in the rhizosphere solution of *Thlaspi caerulescens* and the efficiency for maximal phytoextraction. The Zn uptake was fitted to the Michaelis-Menten equation to be calculated for given external concentrations of Zn at the root surface (Whiting et al. 2003). The Michaelis-Menten function was also successfully applied to demonstrate the uptake of As (arsenite and arsenate) by rice plants (Abedin et al. 2002) or the accumulation of Pb in *Salvinia minima* (Sánchez-Galván et al. 2008).

6.3.2 Transport (Functional) Models

6.3.2.1 Water Flow and Metal Transport Modeling

As mentioned before, these process-based models are solved in the unsaturated media using Richards' equation (3.6) and the convection-dispersion equation CDE (3.7), with the sink term consideration for the root water and metal uptake. Additionally, CDE was modified due to the consideration of metal sorption:

$$\frac{\partial \theta(h)}{\partial t} = \frac{\partial}{\partial x_i}\left[K(h)\left(K_{ij}^{\mathrm{A}}\frac{\partial h}{\partial x_j} + K_{iz}^{\mathrm{A}}\right)\right] - S(h) \qquad (3.6)$$

$$\frac{\partial \theta c}{\partial t} + \frac{\partial \rho_{\mathrm{d}} s}{\partial t} = \frac{\partial}{\partial x_i}\left(\theta D_{ij}\frac{\partial c}{\partial x_j}\right) - \frac{\partial (q_i c)}{\partial x_i} - S'(h,c) \qquad (3.7)$$

where θ is the volumetric water content [L^3 L^{-3}]; h is the soil–water potential [L]; K is the unsaturated hydraulic conductivity; [L T^{-1}], s [M M^{-1}], and c [M L^{-3}] are the concentrations in the solid and liquid phase; ρ_d is the bulk density [M L^{-3}]; q_i is the volumetric flux density [L T^{-1}]; K_{ij}^{A} are components of a dimensionless anisotropy tensor K^A [–]; D_{ij} are components of the effective dispersion tensor [L^2 T^{-1}]; t is time [T]; x_i are spatial coordinates [L]; and S and S' are the sink terms for the root water [L^3 L^{-3} T^{-1}] and metal [M L^{-3} T^{-1}] uptake, respectively. Additionally, the sorption of metals (amount of metal in the solid phase) is governed using linear and nonlinear Freundlich and Langmuir isothermal adsorption, respectively (see Sect. 6.3.1.1).

6.3.2.2 Root Water and Metal Uptake Modeling

Root water and metal uptake are most frequently considered using the macroscopic approach of modeling. As a practical example of such models, the R-SWMS is a numerical model for predicting soil–root water fluxes based on the water potential gradient between soil and root nodes (Lynch 1995). It is a three-dimensional-coupled water flow model for soil and roots with an uptake stress function, which couples the model of Somma et al. (1998) with the model of Doussan et al. (1998). Next, the HYDRUS is a software package for simulating water flow and solute transport in variably saturated porous media. The software package includes computational finite element models for simulating the two- and three-dimensional movement of water and solutes in variably saturated media. The model includes a parameter optimization algorithm for inverse estimation of a variety of soil hydraulic and/or solute transport parameters and an approach that considers a single root to be an infinitely long cylinder of uniform radius and water-absorbing properties (Gardner 1960). Among others, the HYDRUS program was chosen for the study of root metal uptake (Šimůnek and Hopmans 2009; Trakal et al. 2013) and produced a compensated root water and nutrient uptake model, where the total root nutrient uptake is determined from the total of active and passive nutrient uptake. Additionally, all considerations and equations are here presented as the simplest one-dimensional problem. Two- and three-dimensional formulations should then be considered after the expansion of the one-dimensionally used equations.

Root Water Uptake

Root water uptake, the controlling process for root metal uptake, is solved using the assumption of the continuum approach where the water flow through the soil–plant–atmosphere continuum is at a steady state (Hopmans and Bristow 2002). This process, represented by the sink term S in Richards' equation (3.6), is calculated according to the following Eq. (3.8):

$$S(h) = T_{\mathrm{p}}\alpha(h)b(z,t) \qquad (3.8)$$

where T_p is the potential transpiration [L T^{-1}], $\alpha(h)$ is the stress-response function [–], and the parameter $b(z,t)$ represents root water uptake distribution in the rhizosphere [L]. Potential transpiration (T_p), which represents the root water uptake rate, is determined by the atmospheric demand, and its rate is usually calculated from meteorological variables (Šimůnek and Hopmans 2009). The $b(z,t)$ parameter, which is a function of root growth, is functionally described using (1) the linear distribution (Feddes et al. 1978), (2) the exponential distribution (Raats 1974), or (3) the general distribution (Vrugt et al. 2001a, b. Water stress $\alpha(h)$ is determined using two different approaches, such as computing the effective leaf water potential (compensated for water stress) or the zero-to-one stress-response function (uncompensated for water stress; Hopmans and Bristow 2002).

Root Metal Uptake

Metal uptake from soil depends on the plant demand, on the soil supply, and on various soil and plant characteristics. Mathematical models are based on the same principles, where the solute forms of metals (the most frequent being divalent free metal ions M^{2+}) are transported by mass flow and diffusion (Rao and Mathur 1994). The bioavailability of metals is then driven by the activity of free metal ions in solution at the root surface; hence, the metal uptake is assumed to follow a Michaelis-Menten-type relationship (see Sect. 6.3.1.3; Seuntjens et al. 2004; Verma et al. 2006). This model assumes that only free metal ions are taken up. Additionally, root metal uptake modeling allows for both passive and active metal transport; hence, this uptake is not linearly dependent on the metal concentration in the soil solution (Verma et al. 2006).The passive uptake represents the transport of metals into roots associated only with water uptake mechanisms. On the other hand, the active uptake of some essential metals (Cu, Zn, etc.) can be defined as all possible nutrient uptake mechanisms connected with energy-driven processes (Šimůnek and Hopmans 2009). Following these principles, the final sink term for the root metal uptake is solved using Eq. (3.9), according to Somma et al. (1998):

$$S'(h,c) = \delta S(h) + (1-\delta) A(h,c) \tag{3.9}$$

where δ is a partition coefficient with a value from 0 to 1, S describes passive metal uptake (calculated according to the sink term for root water uptake), and A describes active metal uptake. Additionally, as in the case of water uptake, the root metal uptake should be compensated using the metal stress index (Šimůnek and Hopmans 2009). Nevertheless, for phytoremediation modeling, only passive transport (uptake) should be considered since, in the metal-polluted soils to which phytoremediation is applied, metals are present at higher concentrations and hence their uptake is predominantly passive (see Sect. 6.2.2.2).

6.3.2.3 Water and Metal Translocation Modeling

Most of the modeling applied to phytoremediation technologies is focused on identifying appropriate plants for phytoextraction, since models can be used to identify constraints to efficient phytoextraction (whether plant and soil) and to determine whether commercial phytoextraction is feasible (Whiting et al. 2003).

Even though the influx of water at the root surface can be calculated from the transpiration rate of plants [cm^3 d^{-1} g^{-1} shoot DW] (Whiting 1998), studying the transport of metals from soil to aerial parts is complex due to the existence of specific transporters of metals in the roots that control their diffusion. The translocation of metal along the xylem route is equal to the daily transpiration rate from the roots to the shoots (Kramer and Boyer 1995). Metal uptake, via the transporters, from the root surface into the root symplast is concentration dependent and can be fitted to a Michaelis-Menten saturation kinetics (Nissen 1996). So, the metal removal by a plant can be calculated using a saturable Michaelis-Menten-type absorption model, and from that the removal rate can be expressed as a time-dependent parameter (e.g., Juang et al. 2011).

The root absorption power can be determined from the uptake rate of each metal on a fresh weight basis. Using the data for the root dimensions, this rate can be also recalculated per unit root surface area (Whiting et al. 2003).

There are numerous references studying metal transport through the different organs of the plants (roots, stems, leaves, fruits), through xylem and phloem solid and liquid phase tissues (Brennan and Shelley 1999; Chrysafopoulou et al. 2005), or the influxes of metals through the apoplastic and symplastic pathways. Apoplastic metal uptake is calculated using Eq. (3.10):

$$U_{\text{apop}} = K_{\text{F}} [\text{Metal}]^{\beta} \tag{3.10}$$

where U_{apop} is the metal apoplastic uptake using a Langmuir function [M M^{-1} T^{-1}], [Metal] is the metal concentration in the external solution [ML^{-3}], K_F reflects the adsorption capacity of the adsorbent (capacity factor) [L $M^{1-\beta}$ M^{-1} DWT^{-1}], and β measures the affinity of the metal to the adsorbent (intensity factor) [–] (Redjala et al. 2010). Symplastic metal uptake is then calculated using Eq. (3.11):

$$I_{\text{symp}} = \frac{I_{max}[\text{Metal}]}{K_{\text{m}} + [\text{Metal}]} + a[\text{Metal}] \tag{3.11}$$

where I_{symp} is the metal symplastic influx [M L^{-3} T^{-1}]; I_{max} and K_{m} are the Michaelis-Menten constants, the maximum influx [M L^{-3} T^{-1}] and the affinity coefficient [M L^{-3}], respectively; [Metal] is the metal concentration in the external solution [M L^{-3}]; and a is the slope of the linear component [–] (Redjala et al. 2009).

Mechanistic models generally consider only the symplastic influx to be responsible for the root metal sink (Barber 1995). In order to verify the contributions of the apoplast and symplast to the root influx of Cd (Redjala et al. 2009) and Ni (Redjala et al. 2010), different models were employed using the Kaleidagraph™ software. The combined Michaelis-Menten and linear functions were used to model the symplastic influx, while the Freundlich adsorption model was used to describe the apoplastic uptake (Redjala et al. 2009). This approach showed the importance of metal adsorption on the root apoplast, which can contribute significantly to the amount of metal extracted from the soil, and that using only a Michaelis-Menten function may not be sufficient in terms of root uptake modeling (Redjala et al. 2009). However, more investigations are necessary to determine the path of metal movement from the apoplast to plant shoots (Redjala et al. 2009, 2010).

Additionally, the model of Hung and Mackay (1997), originally applied for transport of contaminants from the soil to trees, has been simplified and used to simulate metal transport from the soil to tree seedlings (Baltrënaïtë and Butkus 2007). Generally, it evaluates the transport of metals from the soil and air and computes concentrations of metals in the roots, stem, and leaves. The suitability of the Hung and Mackay model for metal uptake by higher plants needs to be further verified (Baltrënaïtë and Butkus 2007).

6.3.3 Other Models

Furthermore, there are models connected with metal phytoremediation that do not simulate metal root uptake and transport. The following models are considered in this case: (1) empirical fitting models of the bioaccumulation, (2) simulations of root/plant growth connected with the stress response to the presence of metal(s), or (3) predictive models of optimal metal removal rates (see Table 6.2).

6.3.3.1 Empirical Models of BCF Fitting

A plant's ability to accumulate metals from soils can be estimated using the biological concentration factor (BCF), calculated as the metal concentration ratio of plant roots to soil and given in Eq. (3.12) (Yoon et al. 2006). A plant's ability to translocate a metal from the roots to the shoots is measured using the translocation factor (TF), the ratio of the metal in the shoots to that in the roots—as given in Eq. (3.13) (Cui et al. 2007). The biological accumulation coefficient (BAC) is calculated as the ratio of the metal in the shoots to that in the soil and is given in Eq. (3.14) (Li and Luo 2007). The BCF, TF, and BAC are useful parameters to evaluate the potential of the plants in accumulating metals for phytoremediation purposes, because, for example, the aerial part can be harvested during phytoextraction tasks, while the plants can retain metals in their roots during phytostabilization.

$$\mathrm{BCF} = \frac{[\mathrm{Metal}]_{\mathrm{root}}}{[\mathrm{Metal}]_{\mathrm{soil}}} \tag{3.12}$$

$$\mathrm{TF} = \frac{[\mathrm{Metal}]_{\mathrm{shoot}}}{[\mathrm{Metal}]_{\mathrm{root}}} \tag{3.13}$$

$$\mathrm{BAC} = \frac{[\mathrm{Metal}]_{\mathrm{shoot}}}{[\mathrm{Metal}]_{\mathrm{soil}}} \tag{3.14}$$

6.3.3.2 Effects of Metal Uptake on Plant Growth

The effects of metals present in soil on plant growth can be investigated using a nonlinear prediction of the soil–plant interaction. The concentrations of metals in plants (Guala et al. 2010a), and in parts of plants (Guala et al. 2010b), can be predicted by a simple kinetic model based on their relationship with the concentrations of metals in soil. De Leo et al. (1993) modeled the interaction between soil acidity and forest dynamics with respect to the mobilization of Al. Guala et al. (2010a) modified the model of De Leo et al. (1993) and Guala et al. (2009) in order to study the mobility of metals at different soil pH. In addition, Guala et al. (2010a, b) adapted the mathematical equations to involve different plants and different metals in the model. The relationship between the metal in the soil and the total content of the metal in plants is highlighted by Guala et al. (2011). Additional modifications to the abovementioned models have been performed by Guala et al. (2013) and involve changes in the concentrations of metals in the biomass and the accumulation curves of metals in plants as a function of pH. The model relates the dynamics of metal uptake from soil by plants, considering the critical concentration level, the plant resistance, and the accumulation capacity, for the effective design of phytoextraction strategies. In general, the prediction provides information about the effects of different metals on plants, depending on various soil characteristics and the plant species. Thus, the model can be applied to different plants to understand how different levels of metals present in the soil can influence plant growth (Guala et al. 2010a, b, 2011, 2013).

A multielement uptake model was developed to study the accumulation and toxicity of Cu to pea (*Pisum sativum* L.) roots using Visual MINTEQ (Gustafsson 2006, 2011). The types of binding sites and the proton-binding constants were estimated from titration experiments and a series of formation constants were derived. This chemical equilibrium approach can approximate the interactions of various cations with soils and plants, particularly under multi-metal conditions (Wu and Hendershot 2010a, b).

The uptake of a metal ion present in the plant growth medium depends on the plant growth rate and the concentration of the metal ion in the medium. The change in a metal ion concentration with time can be described with a first-order differential equation (Sternberg 2007), as demonstrated by Uysal and Taner (2009).

6.3.3.3 Predictive Models of Optimal Metal Removal Rates

Although other models obviate many of the fundamental processes of metal uptake, some mathematical models are useful for providing information important to the planning and implementation of phytoremediation tasks at the industrial level and for assessing the potential of the plant species used, including economic aspects, for example, the cost and number of harvest cycles that would be needed to reduce the concentration of a metal in the soil to an optimum (Thomas et al. 2005), the identification of the time and concentration conditions for phytoextraction purposes (Gonnelli et al. 2000), the number of years for the decontamination of each metal in a multi-contaminated soil (Jankaite 2009), and the maximum metal content in the biomass yield (Chen et al. 2012).

6.3.4 Drawbacks of Root Metal Uptake Modeling

Not surprisingly, plant uptake varies considerably between types of plant and individual species (Bell 1992). Factors likely to influence uptake include root growth and depth, transpiration rate, active uptake mechanisms, growth period, location of fruits and tubers, and the size and shape of leafy foliage (Buckley 1982). To create models, many assumptions must be accepted (e.g., Brennan and Shelley 1999), and, although each of them has a scientific basis, they may include errors that distort the results. For the additional complexity required to understand such systems, there are few predictive models available (Trapp 2000; Trapp et al. 2003). Accumulation and metabolism can be predicted with satisfying accuracy only for a small fraction of chemicals (Trapp 2004). Another source of errors may be the highly interdisciplinary nature of this research (Trapp 2004).

The behavior of young roots is different from that of adult roots. For example, young roots have no endodermis with a differentiated Casparian band, so that metals can move directly from the soil solution to the xylem without any regulation by membrane transporters. The processes are sensitive to some properties of the soil environment such as temperature, aeration, and pH, while Eh, Ag, Au, Br, Cd, Cu, F, Hg, I, Pb, and U also change the permeability of the cell membrane.

Lynch (1995) pointed out that root architecture is a primary aspect of plant productivity, particularly in environments where water and nutrients are scarce, as occurs in soil contaminated by metals. Water and dissolved solutes (e.g., metals) take the pathway of least resistance to reach the plant vascular tissue from the soil. Therefore, the soil conductivity and root radial and axial conductivities are key parameters affecting model response. Additionally, preferential pathways, created along the roots over the growing season, should be taken into account for the relevant modeling (Trakal et al. 2013). The roots usually extend and consequently shrink as a result of variable water content in the soil (rhizosphere).

Over the growing season, the biomass of the plant increases, diluting the metal concentration within the plant tissues—relative to the flux of chemical uptake—and increasing the aboveground canopy for interception of aerial deposition. The importance of growth dilution has been recognized in several dynamic plant uptake models (Trapp and Matthies 1995; Hung and Mackay 1997; Samsøe-Petersen et al. 2003). However, these models tend to assume that plants are in the middle stage of exponential growth and do not describe biomass accumulation over a season.

Finally, each metal(loid) has its own individual behavior in the soil; hence, the bioavailable forms of each element are dissolved in distinct ways in the soil solution. Due to this, each element is taken up at different rates into the plant. In summary, all these aspects should also be considered; thus, models which couple together both mechanistic and transport (functional) approaches should be the most relevant to the complex phytoremediation modeling of root metal uptake.

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Part II

Planning and Engineering Applications to Phytoremediation

7 Morphophysiological Responses, Heavy Metal Accumulation and Phytoremoval Ability in Four Willow Clones Exposed to Cadmium Under Hydroponics

Valentina Iori, Fabrizio Pietrini, Angelo Massacci, and Massimo Zacchini

7.1 Introduction

The increased presence of heavy metals in the aquatic ecosystem, primarily due to various industrial processes, agricultural practices, mining and other human activities, has resulted in a degradation of water quality and a severe threat to the environment and human health. Among heavy metals, cadmium (Cd) is one of the most hazardous due to its ubiquitous nature, non-biodegradability, high toxicity to biota even at low concentrations and high solubility in water (Da Silva et al. 2012; Xie et al. 2013). Although Cd is not an essential element for plants, it can be readily taken up and accumulated by many plants, representing a possible way to enter the food chain (Prasad 1995; Astolfi et al. 2004).

The reclamation of water is considered as one of the most challenging environmental management issues to address the concern of the water shortage for agricultural purposes. Several techniques have been developed to remove Cd from water and wastewater. However, traditional water remediation methods, such as adsorption, chemical precipitation, flocculation, ion exchange, membrane filtration and solvent extraction, are generally too costly, energy intensive and metal specific (Mishra and Tripathi 2008).

In recent years, considerable attention has focused on the development of alternative methods that are less expensive and more environmentally sustainable than conventional technologies. Phytoremediation is a green technology that uses plants to remediate soils, sediments, surface water and groundwater contaminated by several pollutants. One of the greatest benefits of phytoremediation is that metal removal can occur directly at the sites of pollution (Padmavathiamma and Li 2007; Shi et al. 2012). Most studies concerning water purification have been carried out on aquatic plant species such as *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia herzogii*, *Hydromistia stolonifera*, *Lemna minor* and *Wolffia globosa*. These aquatic macrophytes have been identified as Cd accumulators, suggesting their possible use for the removal of this metal from wastewater (Maine et al. 2001; Xie et al. 2013). In this context, Zacchini et al. (2011a, b) reported the interesting perspectives of the fast-growing, phreatophytic trees for phytoremediation of polluted waters.

Some authors have pointed out that the ideal plant for Cd phytoremediation should concentrate the metal in the above-ground woody, not renewable, organs and have a high biomass production (Vassilev et al. 2005; Shi and Cai 2009; Pietrini et al. 2010a). In this regard, Salicaceae plants have been intensively studied for their ability to accumulate and tolerate heavy metals (Dos Santos Utmazian et al. 2007; Zacchini et al. 2009, 2011a; Drzewiecka et al. 2012; Fernàndez et al. 2012). *Salix* spp. include species that grow along watercourses and rivers and perform an important ecological role in the riparian ecosystem (Cartisano et al. 2013), providing erosion control, water quality improvement and wildlife habitats (Kuzovkina and Quigley 2005). Willows are characterised by many desirable traits for environmental remediation projects: high biomass yield that can be used for biofuel production, fast growth, an extensive root apparatus and a low impact on trophic chains (Marmiroli et al. 2011; Tognetti et al. 2013). Additionally, short rotation coppice management of willow is another suitable trait for phytoremediation programmes, improving the ability to accumulate Cd due to a much higher biomass yield (Dickinson and Pulford 2005; Zhivotovsky et al. 2011).

A large variation in Cd tolerance and accumulation pattern has been observed among willow clones (Landberg and Greger 1996; Vysloužilova et al. 2003; Zacchini et al. 2009). Studies in field trials, greenhouse and hydroponic pot experiments have reported that many species or genotypes of *Salix* have the ability to accumulate high levels of

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Cd in above-ground tissues (Cosio et al. 2006; Meers et al. 2007; Zacchini et al. 2009; Pietrini et al. 2010b). This ability, combined with a valuable metal tolerance and high biomass productivity, is considered a suitable characteristic with which to select an appropriate genotype for the remediation of Cd-polluted water (Vassilev et al. 2005). In this context, the maintenance of photosynthetic activity could be considered as a further indicator to select suitable willow clones for phytoremediation. In fact, Cd alters photosynthetic efficiency and affects growth rate and productivity, which are important factors in selecting high-yielding genotypes (Pietrini et al. 2010a; Flood et al. 2011). In this regard, Pietrini et al. (2010b) outlined a peculiar strategy adopted by willow plants to maintain a high photosynthetic efficiency in leaves in the presence of a high concentration of Cd.

Hydroponics represents a suitable experimental methodology to assess the ability of a plant to tolerate and accumulate metals; hydroponic culture allows a uniform exposure of the root apparatus to the imposed metal concentration and also shortens the treatment period and increases the repeatability of experiments (Pietrini et al. 2010a; Drzewiecka et al. 2012). Furthermore, willow shows high resistance to temporary waterlogging, a fundamental prerequisite to evaluate the remediation potential towards water pollution by Cd (Jensen et al. 2009; Liu et al. 2011).

The aim of this work was to investigate the metal tolerance and accumulation in willow clones by analysing growth responses, photosynthetic performance and Cd content in different organs, in order to assess the Cd phytoremoval ability in polluted waters. To achieve this, we used four clones of *Salix alba* L., a pioneer tree species from the riparian forest (Kuzovkina and Quigley 2005), whose potential for phytoremediation has been previously evaluated under hydroponics (Zacchini et al. 2009).

7.2 Materials and Methods

7.2.1 Plant Material and Growth Conditions

Woody cuttings (20 cm long) of *Salix alba* L. clones SS5, SP3, 6-03 and 2-03 were rooted and grown for 3 weeks in pots filled with 3 L of a continuously aerated one-third strength Hoagland's nutrient solution, pH 6.5. The willow plants used in this experiment were selected from clones of a large Salicaceae collection field, previously assessed for biometric responses to Cd (Zacchini et al. 2009). Cuttings were grown in a controlled climate chamber equipped with metal halide lamps (Powerstar HQI-TS; Osram, Munich, Germany) providing a photon flux density (PPFD) of 300 μmol m^{-2} s^{-1} for 14 h at 25 °C. During the 10 h dark period, the temperature remained at 20 °C. Relative humidity was 60–70 %. At the end of the rooting and acclimation phase, homogeneous grown cuttings were subjected to two treatments: (1) Cd treatment, in which plants were exposed for 3 weeks to Hoagland's solution containing 50 μM of $CdSO_4$ (Sigma, St. Louis, MO, USA) and (2) control treatment, in which plants were maintained in the nutrient solution throughout the experiment. Each treatment group consisted of five cuttings of each clone. Nutrient solutions were completely replaced twice a week to prevent depletion of the metal and nutrients. At the end of the treatment, control and treated plants were harvested and washed with 0.05 M calcium chloride for 30 min in slow agitation without damaging the roots. Plant material was separated into roots, stem and leaves, dried in an oven at 80 °C until a constant weigh was reached.

7.2.2 Cd Content Analysis

Cadmium concentration was measured using an atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT, USA) on digested samples. Dried material was milled to a fine powder (Tecator Cemotec 1090 Sample Mill; Tecator, Hoganas, Sweden), weighed and mineralised. Mineralisation was performed by treating 250 mg of powdered samples with 4 ml of concentrated HNO_3, 3 ml of distilled water and 2 ml of H_2O_2 (30 % vol/vol m/m in water) and heating (EXCEL Microwave Chemistry Workstation, Preekem Scientific Instruments Co., Ltd, Shangai, China) in a four-step procedure: 100 °C for 1 min at 250 PSI, 140 °C for 1 min at 350 PSI, 170 °C for 1 min at 450 PSI and 200 °C for 12 min at 550 PSI.

The metal uptake ratio evaluates the capability of the plant to extract and accumulate Cd and it was calculated as the ratio of the Cd content in the whole plant to the Cd content of the corresponding growth solution (Iori et al. 2013).

The metal translocation index evaluates the ability of the plant to translocate Cd along the plant, and it was measured as the percent ratio of the Cd content of the aerial parts (leaves and stem) to the Cd content of the corresponding roots.

The metal content in plant parts was calculated by multiplying dry weight by metal concentration.

7.2.3 Gas Exchange and Chlorophyll Fluorescence Measurements

At the end of the experiment, net photosynthesis (A, A_{max}), transpiration rate (E), quantum yield ($\Delta F/F_m$) of electron transport through photosystem II (PSII) and quenching coefficient (qP and NPQ) were measured in the cuvette on the last fully expanded leaf with gas exchange system

(HCM 1000, Walz, Germany), configured for simultaneous measurements of chlorophyll fluorescence (MINI-PAM, Walz, Germany). The relative humidity of air entering the cuvette was set at 60 % and the temperature of both air and cuvette was 25 °C. CO_2 partial pressure was set at 370 µbar bar^{-1}. A white light source (KL 1500; Schott, Mainz, Germany) was used to vary the incident photosynthetic photon flux density (PPFD) on the leaf surface between 300 and 700 µmol m^{-2} s^{-1}. The value for net CO_2 assimilation rate (A, A_{max}) was calculated using the equations of von Caemmerer and Farquhar (1981). The fluorescence quantum yield of electron transport through PSII ($\Delta F/F_m$) was calculated as $(F'_m - F_s)/F'_m$ where F'_m is the maximum fluorescence and F_s is the steady-state fluorescence in the illuminated leaf (Genty et al. 1989). Chlorophyll fluorescence quenching parameters were calculated according to Schreiber et al. (1986). The efficiency of PSII (F_v/F_m) was measured with a PEA fluorometer (Plant Efficiency Analyzer, Hansatech, King's Lynn, Norfolk, UK) on 30 min dark-adapted leaves dividing the difference between the maximum (F_m) and the minimum fluorescence (F_o) ($F_v = F_m - F_o$) by F_m.

7.2.4 Pigment Analysis

The analysis of chlorophylls "a" and "b" and total carotenoid contents was performed according to Pietrini et al. (2010a). Two square centimetres of the last fully expanded leaf were ground under dim light in a mortar containing liquid N_2. The leaf was reduced to a fine powder, and 2 ml of methanol were added to extract the pigments. The samples were centrifuged at 12,000 × *g* at 5 °C for 10 min, and the supernatant was collected and used for pigment determinations. Absorbance was measured at 470, 652.4 and 665.2 nm with a spectrophotometer (Perkin Elmer, Norwalk, CT, USA). The chlorophylls "a" and "b" and total carotenoid contents were calculated using the extinction coefficients and the equations reported by Wellburn (1994). Five replicates were done for each measurement.

7.2.5 Statistical Analysis

The data reported refer to a representative experiment with five replicates. Experimental data were processed with a two-way analysis of variance (ANOVA) using the SPSS software supplemented with multiple comparison test of the means using the Duncan's method with a significance level of $P < 0.05$. Statistical significance of the data as resulting by the effects of the two factors (clones and treatment) and their interaction is presented in Table 7.1.

Table 7.1 ANOVA results for the effects of willow clones, cadmium treatment and their interaction on the different analysed parameters

Parameter	Clones	Treatment	Interaction
Cd amount roots	**	–	–
Cd amount roots	**	–	–
Cd amount roots	**	–	–
Cd amount leaf	*	–	–
Cd amount stem	*	–	–
Whole plant uptake	*	–	–
Metal translocation index	**	–	–
Uptake ratio	*	–	–
Leaf Dw	***	ns	ns
Stem Dw	***	ns	ns
Root Dw	***	***	***
Root/shoot	***	***	*
Chl a	***	***	***
Chl b	***	***	***
Chl Tot	***	***	***
Chl a/b	***	***	***
Total carotenoids	***	***	***
A	ns	***	ns
A_{max}	ns	***	ns
E	ns	***	*
*i*WUE	ns	***	*
F_v/F_m	**	***	ns
F_o	ns	ns	ns
ΦPSII	***	***	***
qP	ns	***	*
NPQ	ns	***	**

ns not significant

Significance of the main effects and their interaction are indicated as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

7.3 Results and Discussion

Studies on metal phytotoxicity commonly describe the inhibition of biomass accumulation as one of the main symptoms of Cd stress in plants (Vyslouzilova et al. 2003; Vassilev et al. 2005). In this study, the four willow clones showed a different biomass accumulation and distribution among organs (Fig. 7.1). In particular, clone 2-03 was characterised by the highest dry mass production in all organs, although this was only statistically significant in roots, and also by the highest root-to-shoot ratio. Cadmium treatment differentially affected these growth parameters among clones. While the leaf and stem dry mass did not significantly change due to Cd exposure, root dry mass was clearly reduced in clones 2-03 and 6-03, whereas in clones SP3 and SS5 no change in root biomass occurred.

A similar trend was observed for that regards the root-to-shoot ratio. As a general consideration, Cd exposure caused the highest growth reduction in clone 2-03 and the lowest in

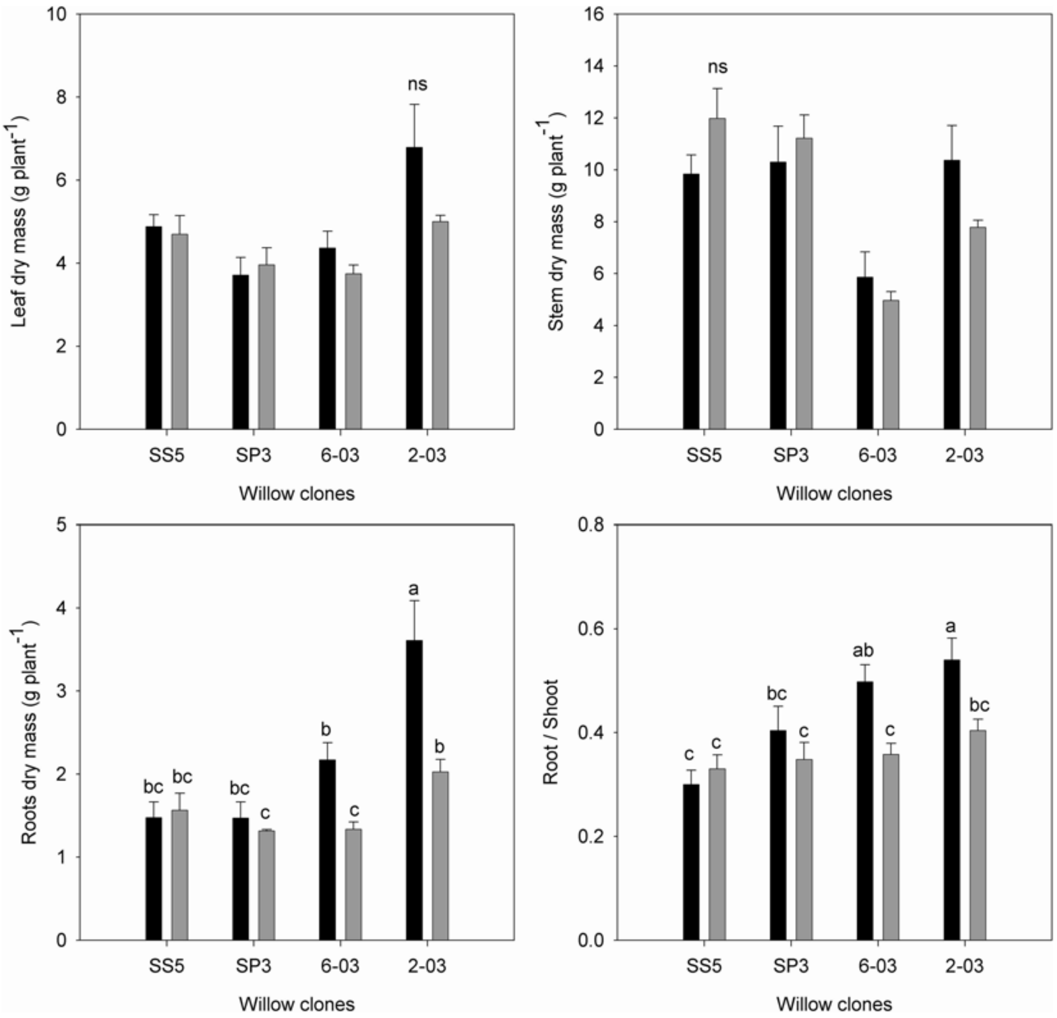

Fig. 7.1 Leaf, stem and root dry mass (g plant^{-1}) and root-to-shoot ratio in willow clones grown for 3 weeks in the presence of 0 µM (control, *black bars*) and 50 µM (*grey bars*) $CdSO_4$ in hydroponics (mean ± S.E., *n* = 5). In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

clone SS5. As the root is the first organ to come into contact with Cd in the nutrient solution and represents the primary route for metal absorption, root growth is considered a suitable parameter for metal tolerance tests (Wong and Bradshaw 1982). The reduction of root biomass observed in clones 2-03 and 6-03 might be due both to competition in the uptake between Cd and some micronutrients essential for plant growth and to the inhibition of root cell division as has been suggested by several authors (Ernst et al. 1992; Gaudet et al. 2011; Zhivotovsky et al. 2011).

The metal bioaccumulation capability is a crucial trait to screen plants for phytoremediation, and the metal uptake parameters are reported as very relevant to the understanding of the plant responses to heavy metal stress (Diwan et al. 2010). Data for Cd accumulation and distribution among organs of willow clones are shown in Fig. 7.2. About Cd accumulation in the leaf and stem, the results showed a similar trend, being the highest accumulation in clone SS5 and the lowest in clone 2-03, with intermediate values in the other two clones.

In roots, a more uniform Cd accumulation was observed among clones, with the lowest value detected in SP3. Consequently, total plant Cd amount was higher in clones SS5 and 6-03 compared to that in the other two willow clones. To better highlight the ability of the selected willow clones to extract Cd from the solution and accumulate it in plant tissues, the metal uptake ratio was calculated (Fig. 7.3). Data confirmed the higher performance of clones SS5 and 6-03 compared to clones SP3 and 2-03.

Phytoremediation efficiency depends not only on the plant ability to tolerate and accumulate high metal amounts, but also on its potential to translocate the metal from the root to the shoot. In this context, metal translocation to the aerial part can be considered as a crucial biochemical process

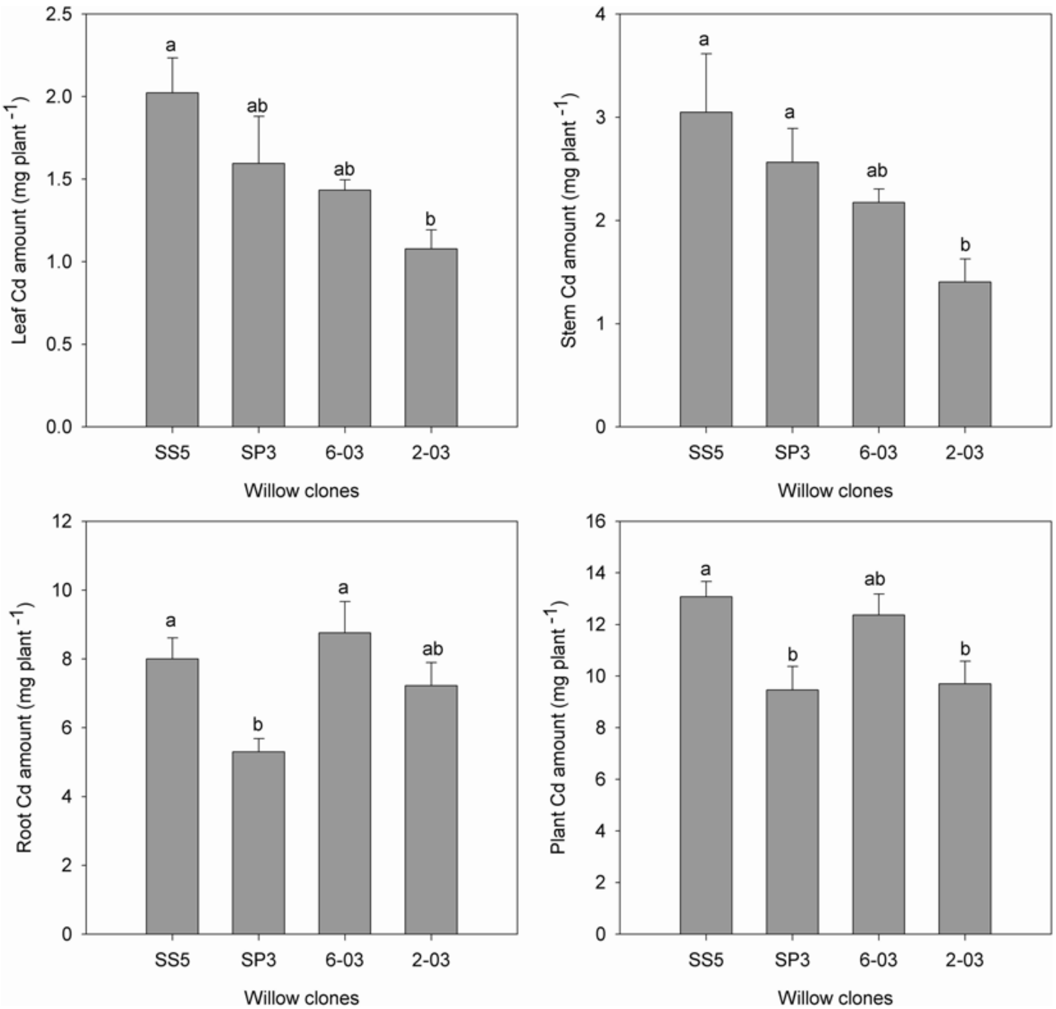

Fig. 7.2 Leaf, stem, root and total plant Cd content (mg plant^{-1}) in willow clones grown for 3 weeks in the presence of 50 µM $CdSO_4$ in hydroponics (mean ± S.E., $n = 5$). In control plants Cd content was undetectable and data were not showed. In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

for an effective utilisation of plants to remediate polluted substrates (Zacchini et al. 2009; Diwan et al. 2010). In fact, the accumulation of Cd in the above-ground woody tissues could reduce the damaging effects exerted by this pollutant on root physiology and biochemistry, resulting in a higher metal removal efficiency, as these plant parts are commonly harvested and stored. In this regard, Pietrini et al. (2010b) put in evidence a peculiar tolerance mechanism carried out in leaves by willow plants to avoid Cd-induced impairment of photosynthetic processes. Regarding metal accumulation in the roots, it is worth to note that in water decontamination technologies, based on plants, also the rooting system can easily be removed by harvesting the whole plant. The ability to translocate the absorbed metal from roots to shoots was shown by calculating the Cd translocation index (Fig. 7.3), which was far higher in clones SS5 and SP3 compared to clones 6-03 and 2-03. These data confirmed the different capability of willow clones to translocate heavy metals in the aerial part, in accordance with findings of several authors (Greger and Landberg 1999; Vyslouzilova et al. 2003; Dos Santos Utmazian et al. 2007; Zacchini et al. 2009).

Cadmium tolerance at leaf level is another important trait to screen plants for phytoremediation (Zacchini et al. 2009; Pietrini et al. 2010a). In leaves, Cd has been reported to interfere with photosynthesis at different levels, i.e. chlorophyll biosynthesis and degradation, stomatal conductance, activities of Calvin cycle enzymes, assembly of pigment protein complexes and thylakoids, electron transport chain and sugar transport and consumption (Seregin and Ivanov 2001; Pietrini et al. 2005). Consequently, photosynthetic performance can be considered as one of the most sensitive indicators of Cd toxicity (Vassilev et al. 2005). In this study, the effect of Cd on the photosynthetic apparatus was evaluated by the analysis of pigment contents, gas exchange measurements

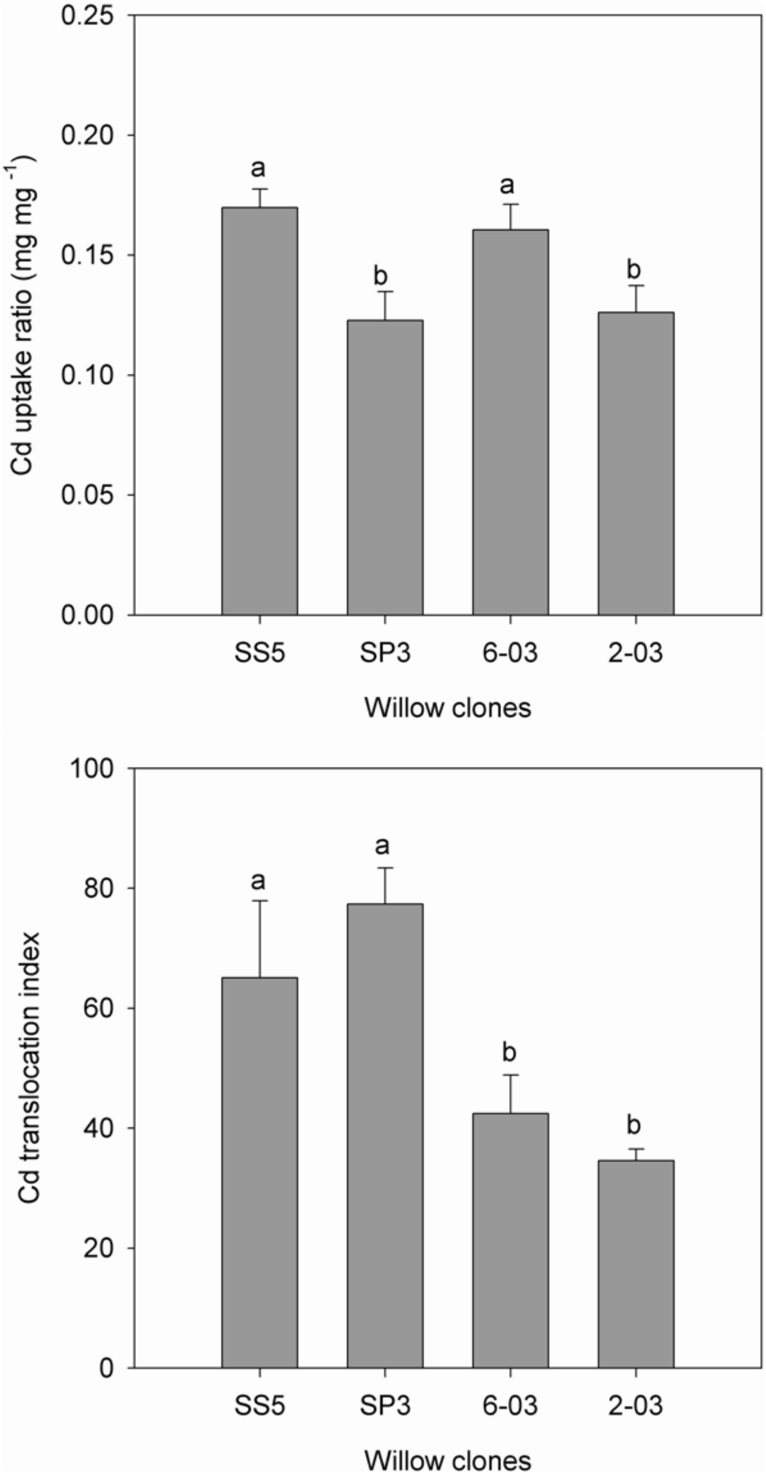

Fig. 7.3 Cadmium uptake ratio (mg mg^{-1}) and cadmium translocation index in willow clones grown for 3 weeks in the presence of 50 µM $CdSO_4$ in hydroponics (mean ± S.E., $n = 5$). In control plants Cd content was undetectable and data were not showed. In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

and chlorophyll fluorescence detection. The effects of Cd treatment on Chl a, Chl b, total Chl, Chl a/b ratio and total carotenoid content in the willow clones are shown in Fig. 7.4. Chl a content was slightly reduced in Cd-treated plants of clone SS5 in comparison to the control, whereas a more pronounced decrease was observed in the other clones, particularly in 6-03. A similar reduction was observed for Chl b content, although the highest reduction caused by Cd treatment was found in clone SP3.

The analysis of total Chl content of the willow clones following Cd treatment showed the lowest and the highest decrease, compared to control, in clones SS5 and 6-03,

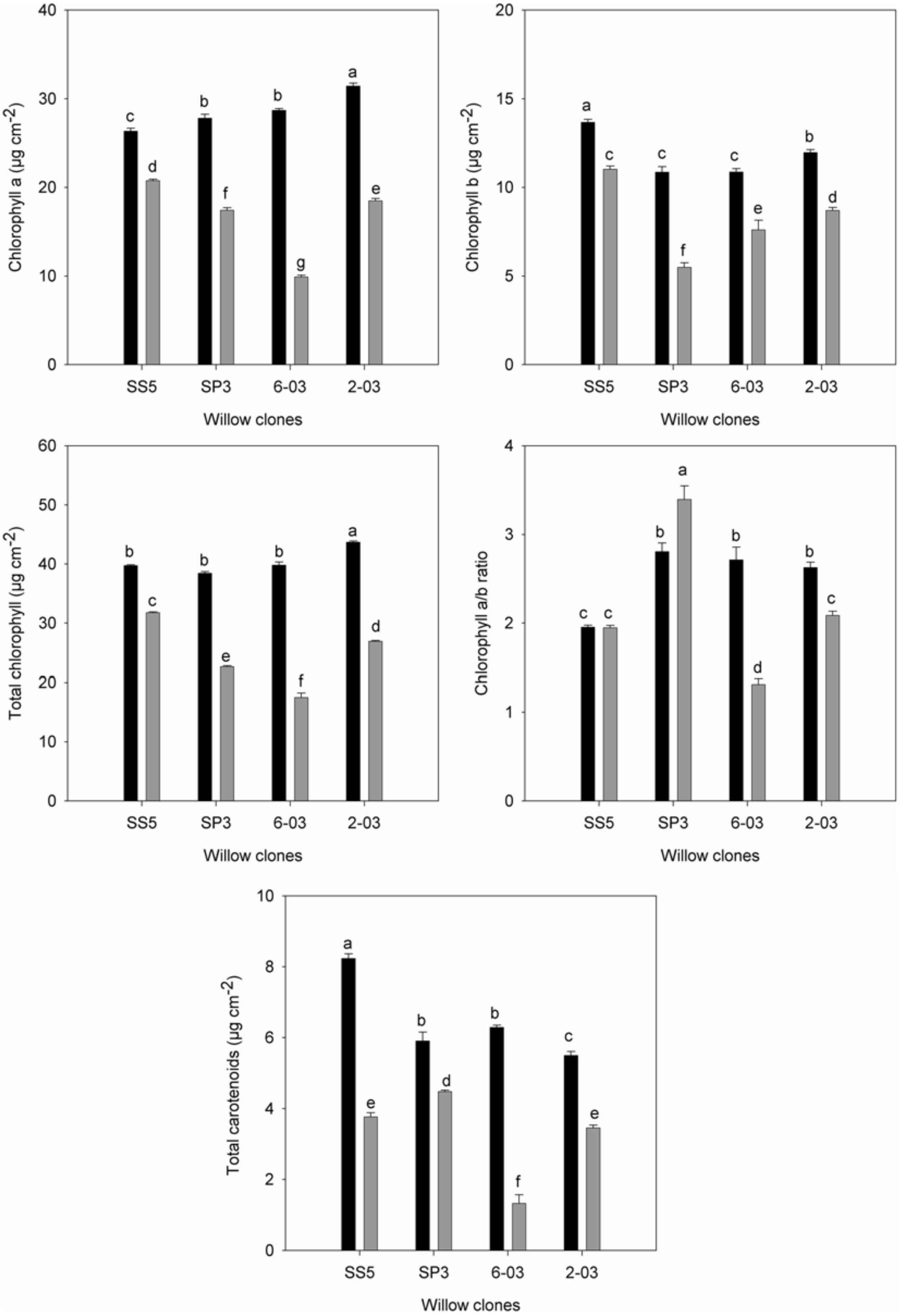

Fig. 7.4 Chlorophyll a, chlorophyll b and total chlorophyll content (µg cm^{-2}), chlorophyll a to chlorophyll b ratio and total carotenoid content (µg cm^{-2}) in leaves of willow clones grown for 3 weeks in the presence of 0 µM (control, *black bars*) and 50 µM (*grey bars*) $CdSO_4$ in hydroponics (mean ± S.E., $n = 5$). In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

respectively, whereas in clones SP3 and 2-03 an intermediate response was observed. As previously suggested by Vassilev et al. (2005) in a similar experiment, the decrease in chlorophyll content in leaves of Cd-treated willow plants might be a consequence of metal-induced enzymatic degradation as well as mineral deficiency. The Chl a/b ratio was notably altered by Cd treatment. Although no effect of Cd treatment was revealed in clone SS5, either a slight or a marked decrease in this parameter was observed in clones 2-03 and 6-03, respectively. This was probably due to a faster degradation of Chl a compared with Chl b under Cd stress (Oliveira et al. 1994; Kummerová et al. 2010). Since the Chl a/b ratio is an indicator of both the functional pigment equipment and the light adaptation/acclimation of the photosynthetic apparatus, monitoring its decrease can be used as an early warning system for the toxic effect of metal accumulation in plants (Li et al. 2009). Conversely, an increase in the Chl a/b ratio was detected in plants of clone SP3. An increase in the Chl a/b ratio has been linked with a change in pigment composition of a photosynthetic apparatus that possesses a lower level of light-harvesting chlorophyll proteins (LHCPs) (Loggini et al. 1999). The reduction in LHCP content is an adaptive defence mechanism of chloroplasts, which allows plants to endure the adverse conditions (Asada et al. 1998). Total carotenoid content (Fig. 7.4) was remarkably and differentially affected by Cd treatment among willow clones. The content of these pigments was dramatically reduced in clone 6-03 but was also markedly decreased in clone SS5. A smaller decrease in pigment content was observed in clones SP3 and 2-03, even though Cd effect in these clones was statistically significant. Carotenoids, other than functioning as light-absorbing pigments, play an important role in the photoprotection of chlorophylls against photo-oxidative damage by quenching reactive oxygen species (ROS) such as singlet oxygen (Behera et al. 2002). In this study, Cd treatment significantly decreased the total chlorophyll and carotenoid content of the tested clones. Similarly, other authors observed a decrease in carotenoid content of Cd-treated plants (Pietrini et al. 2010a; Wang et al. 2013). Previous studies reported that a relationship exists between the maintenance of photosynthesis as close as possible to control rates and the effectiveness of tolerance mechanisms towards Cd toxicity in leaves (Schützendübel and Polle 2002; Pietrini et al. 2003). In this regard, the effects of Cd treatment on leaf gas exchange parameters were measured at the end of the 3-week experiment on the four willow clones, and results are shown in Fig. 7.5. Cadmium treatment reduced net photosynthesis both in growth (A) and in saturating light conditions ($A_{\max}$) in comparison to the control, in all clones. Photosynthesis is a key process for plant growth and biomass production, and it has been used as a bioindicator of stress (Sheoran et al. 1990). Reduced photosynthetic activity is commonly observed in willow plants exposed to Cd treatment (Vassilev et al. 2005; Pietrini et al. 2010b).

This deleterious effect on photosynthetic rates might be a result of both the imbalance in mineral nutrition and direct metal interference in hindering photosynthetic reactions (Chugh and Sawhney 1999). Heavy metals are capable of affecting chlorophyll content, the activity of Calvin cycle enzymes, and even PSII is reported to be extremely sensitive to Cd ions (Di Cagno et al. 1999, 2001).

A high transpiration rate in woody plants represents a useful trait to evaluate their potential for metal phytoextraction (Stomp et al. 1993). Regarding Cd, it has been suggested that its translocation to the shoots can be driven by transpiration (Salt et al. 1995). In this study, a higher leaf Cd concentration in clones SS5 and SP3 fitted well with their higher transpiration rate (E) as compared with that of clones 6-03 and 2-03 (Fig. 7.5). As reported by Hagemeyer and Waisel (1989), a decreased transpiration rate in treated plants implies that Cd also affects water relations. In our study, in Cd-treated plants the values of instantaneous water use efficiency (*i*WUE) were markedly reduced in SP3, slightly decreased in SS5 and 6-03 with respect to the control and not affected in clone 2-03. These results are consistent with Pajević et al. (2009) who pointed out that a stable value or a slight decrease of *i*WUE (with respect to the control), in plants grown under unfavourable environmental conditions, can be considered a symptom of high organic production and an indication of a valuable metal remediation potential.

The measurement of chlorophyll fluorescence parameters has been highlighted as very effective in assessing the metal tolerance strategies at leaf level (Di Cagno et al. 1999; Linger et al. 2005; Pietrini et al. 2010a, b). In the present work, the effects of Cd treatment on leaf chlorophyll fluorescence parameters, measured at the end of the experiment on the four willow clones, are shown in Fig. 7.6. The efficiency of PSII (F_v/F_m) was affected by both willow clones and Cd treatment (Table 7.1; Fig. 7.6). Cadmium treatment did not affect the basal fluorescence emission (F_o) in comparison to the control in all willow clones. These results indicate that there is no clear relationship between F_v/F_m and Cd tolerance, confirming that this parameter, singly evaluated, is unsuitable for assessing Cd tolerance, as already evidenced by Pietrini et al. (2003, 2010a).

Although Cd did not cause a significant decrease in F_v/F_m in all clones, it differentially lowered photosynthetic electron transport, as shown by $\Delta F/F_m$, qP and NPQ parameters. In fact, the fluorescence quantum yield of electron transport through PSII ($\Delta F/F_m$) analysis revealed a differential damage due to Cd exposure of willow clones, with clone SS5 showing the lowest decrease. About photochemical quenching

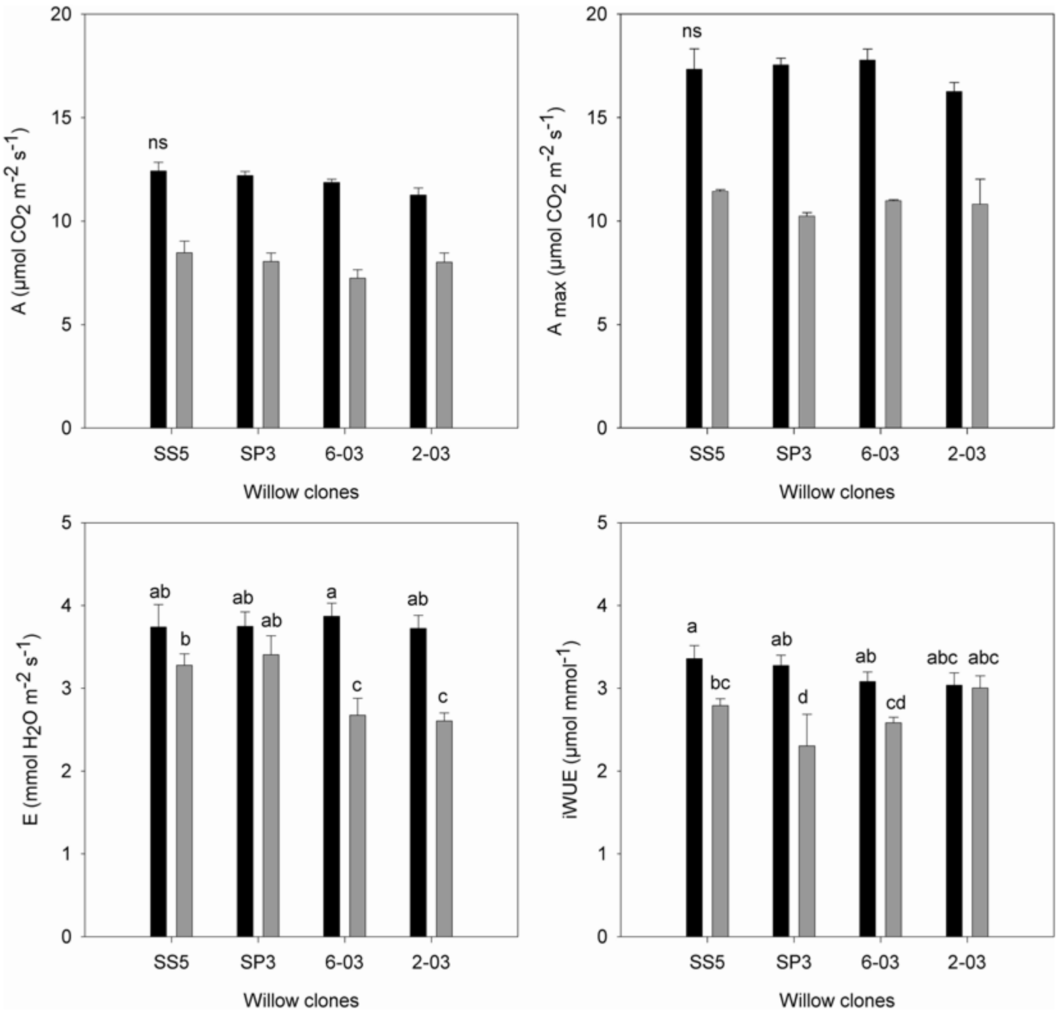

Fig. 7.5 Measurements of net photosynthesis in growth (*A*) and in saturating light conditions (A_{max}), transpiration rate (*E*) and instantaneous water use efficiency (*i*WUE) in leaves of willow clones grown for 3 weeks in the presence of 0 µM (control, *black bars*) and 50 µM (*grey bars*) $CdSO_4$ in hydroponics (mean ± S.E., $n = 5$). In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

(qP), in the presence of Cd, a marked decrease was observed only in clone 2-03. Non-photochemical quenching (NPQ) showed a marked increase in Cd-treated plants of clones SS5, 6-03 and particularly in clone 2-03 with respect to the control, whereas no difference in this parameter was observed in clone SP3. The chlorophyll fluorescence parameters $\Delta F/F_m$ and qP are related to F_v/F_m (Maxwell and Johnson 2000). The changes in these parameters might be associated with the alterations in chlorophyll content described above (Fig. 7.4) and suggest that Cd inhibits PSII activity. In contrast, non-photochemical quenching of maximum Chl fluorescence was increased by Cd treatment, allowing the dissipation of excessive energy within the photosystems (Maxwell and Johnson 2000; Sofo et al. 2009). These results are consistent with data reported by several authors (Di Cagno et al. 1999; Linger et al. 2005; Pietrini et al. 2010a) and highlight that these parameters reflect Cd toxicity effects in leaves better than F_v/F_m does.

7.4 Conclusion

For the successful application of phytoremediation technology, analysis of traits such as metal uptake and the translocation and accumulation in different plant organs is crucial to select plants with higher heavy metal removal efficiencies. In this study, we highlighted that four willow clones exposed to Cd in hydroponic conditions can differentially tolerate,

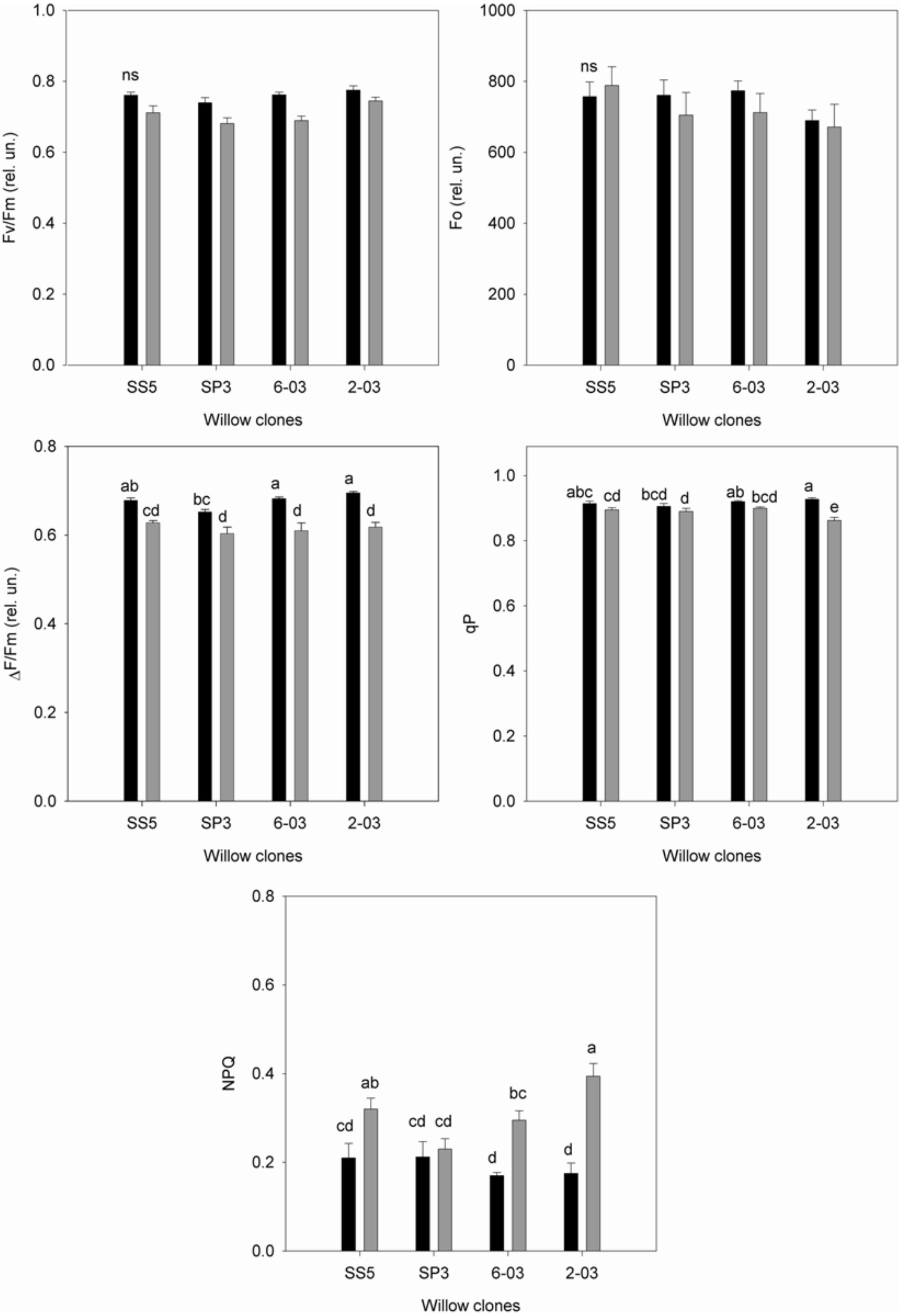

Fig. 7.6 Measurements of leaf chlorophyll fluorescence parameters: maximal quantum efficiency (F_v/F_m), minimum (F_o) and maximum (F_m) fluorescence yields, quantum efficiency of PSII photochemistry ($\Delta F/F_m$), photochemical quenching (qP) and non-photochemical quenching (NPQ), in leaves of willow clones grown for 3 weeks in the presence of 0 µM (control, *black bars*) and 50 µM (*grey bars*) $CdSO_4$ in hydroponics (mean ± S.E., *n* = 5). In column, *different letters* correspond to statistical different values (Duncan's test, for the *P* value see Table 7.1)

transport and accumulate the metal. Among the tested clones, SS5 revealed a valuable potential for Cd phytoremediation and deserves further attention to better characterise its metal removal capability in waters. In fact, this clone showed the highest ability to extract and accumulate Cd in roots and above-ground organs, maintaining a more efficient photosynthetic activity compared to the other clones. In this regard, based on the data about Cd content, plants of clone SS5 showed the ability to remove approximately 65 mg of Cd from the solution present in each pot, representing about the 40 % of the total metal amount added in the nutrient medium during the treatment. Moreover, the capability of this clone to tolerate Cd presence in the solution, showed through physiological and growth parameters, allows supposing that Cd removal efficiency can be maintained over time. Further investigations will be focused on testing the Cd phytoremoval ability observed in this study by the *S. alba* clone SS5 in a scaled-up system supplied with actual metal-contaminated wastewater.

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8 Overview and New Insights of Genetically Engineered Plants for Improving Phytoremediation

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8.1 Introduction

Phytoremediation is a green, eco-friendly, and emerging technology that uses plants and enzymes derived from them for the treatment of soil, water, and groundwater contaminated by toxic pollutants (Ali et al. 2013). The concept of using plants for these purposes emerged few decades ago with the understanding that plants were capable of accumulating high quantities of toxic metals and/or metabolizing organic compounds in their tissues or organs. However, the term "phytoremediation" has been used since the beginning of the 1990s, when scientists focused their research interests on plant-based technologies, as suitable alternatives to traditional cleanup procedures because of their low capital costs, low maintenance requirements, end-use value, and aesthetic nature. Since then, a number of related technologies were developed, and the definition later evolved in "phytotechnologies" (ITRC 2001) that includes a wide range of processes such as phytoextraction, phytostabilization, phytotransformation, phytovolatilization, rhizofiltration, and phytostimulation, which have been extensively described (Pilon-Smits 2005; Abhilash et al. 2009). In addition to the well-known advantages of phytoremediation over other remediation strategies, it offers potential benefic side effects, such as erosion control, site restoration, carbon sequestration, and feedstock for biofuel production (Van Aken et al. 2010).

Even though these technologies have shown to efficiently reduce the chemical hazard associated with various classes of inorganic and organic pollutants, they also have several limitations related to the metabolism of recalcitrant xenobiotic compounds. Thus, to improve the phytoremediation abilities of selected plants, research was oriented to the obtainment of genetically modified plants (Macek et al. 2008; Novakova et al. 2010). Genes involved in multistep degradation pathways of pollutants, transport, and sequestration can be isolated from bacteria, fungi, animals, or plants and introduced into candidate plants. Therefore, three main strategies have been employed: (a) transformation with genes from other organisms, (b) transformation with genes from other plant species, and (c) overexpression of genes from the same plant species. The target is to obtain a plant with high ability to tolerate, accumulate, detoxify, or degrade pollutants and with suitable agronomical characteristics. In fact, the possibility of using transgenics in phytoremediation depends on the availability of gene sequences, which can improve, modulate, or radically change plant metabolism, conferring to the plant a phenotype which does not naturally possess. The main objective of inorganic remediation is to enhance metal accumulation and tolerance by overexpressing in transgenic plant genes involved in homeostasis, metabolism, uptake, and/or translocation of the toxic elements (Kotrba 2013). On the contrary, for organic compounds, this approach could lead, in an optimal case, to the complete mineralization of the pollutants or the formation of less toxic metabolites than those produced by the existing pathway. Another potential advantage of the transgenic approach is the possibility of producing enzymes in root exudates through the expression (or overexpression) of secretory enzymes involved in pollutant removal or through the expression of heterologous enzymes with signal sequences to drive them to the secretary pathway.

In the case of a heterologous gene introduced with genetic engineering, a critical issue is its expression level in the new genetic and cellular context. For this reason, the introduced genes are frequently embedded in an "expression cassette" which can ensure an efficient transcription and translation (Maestri and Marmiroli 2011).

Since the first work describing transgenic plants modified for pollutant metabolism (Feng et al. 1997), a lot of research

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has been performed in this way. However, works dealing with inorganic contaminants have always been more abundant, as compared to works on organic contaminants, and there is only a small amount of papers addressing simultaneously with both compounds.

Regarding plant species used for transformation experiments, *Arabidopsis thaliana* and *Nicotiana tabacum* are among the most common model organisms due to well-developed and efficient protocols for DNA delivery and recovery of transformants (Meyers et al. 2010). However, it is important to note that for the past few years, the use of transgenic plants has evolved from transformation of model plants and laboratory tests to clarify the roles and functions of genes to transformation of plants effectively useful for phytoremediation in the field (Maestri and Marmiroli 2011). Such plants would posses some suitable properties like high biomass and deep roots, and they would also be amenable to easy growth in different climatic and soil conditions.

Despite its promising potential, plant genetic engineering still faces considerable technical challenges. Thus, further research into mechanisms underlying nuclear and/or plastid transformation, more efficient DNA delivery systems as well as plant tissue culture and regeneration protocols are essential for progressing in the field of plant genetic engineering. In addition, certain drawbacks related to the applicability of transgenic plants for phytoremediation in the field should be saved in order to successfully apply this technology, as it will be described in the present chapter.

8.2 Phytoremediation of Inorganic Contaminants

Inorganic pollutant phytoremediation involves mechanisms, enzymes, and processes different from those concerning organic pollutants. This is mainly due to the fact that metal(loid)s are not degradable. Thus, phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration are suitable phytotechnologies for inorganic pollutant removal.

There are several factors that affect metal phytotoxicity such as metal type, concentration, redox state, and solubility. Metal type is the first aspect that needs to be considered. Some metals are essential for plants as micronutrients like Fe, Mn, Zn, Cu, Mg, Mo, B, Cl, and Ni, while others have no biological role such as As, Pb, Cd, Hg, Ag, and U and are toxic even at very low concentrations, being classified as nonessential metals. However, when some essential metals occurred at high concentrations, they can become toxic to plant cellular processes. Plants evolved several transport mechanisms to take up micronutrients from soil matrix. Contrarily, toxic metal(loid)s are not taken up by specific transpoter mechanisms and they are taken up by plants along with other micronutrients (Kabata-Pendias 2011).

Plants have developed defense mechanisms to minimize metal toxicity. For instance, plants can make metals insoluble mostly by the aid of chelating agents and after being accumulated. Besides, there are plant enzymes that can change the redox state to a less toxic form, whereas others can transform metal(loid)s to make them more volatile.

In this work, we are going to focus on plant genetic engineering directed to increase accumulation of nonessential metals since hyper-accumulation of essential metals is linked with food bio-fortification and it needs a different consideration and discussion. In this sense, food bio-fortification and phytoremediation are two sides of the same coin, although, as we already mentioned, essential metals in excess also become an environmental problem (Guerinot and Salt 2001). It is important to note that many transgenic plants have also been developed with the aim of studying both the function of metal(loid) transporter proteins and its relation with accumulation in edible parts to asses food security issues.

Transgenic approaches for improving inorganic phytoremediation include:

- *Increasing mobilization and uptake of metal(loid)s from the surrounding environment*
- *Increasing sequestration of metals within cells*
- *Increasing metal translocation to the aboveground tissues*
- *Increasing or adding ligands to allow phytovolatilization*

In this section and considering that each plant-metal system is so particular, i.e., it has specific molecular mechanism for uptaking, transporting, and sequestrating, we will review recent advances separately by metal(loid). However, most current findings provide evidence that the relationship between genetic engineering and the effect of the genetic modification is quite complex. In some cases, an increase in phytochelatins (PCs)/thiol peptide concentration has not always been correlated with an increase in metal(loid) accumulation and thus with plant resistance or tolerance. In other studies, it has been true for one metal and not for others.

8.2.1 Genetically Engineered Plants for Improving Phytoremediation of the Nonessential Metal(loid)s: As, Cd, Pb, and Hg

8.2.1.1 Arsenic

Arsenic (As) is a very toxic and ubiquitous metalloid. Although it has a geological origin, its concentration can be increased by anthropogenic activities such as pesticide application and wood preservatives, mining and smelting operations, and coal combustion (Wang and Mulligan 2006).

Consequently, elevated levels of As have been reported in soils and groundwater worldwide. In soils and groundwater, inorganic arsenic is present mainly as As^{+5} and As^{+3}. Arsenic is a nonessential element for plants. Thus, they do not have specific transporter systems for arsenic species. The lines below describe the targets for genetic engineering directed toward the improvement of arsenic phytoextraction and phytostabilization from soils and sediments.

Increasing Sequestration of As Within Cells

Phytochelatins play a central role in metal(loid) detoxification by chelating those toxic ions. Most research has focused in some of the three enzymes that constitute the PCs biosynthetic pathway: gamma-glutamylcysteine synthase (ECS), glutathione synthase (GS), and phytochelatin synthase (PCS). The expression of these genes from different organisms has contributed to the knowledge about tolerance and removal of As and other metals (Dhankher et al. 2002; Li et al. 2004, 2005, 2006). Moreover, the simultaneous overexpression of yeasts PCS and GS (*AsPCS1* and *GSH1*) in *A. thaliana* successfully led to elevated total PCs production and increased tolerance and accumulation of As and also Cd (Guo et al. 2008). Furthermore, independent overexpression of different PCS enzymes (*AtPCS1* gene from *Arabidopsis* or *CePCS* from *Caenorhabditis elegans*) in the same plant species (*N. tabacum*) resulted in distinctive metabolic changes accompanied by differences in Cd and As tolerance between the transgenic plants (Wojas et al. 2010). Recently, enhanced As tolerance of transgenic poplar plants was achieved by expressing the bacterial ECS (from *E. coli*) (LeBlanc et al. 2011). In addition, transgenic tobacco plants expressing *PCS* gene from *Ceratophyllum demersum* (*CdPCS1*), an aquatic macrophyte, showed severalfold increased PCs content and precursors of thiol peptides, with enhanced accumulation of Cd and As (Shukla et al. 2012).

Metallothioneins (MTs), another type of metal-binding ligands, are also targets of genetic engineering. MTs are low molecular mass proteins (from 2 to 16 kDa) with unique abundance of cysteine residues (more than 30 % from all amino acids). Even though the role of MTs in plant protective mechanisms against metals is not fully understood, they are known as effective free radical regulators by binding metals. Although As is not in the list of metals reacting with MTs, the expression of the MT 2b (*AtMT2b*) from *A. thaliana* in tobacco led to enhanced As^{+3} sensitivity and translocation (Grispen et al. 2009).

All current publications seem to indicate that multigene approach directed to increase sequestration of As within cells led to better results than simple gene transformation. This is a very important consideration since most contaminated sites, such as mining or industrial areas, contain a mixture of metals, and thus transgenic plant with phytoremediation capabilities must be able to cope with this situation.

Increasing As Translocation to the Aboveground Tissues

The mechanisms involved in As translocation are not fully understood; consequently the development of transgenic plants with genes involved in this process has been delayed. Recently, transgenic *A. thaliana* plants expressing an As^{+3} antiporter gene from *P. vittata (PvACR3)* accumulated approximately 7.5-fold more As in the aboveground tissues than WT plants (Chen et al. 2013). These results suggest the involvement of *PvACR3* in As translocation.

Adding Ligands to Allow As Volatilization

Regarding As volatilization, no genes for methyltransferase activity have been identified in plants. However, an *arsM* gene from the soil bacterium *Rhodopseudomonas palustris* was expressed in rice (*Oryza sativa*), and the transgenic rice produced volatile methylated arsenic species (MMA^{+5} and DMA^{+5}) after exposure to As^{+3}, theoretically providing a potential strategy for phytoremediation (Meng et al. 2011).

8.2.1.2 Lead

Lead (Pb) contamination of soils is widespread at many industrial and mining sites throughout the world. In addition, Pb contamination also derives from the past use of lead pesticides, leaded paints, leaded gasoline, and some types of pressure-treated wood. Because of the immobility of Pb in soils, historical Pb contamination in urban, industrial, and high-traffic areas persists today despite the phase out of leaded gasoline and paints beginning in the 1970s (McBride et al. 2012). In a similar way as other nonessential and highly toxic elements, plant cells are not likely to possess specific Pb transporters (Arazi et al. 1999), but some cation transporters in the plasma membrane offer potential entry pathways into plant cells. There has been a lack of specific genes identified as conferring capacity for Pb resistance and accumulation (Song et al. 2003), which has delayed genetic engineering for the obtainment of Pb-extracting plants. However, many advances have taken place in the last years.

Increasing Pb Uptake from Soil

NtCBP4 gene isolated from *N. tabacum* codifies a membrane protein channel which can carry Pb ions through the plasma membrane into the plant cell. Transgenic plants overexpressing *NtCBP4* exhibited increased accumulation of Pb (Arazi et al. 1999). Gupta et al. (2013) mentioned this strategy as the first example of a plant gene that can modulate Pb tolerance and accumulation, after which there have not been other advances in this aspect.

Increasing Sequestration of Pb Within Cells

This approach has been the most applied concerning lead phytoextraction. Several studies have evaluated Pb accumulation and translocation in plants transformed with genes involved in general processes of metal sequestration. For instance, three different plant species (*A. thaliana*, *Brassica juncea*, and *Populus alba*×*P. tremula var glandulosa*) overexpressing a yeast Cd factor 1 (*ScYCF1*, from *Saccharomyces cerevisiae*), a transporter that pumps GSH-conjugated Cd into the vacuole, tolerated and accumulated increased amounts of Pb conjugated with glutathione (GSH) from cytoplasm to vacuoles (Song et al. 2003; Bhuiyan et al. 2011b; Shim et al. 2013). In a similar approach, directed to enhance the expression of PCs, aspen transgenic lines expressing a *PCS* gene (*TaPCS1)* from wheat reached total biomass and Pb accumulation significantly greater than in the control plants (Couselo et al. 2010). These studies evidence the utility of overexpressing ligands able to bind Pb ions and transport them conjugated into vacuoles.

A different and successful approach was overexpression of *AtATM3*, a member of the ATP-binding cassette (ABC) transporter family localized at the mitochondrial membrane, under the control of the CaMV35S in *B. juncea.* This genetic modification conferred enhanced tolerance not only to Pb^{+2} but also to Cd^{+2} (Bhuiyan et al. 2011a).

8.2.1.3 Cadmium

Cadmium (Cd) is widespread in soils, water, and atmosphere. The main sources of Cd contamination into the environment are metallurgic industries, waste incinerators, urban traffic, cement factories, and phosphate fertilizers (Gratao et al. 2005).

The effect of Cd toxicity on plants has been largely explored (Gallego et al. 2012). The metal often produces plant growth inhibition and decrease of photosynthetic activities; thus, strategies of obtaining transgenic plants with different candidate genes have been used in order to improve plant tolerance and/or Cd phytoextraction and/or phytostabilization.

Increasing Sequestration of Cd in Vacuoles

As it was pointed out to other metal(loid)s, an efficient Cd detoxification is related to binding free ions in the cytoplasm, and their sequestration into vacuoles (Clemens 2006), one possible approach to generate plants suitable for Cd phytoremediation, might consist of introducing gene coding proteins able to transport heavy metals or their complexes to appropriate storage compartments. Vacuolar sequestration of Cd can be achieved through either PC-dependent or PC-independent pathways (Hirschi et al. 2000; Song et al. 2003; Korenkov et al. 2007; Martinoia et al. 2007).

Multidrug resistance-associated proteins (MRPs), a subfamily of ABC transporters, catalyze the export of substrates out of the cytosol in an ATP-dependent manner (Verrier et al. 2008), and they have been related with Cd sequestration (Klein et al. 2006). Wojas et al. (2009) showed that *At*MRP7 overexpression in tobacco increased Cd tolerance by an efficient storage of the metal in vacuoles and higher Cd retention in roots, suggesting a contribution to the control of Cd root-to-shoot translocation.

Another strategy for Cd accumulation into vacuoles includes cations/H^+ exchangers, such as CAXs (from calcium exchangers). Recently, Wu et al. (2011) demonstrated that the expression of a CAX1 protein in petunia enhances Cd accumulation and tolerance. The transport of Cd into vacuoles by cation/H^+ antiporters is energized by the pH gradient established by proton pumps. Thus, Khoudi et al. (2012) studied the potential for enhancing proton pump expression as a strategy to improve Cd accumulation in plants. They found that transgenic tobacco plants which expressed *TaVP1* cDNA, encoding wheat vacuolar H^+-pyrophosphatase (V-H-PPase), were both more tolerant to Cd compared to wild-type (WT) plants and accumulated higher Cd concentration.

Recently, *ScYCF1* has been overexpressed in poplar trees. Transgenic plants exhibited enhanced growth, reduced toxicity symptoms, and increased Cd content in the aerial tissue compared to WT plants (Shim et al. 2013). Furthermore, these plants established an extensive root system in mine tailing soil and accumulated high amounts of Cd, Zn, and Pb. Thus, YCF1-expressing poplar may be useful for phytostabilization, especially in highly contaminated regions, where WT plants cannot survive.

In addition, other authors have used transgenic plants with simultaneous expression of two genes to increase Cd tolerance and accumulation. Guo et al. (2012) assembled in transgenic *A. thaliana* plants *AsPCS1*and *ScYCF1* genes for an effective metal chelation by thiols and the following inclusion in vacuoles.

Even though the overexpression of genes involved in Cd-tolerance mechanisms gives positive results, there are some cases which are not successful. For example, overexpression of *AtPCS* in the same plant species paradoxically produced hypersensitivity to Cd stress (Lee et al. 2003).

Regarding strategies that involve Cd and MTs, Krystofova et al. (2012) found that the expression of a yeast MT was responsible for higher Cd accumulation in roots of transgenic *N. tabacum* plants and its limited transport to aerial parts.

8.2.1.4 Mercury

Mercury (Hg) exists in different forms (HgS, Hg^{2+}, Hg^0, and methyl-Hg); however, in agricultural soil ionic form (Hg^{2+}) is predominant (Han et al. 2006). Inorganic Hg forms are usually less harmful than organic forms, because the last ones are hydrophobic and move across cell membranes. In plants, ionic Hg tends to affect the plasmatic membrane producing damage to transporters such as aquaporins, leading to nutrient and water disruption (Zhang and Tyerman 1999), while organomercurials rapidly localize into plastids where they accumulate and disrupt important metabolic functions (Bernier and Carpentier 1995).

Since plants cannot successfully detoxify or interconvert Hg to less harmful forms, genetic engineering is directed to integrate foreign genes from other organisms to enhance their phytoremediation capabilities (Ruiz and Daniell 2009).

Increasing Hg Volatilization

A well-characterized Hg-phytoremediation system is the use of the bacterial *mer*A (mercuric ion reductase) and *mer*B (organomercurial lyase) genes to genetically engineer plants for the remediation of this metal (Bizily et al. 2003; Che et al. 2003; Lyyra et al. 2007). This mechanism is based in protonolysis of organic Hg to Hg^{2+} by the lyase enzyme and the following reduction of Hg^{2+} to Hg^0 by the mercuric ion reductase, which is volatilized from plants. Despite the fact that the first attempts failed to express bacterial genes in eukaryotic organisms, the use of preferred codons for plants allowed obtaining transgenic plants highly Hg resistant (Rugh et al. 1996; Yang et al. 2003). For *mer*A the best results were obtained with its root-specific expression, indicating that root is the main organ affected and that its protection is important for phytoremediation. These gene expressions have been directed to nuclei but also to chloroplast. Ruiz et al. (2003) hypothesized that expressing *mer*A and *mer*B genes within plant chloroplasts would confer protection for essential metabolic reactions occurring within plastids, since chloroplast has shown to be the main target for Hg poisoning. More recently, considering these explored aspects, Hussein et al. (2007) obtained transgenic tobacco plants with the combined expression of *mer*AB via the chloroplast genome and showed enhanced conversion of Hg^{+2} into Hg^0, rapid volatilization, and increased shoot accumulation of different forms of Hg, even surpassing the concentrations found in soil.

Increasing Hg Scavenging by Chelation

One limitation regarding the use of the *mer*AB system is the release of Hg^0 into the atmosphere. Therefore, an alternative approach would be the chelation of ionic Hg inside the cell by molecules negatively charged, as polyphosphates. Tobacco plants expressing the bacterial *ppk* gene, which codifies for the enzyme involved in polyphosphate synthesis (polyphosphate kinase), showed enhanced tolerance and accumulation of Hg^{2+} (Nagata et al. 2006a, b). With the same purpose of Hg chelation, Hsieh et al. (2009) expressed the bacterial *mer*P gene in plants that codified for a cell membrane protein, providing enhanced resistance to $HgCl_2$. Then, Nagata et al. (2009) reported that the expression of bacterial *mer*T (Hg transporter gene) for a in *ppk*-transgenic tobacco resulted in accelerated and enhanced Hg uptake and accumulation. More recently, Nagata et al. (2010) combined several of the genes mentioned below and obtained tobacco plants that coexpressed three bacterial genes: *ppk* from *Klebsiella aerogenes* and *mer*T and *mer*B, both from *Pseudomonas* K-62. The *ppk*/*mer*T/*mer*B-transgenic tobacco callus showed more resistance to methylmercury (CH_3Hg^+) and accumulated more Hg from CH_3Hg^+-containing medium than the *ppk/mer*T-transgenic and WT progenitors. These results indicate that the MerB enzyme degraded the incorporated CH_3Hg^+ to Hg^{2+}, which then was accumulated as a less toxic Hg-polyP complex in tobacco cells. Hence, it is believed that these engineered *ppk/mer*T/*mer*B-transgenic plants would have more public acceptance since they prevent the release of volatile Hg into the atmosphere.

Another possible Hg-chelation strategy could be mediated by disulfide compounds (copper-zinc superoxide dismutase, Cu/Zn SOD) and sulfhydryl compounds (GSH, PCs, and MTs), since Hg cations have a high affinity for sulfhydryl groups. Thus, Chen et al. (2012) studied the relationship between Hg detoxification and a disulfide isomerase-like protein (PDIL) with a chaperone function and disulfide isomerase activity. For that reason, the authors expressed *MTH1745*, a gene that codifies a PDIL from thermophilic archaea *Methanothermobacter thermoautotrophicum* in *Oryza sativa* L. cv. Nipponbare. The transgenic rice seedlings showed more effective photosynthesis, lower levels of ROS and malondialdehyde, as well as higher levels of antioxidant enzymes than WT plants indicating an enhanced Hg tolerance.

In order to avoid releasing volatile Hg into the environment, Hg accumulation inside plants would be a useful strategy. Ruiz et al. (2011) reported the development of a transplastomic approach, which consists in the insertion of foreign DNA in chloroplasts, to express the mouse MT gene (*mt1*). The transplastomic lines accumulated high Hg concentrations and maintained high chlorophyll content. This study reported the usefulness of chloroplast genetic engineering approach to express Hg-scavenging proteins.

As it could be noted, transgenic plants with increased metal tolerance and accumulation rely on overexpressing genes involved in the biosynthesis pathways of metal-binding proteins and peptides, genes that can convert a toxic ion into a less toxic or easy to handle form, as well as genes coding transport proteins involved in vacuolar accumulation or a combination of some of them. There are no reports about improving a more efficient translocation of metal(loid)s from root to shoot, constituting a challenge since this is the more interesting character of hyperaccumulator plants. In this sense, studies based on genomic and functionality of target elements related to metal tolerance of hyperaccumulator plants would allow interesting advances in this aspect.

8.3 Phytoremediation of Organic Compounds

The efficiency of transgenic plants in the phytoremediation of organic contaminants has been investigated. For that, two main strategies have been pursued: (1) the manipulation of metabolic activities to enhance in planta degradation rates or to impart a novel metabolic activity and (2) the enhanced secretion of enzymes from roots leading to accelerated ex planta degradation of organic contaminants. One of the main processes involved in organic pollutant phytoremediation is phytotransformation, also known as phytodegradation. In this process, plants uptake pollutants and subsequently metabolize or transform them into less toxic metabolites. Once taken up, the organic chemicals generally undergo three transformation phases: (I) chemical modification (oxidations, reductions, hydrolysis), (II) conjugation, and (III) sequestration or compartmentalization (Ohkawa et al. 1999; Cherian and Oliveira 2005). Plant enzymes that typically catalyze the reactions of phase I are cytochrome P450 monooxygenases, carboxylesterases, peroxidases, and laccases (Coleman et al. 1997; Burken 2003). The second phase involves conjugation with GSH, sugars, or amino acids, catalyzed by glutathione, glucosyl, and malonyl transferases, resulting in more soluble, polar compounds (Marrs 1996). The third phase of plant metabolism is compartmentalization and storage of soluble conjugates either in vacuoles or in the cell wall. The conjugates are actively transported to the vacuole or apoplast by ATP-dependent membrane pumps (Martinoia et al. 1993).

A great diversity of organic pollutants has been introduced into the environment by human activities. However, phytoremediation using transgenic plants of only some of these compounds has been extensively studied. The first attempts for these purposes were targeted to herbicides, explosives, and halogenated organic compounds (Feng and Kennedy 1997; French et al. 1999; Doty et al. 2000). In the following sections, the more recent examples related to the use of transgenic plants for phytoremediation of these contaminants and others will be presented.

8.3.1 Pesticides

Pesticides include a wide range of chemicals used to kill, repel, or control pests and weeds. Among them, DDT [2,2-bis(chlorophenyl)-1,1,1-trichloroethane] is one of the 21 POPs that require immediate elimination, according to the 2010 Stockholm Convention (Sudharshan et al. 2012). Since World War II DDT has accumulated in the environment because of its use against forest and agricultural pests and against insect vectors of typhus and malaria (Lunney et al. 2004). Cytochrome P450 enzymes from pig, human, and other living organisms have been expressed in different genetically engineered plant species for pesticide removal (Hussain et al. 2009). In this sense, Mouhamad et al. (2012) evaluated the phytoremediation of TCE and DDT polluted water using transgenic *Sesbania grandiflora* and *A. thaliana* plants harboring rabbit cytochrome P450 2E1. *Arabidopsis* transgenic plants exposed to both contaminants accumulated more DDT and TCE compared with WT plants.

Lindane (g-1,2,3,4,5,6-hexachlorocyclohexane), also known as gammaxene or benzene hexachloride (BHC), is another organochlorine insecticide used worldwide in agriculture, as well as to control insect-borne diseases relevant to human and animal health (Singh et al. 2011). Although lindane has been banned from the European Community and United States markets due to its toxicity and recalcitrance, there are still several areas polluted with this insecticide (Rigas et al. 2009). Therefore many research studies focused on its remediation. In this context, Singh et al. (2011) showed that stable integration and expression of human cytochrome P450 2E1 (*CYP2E1*) in tobacco plants produced great tolerance as well as enhanced removal of this compound from liquid solution and soil.

Herbicides play an important role in agriculture worldwide but have negative effects on the environment (Dowling and Doty 2009). Even though phytoremediation of herbicides has been well studied using conventional plants, field trials suggested that the rate of contaminant removal was inadequate. Transgenic technology has been used with some success considering that two enzymes play main roles in the increased degradation of pesticides: cytochrome P450 monooxygenases (P450s) and glutathione S-transferases (GSTs) (Inui and Ohkawa 2005; Kawahigashi et al. 2005, 2006; Karavangeli et al. 2005). For example, Kawahigashi et al. (2008a) engineered rice plants expressing human cytochrome P450 genes (*CYP1A1*, *CYP2B6*, and *CYP2C19*) that were more tolerant to several herbicides than WT plants. Besides, transgenic plants were able to remove atrazine and metolachlor from soil. In addition, the accumulation of the OsGSTL1 protein (with GST activity) in the vegetative tissues of transgenic rice plants enhanced their tolerance to chlorsulfuron and glyphosate (Hu et al. 2009).

8.3.2 Explosives

The most widespread explosives are 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine, hexogen (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX) (Octogen). These toxic and mutagenic explosives are stable in the environment and recalcitrant to remediation (Panz and Miksch 2012).

As it was mentioned before, the use of genetic engineering is also a powerful tool for enhancing the efficiency of explosive phytoremediation (French et al. 1999; Hannink et al. 2001, 2007). In particular for TNT remediation, the overexpression of two glycosyltransferases in *Arabidopsis* resulted in an increase of conjugate production and detoxification (Gandia-Herrero et al. 2008). Bacterial nitroreductases efficiently reduce the nitro side groups of TNT to different isomers of aminonitrotoluene. Accordingly, Van Dillewijn et al. (2008) showed that the expression of the bacterial nitroreductase gene (*pnrA*) improved the natural capacity of transgenic hybrid aspen (*Populus tremula* × *tremuloides* var. Etropole) to tolerate, grow, and more importantly eliminate TNT not only from contaminated hydroponic medium but also from contaminated soil where its bioavailability is reduced.

RDX is a nitramine, often found along with TNT (Rylott and Bruce 2009). The first study demonstrating the use of transgenic plants to remove simultaneously TNT and RDX was carried out by Rylott et al. (2011) using *A. thaliana.* These plants were transformed with the bacterial genes *xplA* and the associated reductase *xplB*, an unusual explosive-degrading P450 system, (RDX degrading) from *Rhodococcus rhodochrous* strain 11Y, in combination with the gene *nfs*I (TNT-detoxifying nitroreductase) from *Enterobacter cloacae*. The transgenic plants obtained, removed RDX from soil leachate, and grew on soil contaminated with both explosives at inhibitory concentrations for plants that only expressed XplA.

On the other hand, HMX is less susceptible to phytoremediation than RDX and TNT. Since this octogen has a chemical structure that is similar to the hexogen, there have been attempts to remove this compound using genetically modified plants expressing the *xpl*A gene. However, transgenic lines did not assimilate more HMX than WT plants (Rylott and Bruce 2009; Panz and Miksch 2012). Thus, it constitutes a challenge that should continue under investigation.

8.3.3 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs), a group of chemical with long half-life in the environment and potential bioaccumulation through the food chain. PCBs are characterized by two linked aromatic rings substituted by 1–10 chlorine atoms. There are about 209 of their congeners and are identified by chlorine numbers and position (Anyasi and Atagana 2011). PCBs have been used for a variety of industrial applications, including lubricants, dielectric fluids, and plasticizers. Due to their hydrophobicity and chemical stability, PCBs are slowly taken up and degraded by plants, resulting in an incomplete metabolism and potential release of toxic metabolites into the environment. In order to overcome these limitations, bacterial genes involved in PCB metabolism, such as biphenyl dioxygenases, enzymes that catalyze the first steps in their degradation, have been introduced into plants (Mohammadi et al. 2007; Sylvestre et al. 2009; Van Aken et al. 2010). Among these enzymes, the 2,3-dihydroxybiphenyl-1,2-dioxygenase (BPHC) is the third enzyme in the biphenyl degradation pathway, and its function is the cleavage of biphenyl ring. Chrastilová et al. (2008) and Novakova et al. (2009) obtained 12 lines of transgenic *N. tabacum* plants expressing *bphC* gene from *Comamonas testosteroni* B356. The presence and expression of the *bph*C gene as well as the enzyme were detected in transgenic plants. One transgenic line, namely, H2, showed high biomass, high viability on toxic substrates, and increased phytoremediation of high 2,3-dihydroxybiphenyl (2,3-DHB) concentrations (Novakova et al. 2010).

8.3.4 Volatile Organic Compounds

Another group of organic contaminants called volatile organic compounds (VOCs) includes trichloroethylene (TCE), carbon tetrachloride (CT), vinyl chloride (VC), benzene, chloroform, toluene, and bromodichloromethane (BDCM). Many VOCs are used and produced in the manufacture of paints, adhesives, petroleum products, pharmaceuticals, and refrigerants. James et al. (2008) developed transgenic tobacco plants capable to remove VOCs expressing CYP2E1, a key enzyme in the mammalian metabolism of several low molecular weight VOCs. These transgenic plants showed increased removal of TCE, VC, CT, benzene, toluene, chloroform, and BDCM, compared to WT plants but not of perchloroethylene or 1,1,1-trichloroethane. In a similar way, transgenic petunia plants expressing the same enzyme showed a significant increase in absorption capacity of benzene and toluene and improved resistance to formaldehyde (Zhang et al. 2011). This study revealed that the *CYP2E1* gene enhances plant resistance to formaldehyde and also provides a method for reducing VOCs, by using transgenic flowering horticultural plants.

In view of the large size and extensive root systems of trees, transgenic poplars would constitute a useful tool to effectively remediate sites contaminated with a variety of pollutants at faster rates and lower costs. In this sense, Doty et al. (2007) obtained transgenic poplars expressing CYP2E1 that showed enhanced metabolism and removal of TCE, chloroform, and benzene. More recently, these transgenic poplars were evaluated in field conditions. They showed enhanced degradation of TCE in the field but in a lesser extent than that observed in laboratory studies (Legault 2013). All the studies carried out until now have shown that *CYP2E1* would be the most common gene used for engineering plants with enhanced VOC phytoremediation ability.

8.3.5 Phenol Compounds

Phenol and its derivatives are widely distributed in the environment due to their multiple applications in petrochemical and pharmaceutical industries, in the synthesis of resins, perfumes, solvents, and lubricating oils, as well as in the preparation of other chemicals (e.g., plastics, drugs, explosives, pesticides, and detergents) (Iurascu et al. 2009). Different strategies were used to enhance phenol remediation capabilities, such as the overexpression of enzymes involved in phase I or in phase II of plant xenobiotic metabolism.

For many years our research group has developed expertise in phenol phytoremediation using mainly hairy roots (HRs) as model system from different plant species. Removal efficiencies and optimization of the process were determined, as well as the involvement of different peroxidase isoenzymes (Agostini et al. 2003; González et al. 2006; Coniglio et al. 2008; Talano et al. 2010). In order to increase the efficiency and to evaluate physiological and biochemical mechanisms involved in phytoremediation of phenols, transgenic tomato and tobacco plants and HRs were developed (Wevar Oller et al. 2005; Sosa Alderete et al. 2009, 2012; Talano et al. 2012). For instance, the involvement of basic peroxidase isoenzymes, TPX1 and/or TPX2, in phenol removal was evaluated by overexpression in tomato and tobacco (Wevar Oller et al. 2005; Sosa Alderete et al. 2009). The increased removal efficiency obtained with transgenic HRs contributed to give more evidence that reinforces the hypothesis that basic peroxidases would be the main isoenzymes involved in phenol removal process. In addition, an increase in removal efficiency of 2,4-DCP and a decrease in toxicity of treated solutions were obtained using double transgenic (*tpx1* and *tpx2*) tobacco plants (Talano et al. 2012). Recently, the effects on the phospholipid turnover and phospholipase D activity after phenol treatment were also studied, using WT and double transgenic tobacco HRs. The results obtained suggest that the pollutant may induce changes of lipid kinase activities, involved in the synthesis of signaling phospholipids (Sosa Alderete et al. 2012).

Sonoki et al. (2012) carried out studies to enhance the remediation of bis-phenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane), a widely distributed alkyl phenol. This compound is one of the major chemicals used in plastics and resins, and it is well known that it disrupts endocrine systems in humans and animals. Tobacco plants were genetically modified with fungal enzymes such as lignin peroxidase (LiP), laccase (Lac), and manganese peroxidase (MnP) that can degrade and polymerize BPA (phase I). An increase of BPA removal efficiency by fungal peroxidase expression in these plants was observed.

Another strategy has been to obtain plants with an enhanced ability to secrete detoxifying enzymes. In this context, Wang et al. (2004) overexpressed a secretory laccase to enhance ex planta phytoremediation of phenolics leading to more competitive plants. Recently, Chiaiesea et al. (2011) cloned the fungal laccase gene *poxA1b* that codifies for an enzyme involved in phenol metabolism and transformed tobacco plants and microalgae cells of *Chlamydomonas*, *Ankistrodesmus*, and *Chlorella* genera. Transgenic plants and microalgae were able to secrete the laccase and to remove high phenol concentrations from an olive oil mill wastewater. These authors suggest further studies to evaluate the application of a consortium of algae or a combination of plants and microalgae expressing fungal laccase for phenol removal.

On the other hand, the xenobiotic glycosylation ability of uridine diphosphate-glucose-dependent glucosyltransferase (UGTs) is known to function in phase II of plant detoxification pathway. In this sense, previous works have reported that PtUGT72B1 enzyme from *Populus trichocarpa* has high activity in detoxifying trichlorophenol by conjugation with glucose. Xu et al. (2013) analyzed the substrate specificity of PtUGT72B1 toward phenols and determined that this enzyme was able to catalyze the *o*-glucosylation of phenol, hydroquinone, and catechol when it was expressed in *A. thaliana*. Transgenic plants removed these compounds more efficiently than WT plants.

8.3.6 Hydrocarbons

Other important targets for plant-based decontamination are hydrocarbons that are mainly produced during fuel combustion. Many plants have been transformed with foreign genes aimed to remove different kinds of hydrocarbons. For instance, tobacco plants overexpressing fungal GSTs from *Trichoderma virens* showed enhanced tolerance to anthracene (Dixit et al. 2008, 2011). Two bacterial enzymes, haloalkane dehalogenase (DhlA) and haloacid dehalogenase (DhlB) from the bacterium *Xanthobacter autotrophicus* GJ10, with ability to dehalogenate a range of halogenated aliphatic hydrocarbons, including 1,2-dichloroethane (1,2-DCA), were also studied. Focused on these enzymes, Mena-Benitez et al. (2008) expressed *dhl*A and *dhl*B genes into *N. tabacum* plants and used 1,2-DCA as a model substrate to demonstrate the ability of transgenic tobacco to remediate a range of halogenated aliphatic hydrocarbons.

8.3.7 Textile Dyes

Dyes are recalcitrant, and thus they remain in the environment for a long period without being degraded. Although dye wastewaters are usually treated by physicochemical processes, these technologies are generally ineffective in color removal, expensive, and less adaptable to a wide range of dye wastewater (Vinayak et al. 2012). Few studies are reported of textile dye phytoremediation with conventional plants (Ghodake et al. 2009; Kagalkar et al. 2009; Khandare et al. 2011; Telke et al. 2011; Vinayak et al. 2012; Kabra et al. 2012) and even fewer using transgenic plants. Several microorganisms have been reported to be capable of decolorizing triphenylmethane dyes. Recent studies using *A. thaliana* transgenic plants showed that overexpression of a triphenylmethane reductase from *Citrobacter* sp. enhanced plant tolerance to crystal violet (CV) and malachite green by

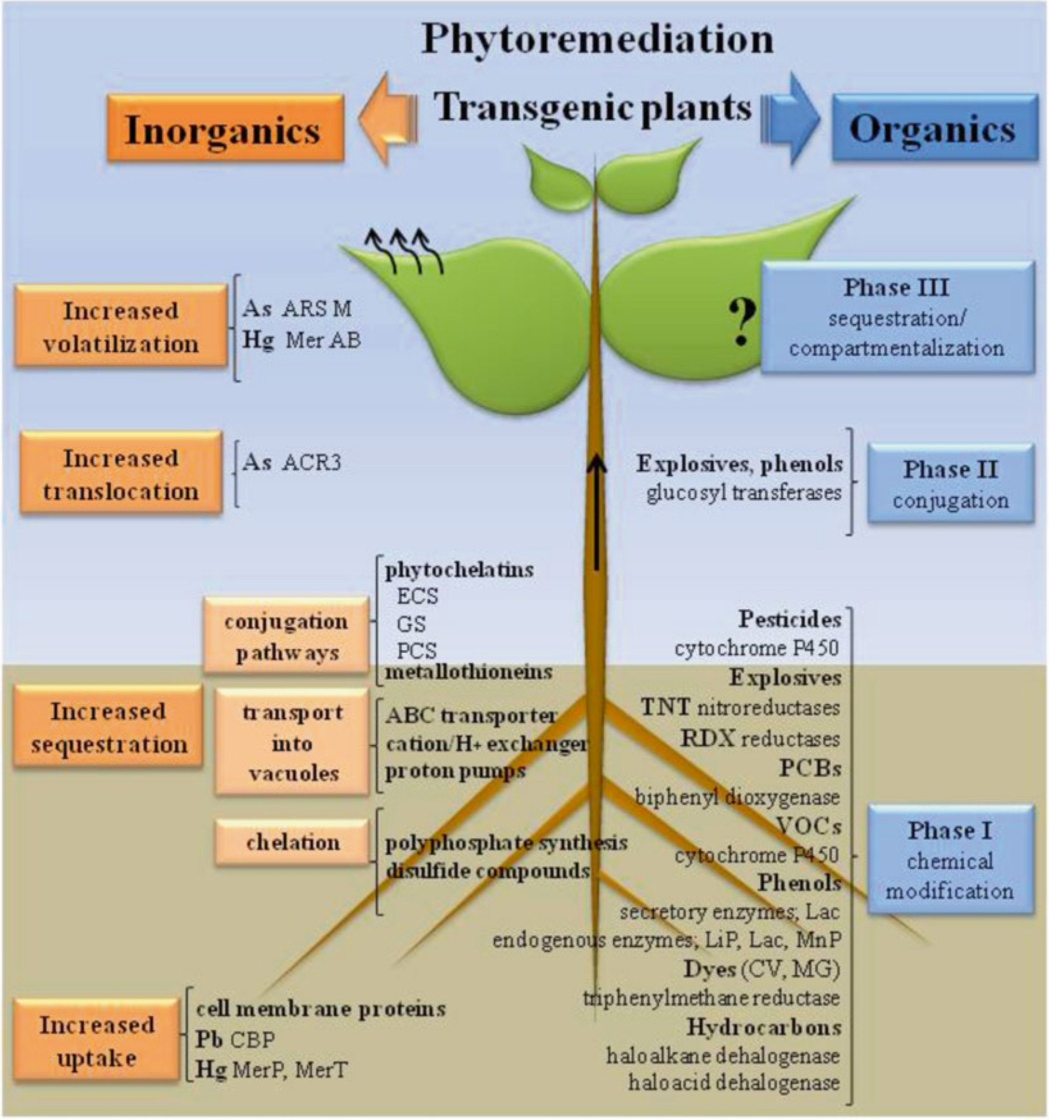

Fig. 8.1 Scheme with different strategies of transgenic plants with improved abilities for inorganic (As, Pb, Cd, Hg) and organic phytoremediation. Increased uptake of metal(loid)s in transgenic plants has been possible for Pb and Hg incorporation through overexpression of different cell membrane proteins. Regarding increased sequestration, it has been realized by increasing the expression of enzymes from PC synthesis pathway like ECS (gamma-glutamylcysteine synthase), GS (glutathione synthase), and PCS (phytochelatin synthase) or through the expression of genes coding for metallothioneins. Also, metal(loid) transport into vacuoles has been improved by overexpressing ABC transporter genes, cation/H+ exchanger, and proton pumps. In addition, an efficient chelation of metal(loid)s has been achieved by the expression of genes involved in polyphosphate synthesis or disulfide compounds. Increased volatilization in transgenic plants has been attained for As and Hg. Increasing translocation from roots to shoots has been less explored. For organic compound phytoremediation, the more deepened strategies have been those related with phase I (chemical modification), and there have been some examples involving phase II, such as the overexpression of glucosyltransferases. Phase III has not been explored for developing transgenic plants with improved organic phytoremediation

converting CV to nontoxic leucocrystal violet (Fu et al. 2013). This finding is an important contribution to this area of research and will surely lead to further studies in this topic.

As it was already described, the development of transgenic plants for organic compound remediation is mainly based on overexpression of genes codifying enzymes involved in xenobiotic transformation (phase I) or conjugation (phase II). To our knowledge, there are no genetic engineering studies that involve sequestration or compartmentalization (phase III). Moreover, genetic engineering of plants is important and necessary since they rarely mineralize hazardous organic compounds; thus, transgenic plants will be necessary to achieve this goal.

Figure 8.1 summarizes different strategies used to develop transgenic plants with enhanced abilities for inorganic (As, Cr, Cd, Hg) and organic phytoremediation.

8.4 Limitations of Using Genetically Modified Plants to Clean Up the Environment

It is clear that biotechnology has opened new gateways in phytoremediation allowing plants to be genetically modified to enhance their pollutant remediation capabilities. Thus, plants with high biomass, rapid growth rate, and climatic adaptability can be genetically engineered to produce elite plants with enhanced remediation abilities (Czakó et al. 2006). It is noteworthy that although transgenic plants used in phytoremediation will not be intended as human and animal food, so food safety, allergenicity, and labeling are not relevant issues, they still have a number of drawbacks to be widely used (Davison 2005).

One of these limitations is related with the increased invasiveness of transgenic plants and decreased genetic variability of native plants due to interbreeding or cross-pollination (Davison 2005). To minimize the risk of interbreeding to WT relatives, it is better to choose transgenic plant species that have no compatible WT relatives. Other gene flow containment measures are using male sterility, planting away from WT relatives, and/or harvesting the plants before flowering (Pilon-Smits and Pilon 2002; Ruiz and Daniell 2009; Kotrba 2013). In addition, an alternative to contain transgenes is integrating them into the chloroplast genome instead of the nucleus, since plastid inheritance is almost entirely maternal and its transmission via pollen rarely occurs (Hails 2000; Davison 2005; Kotrba 2013). Another suitable technique is the use of several constructions that confer conditional lethality on transgenic plants. One of the constructions that have been proposed is based on poison/antidote idea and employs lethal ribonuclease barnase of *Bacillus amyloliquefaciens* as poison and protein barstar as antidote. The barnase gene is expressed from a sulfhydryl endopeptidase promoter, active at the time of seed pod development and preventing, consequently, seed germination. The "antidote" is the expression of barstar gene, which is placed under the control of a heat shock promoter. Seed development and germination is only possible when the barstar is produced due to the controlled heating of developing seeds to 40 °C. Such conditions are unlikely in the field, making the germination of progeny likely to fail there (Davison 2005; Kotrba 2013).

Another drawback regarding the use of transgenic plants is that most data on the performance of phytoremediating transgenic plants are based on observations made in controlled conditions, rather than in the field (Abhilash et al. 2009; Ruiz and Daniell 2009; Bhargava et al. 2012). One of the reasons for the discrepancy between the number of scientific papers based on laboratory test over those dealing with field conditions is the high cost for maintenance, monitoring of installations, and waste disposal (Maestri and Marmiroli 2011). In this sense, several methods of contaminated plant disposal after phytoremediation process have been researched, including ashing, incineration, and liquid extraction (Sas-Nowosielska et al. 2004). Currently, incineration is proposed as the most feasible, economically acceptable, and environmentally thorough disposal method (Rayu et al. 2012).

Public acceptance is another barrier to the use of genetically engineered plants for phytoremediation. In this sense, although the creation of the first transgenic organisms took place during the early 1970s, the debate about their risks continues today. The public receive scientific information through the massive media and depending on their financial and political influence; the media can manipulate the public, causing scientific controversies that are rarely about science (Farre et al. 2011). Media involvement can also affect government decisions and policy. The impact of phytoremediation with transgenics should be carefully evaluated and weighed against the risks of doing nothing and with the known disadvantages of traditional remediation techniques (Pilon-Smits and Pilon 2002; Bhargava et al. 2012; Kotrba 2013; Pathak et al. 2013).

It is important to note that after more than twenty years of research and after different transgenic plants with enhanced phytoremediation capabilities have been developed, none of them reached commercial existence (Maestri and Marmiroli 2011). This fact is related with time and money and with the strict regulations necessary to bring a genetically modified organism to market compared to a nongenetically modified one. The regulatory process is bureaucratic and unwarranted by science: despite rigorous investigation over more than a decade of the commercial use of genetically engineered plants, environmental or health risks have not been noticed (Potrykus 2010a). Meanwhile, a new plant created by traditional breeding methods, which also modify the genome, requires less or no safety data, only the demonstration that it performs at least as well as others (Potrykus 2010b). In some countries, like the United States, Canada, the United Kingdom, Germany, and Italy, among others, there are companies specialized in conducting air, soil, sediment, and groundwater phytoremediation protocols. Some of them are supported by more than 10 years of experience offering effective solutions to environmental pollution. Thus, although nowadays the use of transgenic plants is still associated to perceived risks for ecosystems, their application could be perceived more favorably in the future, allowing their large-scale application and leading to cleaning up the environment more efficiently.

8.5 Future Trends Using Genetically Engineered Plants

Most of the research till now has been focused with single or double traits/genes to introduce or enhance a phytoremediation capacity of a vegetal species. However, the expression of complete pathways for metabolism, including the uptake, translocation, and sequestration, needs to be developed (Dowling and Doty 2009; Seth 2012). Although the major problem encountered with plants transformed with multiple traits is gene silencing, plants for agricultural applications have been successfully developed (James and Strand 2009).

Since the majority of polluted sites contain complex mixtures of chemicals, including both inorganic and organic compounds, thus it is important to develop plants that can cope simultaneously with multiple contaminants. Even though only a small proportion of papers have dealt with both types of pollutants, they have obtained promising results (Maestri and Marmiroli 2011; Zhang and Liu 2011; Zhang et al. 2013).

Although phytotechnologies have greatly contributed to reduce and control environmental pollution, there are still many challenges to overcome. Thus, scientific community should make an effort to address the most important questions that limit the application of transgenic plants for phytoremediation.

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9

Phytomanagement: Phytoremediation and the Production of Biomass for Economic Revenue on Contaminated Land

Michael W.H. Evangelou, Eleni G. Papazoglou, Brett Harvey Robinson, and Rainer Schulin

9.1 Introduction: From Remediation to Management of Trace Element-Contaminated Land

The term phytoremediation refers to the use of plants and associated microorganisms to eliminate environmental damage or threats posed by environmental pollution. While this includes the use of plants in soil conservation such as protection against erosion or regeneration of compacted soils, the term phytoremediation is primarily used in conjunction with the decontamination/redevelopment of soils, which are contaminated by pollutants. Raskin et al. (1997) defined phytoremediation as the use of green plants to remove pollutants from the environment or to render them harmless. Phytoremediation may be applied to soils that are contaminated with toxic trace elements (TE) or organic pollutants. Depending on the targeted pollutants and the mechanisms, phytoremediation can be divided into: (a) phytoextraction, (b) phytotransformation, and (c) phytostabilization. This chapter will focus on TE as they are the most widespread and intractable soil contaminants.

Phytoextraction describes the use of plants to remove/extract pollutants from soil. In 1583 the botanist Cesalpino, the author of the renowned book *De plantis Libri, which* contains descriptions of about 1,500 plant species, described an “alyson” that appeared to be confined to serpentine soils, which are rich in nickel (Ni), in the vicinity of Florence, Italy. In 1885, Baumann, a German botanist working near the border of Germany and Belgium, discovered that certain plant species growing on soils naturally enriched in Zn were capable of accumulating uncommonly high Zn levels. Brooks et al. (1977) coined the term hyperaccumulator for plants that accumulate >1,000 mg kg^{-1} Ni on a dry matter basis. Currently, a plant is defined as a hyperaccumulator if it reaches concentrations of at least 100 mg kg^{-1} (0.01 % dry wt.) Cd and As; 1,000 mg kg^{-1} (0.1 % dry wt.) Co, Cu, Cr, Ni, and Pb; and 10,000 mg kg^{-1} (1 % dry wt.) Mn and Zn in their aboveground tissues (Reeves and Baker 2000; Watanabe 1997). To date more than 500 plant species have been identified as natural metal hyperaccumulators, representing <0.2 % of all angiosperms most of which are Ni hyperaccumulators (450 species) (Ent et al. 2013).

In 1993, McGrath et al. proposed that hyperaccumulators could be used for the removal of TE pollutants from soil. Unfortunately, most hyperaccumulator species are slow growing and have limited biomass production. As total metal extraction is the product of biomass and tissue concentration, the speed of metal removal is accordingly limited. Field experiments by Robinson et al. (1998), Lombi et al. (2000), and McGrath et al. (2000) highlight this problem, showing that metal removal efficiency is in general not high enough to remediate contaminated soils. Subsequently, research focused more on high biomass plants, such as tobacco (*Nicotiana tabacum*) (Evangelou et al. 2004; Fässler et al. 2010; Kayser et al. 2000), maize (*Zea mays*) (Fässler et al. 2010; Keller et al. 2003), Indian mustard (*Brassica juncea*) (Keller et al. 2003; Quartacci et al. 2006), poplar (*Populus* spp.) (Mertens et al. 2004; Robinson et al. 2003b, 2006), willow (*Salix* spp.) (Cosio et al. 2006; Dickinson and Pulford 2005; Jensen et al. 2009; Klang-Westin and Eriksson 2003;

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Mleczek et al. 2009), and sunflower (*Helianthus annuus*) (Fässler et al. 2010; Madejon et al. 2003), which are fast growing, deep rooted, and easily propagated and cultivated and have a high biomass production and a relatively high metal uptake capacity. It was soon realized though that regardless of the plants used, the rate of contaminant accumulation was insufficient and thus would have to be considerably increased without diminishing their yield.

One approach to achieve this was increasing the availability of contaminating TE in soil for plant uptake, e.g., by artificial soil acidification or solubilization by means of chelating agents. Various synthetic aminopolycarboxylic acid (APCA) such as ethylene diamine tetraacetic acid (EDTA), diethylene triamino pentaacetic acid (DTPA), trans-1,2-cyclohexylene dinitrilo tetraacetic acid (CDTA), ethylenediamine-*N*, *N*´-bis (2-hydroxyphenyl) acetic acid (EDDHA), and others displayed potential to significantly increase TE uptake by plants (Evangelou et al. 2007; Lai and Chen 2004; Wu et al. 2004). However, as more and more research was put into **chelant-assisted phytoextraction**, various drawbacks arose such as their toxicity to soil microorganisms (Grcman et al. 2001) and to plants (Chen and Cutright 2001; Epstein et al. 1999), and in particular the risk that mobilized TE could leach into groundwater or surface water (Evangelou et al. 2007; Lai and Chen 2005; Luo et al. 2005; Meers et al. 2005). To reduce this risk the use of biodegradable chelating agents such as ethylene diamine disuccinate (EDDS) or nitrilotriacetic acid (NTA) was suggested. However, the degradation rates of biodegradable chelating agents such as EDDS and NTA were still too low to significantly reduce the risk of leaching (Evangelou et al. 2007; Meers et al. 2005). The risk of TE leaching was caused by the fact that in order to achieve plant shoot concentration of >1,000 mg kg^{-1}, chelants have to be applied (a) in a single large dose, to break down the endodermis in order to increase the uptake via the limited apoplastic pathway, and (b) to large excess, as most chelants are nonspecific; hence, soil components such as Ca and Fe compete with targeted TE, thus reducing the efficiency of the applied chelants (Nowack et al. 2006).

The numerous setbacks in the development of phytoremediation led to a change in focus from phytoextraction, i.e., the removal of pollutants to **phytostabilization**. The aim of phytostabilization (a) is to prevent the dispersal of particle-bound pollutants by wind and water erosion and to reduce the export of dissolved contaminants by reducing surface runoff and water flow into the subsurface and (b) to minimize the transfer of contaminants into the food chain by using plants with minimal uptake of contaminants (Collins et al. 2006). This change of concept means that quite different plant characteristics are desired compared to phytoextraction. In phytoextraction, high accumulation of contaminants was desirable, whereas in phytostabilization plants should preferably exclude contaminants from their aerial parts. In recent years, the perception of contaminated soils has changed. For decades, such soils were regarded only as a source of hazard, which required remediation. Nowadays, contaminated soils are increasingly considered as a valuable resource that can sustain plant growth, biodiversity, and other ecosystem functions. Contaminated land is an extensive underutilized resource, which could and should be used in a sustainable way to grow plants for a large variety of profitable purposes. From this new perspective, the idea of phytomanagement emerged. Phytomanagement describes the engineering or manipulation of soil–plant systems to control the fluxes of TEs in the environment, maximizing economic and/or ecological benefits while minimizing risks. Thus, the goal of phytomanagement may be to alleviate deficiencies of crops in essential TEs or to reduce the environmental risk posed by contaminating TEs. A key component of phytomanagement is that it should either cost less than other remediation or fortification technologies or be a profitable operation, by producing valuable plant biomass products (Robinson et al. 2009).Thus, the aim of phytomanagement is to produce economic revenue on a contaminated land without causing detrimental effects on human health and nature.

9.2 Contaminated Land: An Extensive but Underutilized Resource

Growth in global population, high—and growing—consumption levels in industrialized countries, rapidly increasing middle classes, related increased consumption levels, expanding urban areas, and changing diets in emerging countries combined with the increasing energy consumption are some of the key drivers behind the increasing demand for land. Demographers project that the world population will rise to 9 bn by 2050 and level off somewhere between 9 and 12 bn people by the end of the century. Accompanying the population growth is an increase in personal income. Globally, the size of the middle class could increase from 1.8 bn people to 3.2 bn by 2020 and to 4.9 bn by 2030. This results in changes in lifestyle, diets, and demographics, with meat consumption playing an important role (OECD 2010). As a consequence, the global crop demand will increase by 100–110 % from 2005 to 2050 (Tilman et al. 2011); thus, according to the OECD and UN-FAO, agricultural production has to increase by about 60 % globally and nearly 77 % in developing countries by 2050 (OECD-FAO 2012). The potential to expand the arable land areas is not great. According to the FAO, out of the world's 13.5 bn ha of total land surface, the area of land that is potentially available for expanded rain-fed crop production is ca. 2 bn ha. Of this, 1.4 bn ha are currently used for agriculture and at least

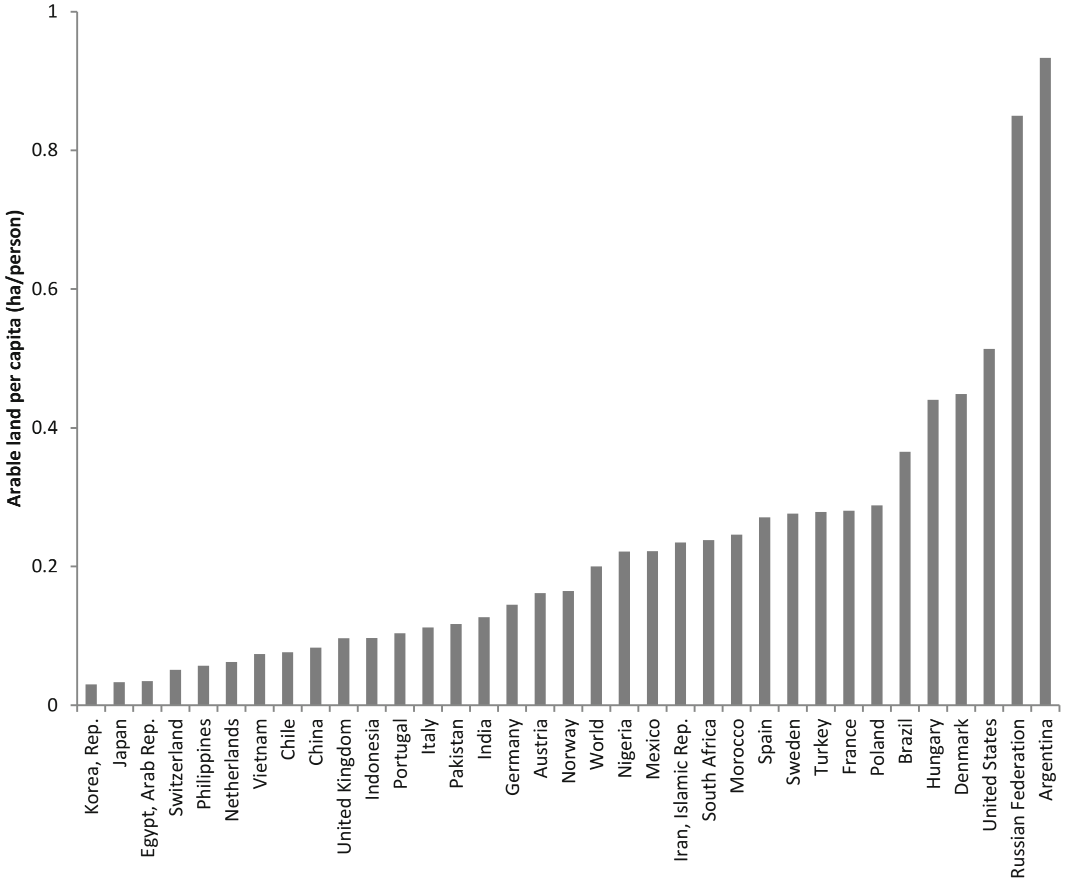

Fig. 9.1 Arable land per capita in selected countries for the year 2011 (TWB 2012)

500 M ha should remain protected from agriculture for environmental reasons (Haralambous et al. 2009). Additionally, built-up areas (currently 150 M ha) will further expand at the expense of arable or potentially arable land (FOE 2013).

Land demand for the production of food, fodder, and its use for housing will increasingly compete with the energy supply demands, in the form of bioenergy/biofuels. Biofuel growth is driven by policies and targets from over 50 countries, among them China, the EU, the United States, Brazil, the Philippines, India, and Uruguay who strive to become less reliant on foreign oil. In order to reach their biofuel demand of approximately 65 bn US gallons vast swathes of land would have to be converted from food to energy crop production (Evangelou and Schulin 2013). The OECD/FAO (2011) estimated that by 2020, 12 % of the global coarse grain production as well as 33 % of the sugar production will be used to produce ethanol. Additionally, 16 % of the global production of vegetable oil will be used to produce biodiesel. According to the International Energy Agency (IEA), in 2006 about 14 M ha of land—ca. 1 % of the world's currently available arable land—were used for the production of biofuels (IEA 2006). The FAO projects that these figures will increase up to 3.5 % by 2030 (Haralambous et al. 2009). These estimations have brought up concerns about food security and affordable food prices. Increasing population and soil loss is resulting in reduced arable land per person (Figs. 9.1 and 9.2).

Pointing to threatened food security, it has been argued that biomass production for biofuels is unsustainable (Friedemann 2007). As a result it has been suggested to use lands that are marginal, "underutilized," or "unused." However, such land, especially in developing countries, is often important for the livelihoods of poor rural communities, as it is used for grazing; as livestock transit routes; for collection of fuel wood, wild fruits and nuts, medicinal plants, and other plant products; and for access to water sources (Haralambous et al. 2009).

Contaminated land, which is not suitable for food production, is in contrast to "marginal" land often not used economically at all; thus, it could also be considered as a suitable alternative, not only for biofuels but timber and fodder as well, which would otherwise be grown on fertile non-contaminated soil that could be used to produce food. The global area of TE-contaminated soils is approximately 33 M ha (Evangelou et al. 2012). This estimation is conservative, as the extent of contaminated land in poor countries

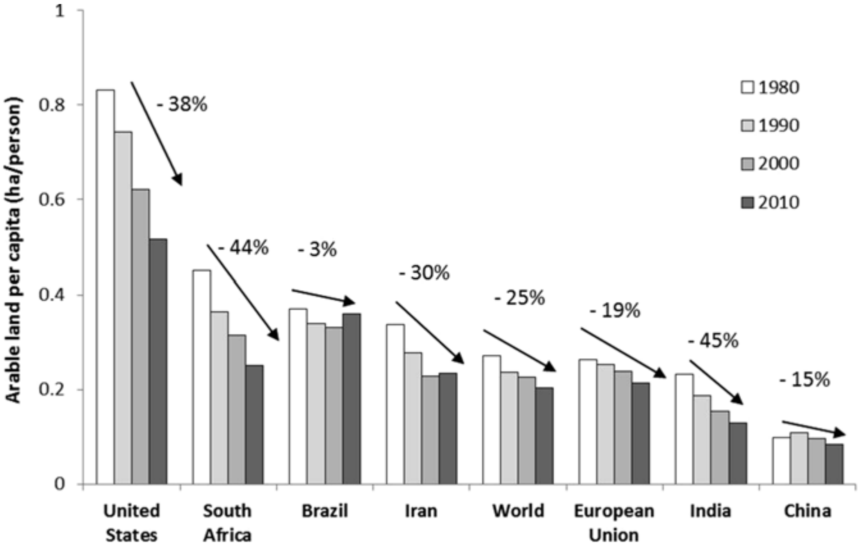

Fig. 9.2 Decrease of arable land in the years 1980–2010 (TWB 2012)

is difficult to assess due to a lack of published data. Nevertheless, it is well documented the area of contaminated land is increasing due to industrialization and lax environmental regulations in poor countries. The use of these lands would open to the affected countries new economic possibilities as most countries lack the wherewithal to remediate or secure contaminated land.

9.3 Potential Plant Species for Phytomanagement

Plants used for phytomanagement should be fast growing, deep rooted, and easily propagated and have a high biomass production as well. Their TE accumulation characteristics depend on the goal of phytomanagement. Plants that accumulate high concentrations of Se or Zn may be usefully employed on soil contaminated with these elements to provide supplementary fodder for stock in deficient areas (Banuelos and Dhillon 2011; Fässler et al. 2010). In some other cases, where accumulation of TEs may present a risk to the food chain, excluder species are desirable. In all cases the biomass should have economic or ecological value. The plants should additionally mitigate the risk originating from that soil, by, e.g., stabilizing the soil, reducing leaching, etc. Furthermore, their cultivation must be practically feasible and economically attractive under the given site and land use conditions (Robinson et al. 2009).

9.3.1 Trees

Various tree species can be used to produce biomass on contaminated land. Willow (*Salix* spp.) and poplar (*Populus* spp.) are used worldwide for bioenergy production, due to their fast growth and their capability to be coppiced (e.g., short-rotation coppice). Hardwood species such as eucalyptus (*Eucalyptus* spp.), beech (*Fagus* spp.), maple (*Acer* spp.), and birch (*Betula* spp.) as well as softwood species such as spruce, pine, fir, larch, and hemlock are used for the production of pulp, timber, and firewood. The trees should not accumulate high TE concentrations (a) in the wood, as risks could arise from the release of these TE during processing (e.g., bioenergy, pulp, burning) as well as during its use (e.g., wooden furniture, use of paper), and (b) in the leaves as TE could be spread through the dispersion of foliage into the surrounding ecosystems. Leaves usually accumulate the highest TE concentrations followed by bark and wood, as shown by Unterbrunner et al. (2007) for *Salix caprea*, *Salix purpurea*, *Salix fragilis*, *Salix* sp., *Populus tremula*, *Populus nigra*, and *Betula pendula*. Lead is an exception, as it often accumulates more in stems than in leaves (Migeon et al. 2009; Evangelou et al. 2013).

Willow (*Salix* spp.) and poplar (*Populus* spp.) are known to accumulate high concentrations of Cd and Zn (Dickinson and Pulford 2005; Migeon et al. 2009; Vamerali et al. 2009; Evangelou et al. 2012, 2013) (Table 9.1). Birch is a pioneer tree characterized by fast growth and low demand for soil nutrients. Thus, it can be a suitable candidate for the phytomanagement of contaminated soils with low nutrient contents. It does, however, take up more Pb than other tree genera such as willow (*Salix* spp.), poplar (*Populus* spp.), oak (*Quercus* spp.), beech (*Fagus* spp.), and maple (*Acer* spp.) (Migeon et al. 2009; Evangelou et al. 2012, 2013). Compared to willow (*Salix* spp.) and poplar (*Populus* spp.), birch (*Betula* spp.) usually accumulates less Cd (French et al. 2006; Hermle et al. 2006; Unterbrunner et al. 2007). Van Nevel et al. (2011) found that leaf Cd and Zn accumulation decreased in the order aspen (*Populus tremula*) > silver birch (*Betula pendula*) >> Scots pine (*Pinus sylvestris*) ≈ oak (*Quercus robur* and

Table 9.1 Bioaccumulation factors of potential trees for phytomanagement

Species	Plant part	TE	Bioaccumulation factor	References
Poplar (*Populus* spp.)	Wood	As		Laureysens et al. (2004); Mertens et al. (2007); Unterbrunner et al. (2007); Migeon et al. (2009); Vamerali et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.25–2.35	
		Cr	0.22–0.26	
		Pb	0.004–0.02	
		Zn	0.02–0.74	
	Leaves	As	0.005	Laureysens et al. (2004); Mertens et al. (2007); Unterbrunner et al. (2007); Migeon et al. (2009); Vamerali et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.56–4.63	
		Cr	0.21	
		Pb	0.006–0.01	
		Zn	0.2–1.47	
Willow (*Salix* spp.)	Wood	As		Klang-Westin and Eriksson (2003); Rosselli et al. (2003); Jensen et al. (2009); Migeon et al. (2009); Mleczek et al. (2009); Vamerali et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.72–6.5	
		Cr	0.16–0.22	
		Pb	0.002–4.5	
		Zn	0.28–1.62	
	Leaves	As	0.01	Unterbrunner et al. (2007); Reglero et al. (2008); Migeon et al. (2009); Vamerali et al. (2009); Evangelou et al. (2012, 2013)
		Cd	2.5–12.2	
		Cr	0.18–0.24	
		Pb	0.01–0.29	
		Zn	0.28–4.00	
Birch (*Betula pendula*)	Wood	As		Kozlov et al. (2000); Rosselli et al. (2003), Margui et al. 2007; Unterbrunner et al. (2007); Migeon et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.11–0.3	
		Cr	0.16	
		Pb	0.001–0.05	
		Zn	0.32–0.86	
	Leaves	As		Kozlov et al. (2000); Margui et al. (2007); Unterbrunner et al. (2007); Migeon et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.9	
		Cr	0.18	
		Pb	0.01–0.03	
		Zn	0.01–3	
Eucalyptus	Wood	As	0.01	Marchiol et al. (2013); Mok et al. (2013)
		Cd	0.1–0.90	
		Cr	0.1	
		Pb	0.03	
		Zn	0.05–7.61	
	Leaves	As		Shukla et al. (2011); Marchiol et al. (2013); Mok et al. (2013)
		Cd	0.32–0.94	
		Cr	0.1–1.2	
		Pb	0.3	
		Zn	0.37–6.14	
Oak (*Quercus* spp.)	Wood	As		Migeon et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.05–1.62	
		Cr	0.16	
		Pb	0.002–0.1	
		Zn	0.01–0.07	
	Leaves	As		Migeon et al. (2009); Evangelou et al. (2012, 2013)
		Cd	0.05–0.2	
		Cr	0.21	
		Pb	0.01–0.04	
		Zn	0.05–0.28	

Quercus petraea), while for the stem the order was aspen (*Populus tremula*) ≈ silver birch (*Betula pendula*) > Scots pine (*Pinus sylvestris*) > oak (*Quercus robur* and *Quercus petraea*). Scots pine (*Pinus sylvestris*) is a good bioindicator because it is sensitive to industrial pollution (Kosinska and Baaga 2007). Meeinkuirt et al. (2012) found a low ability of eucalypt (*Eucalyptus camaldulensis*) to accumulate Pb, while Mok et al. (2013) showed that the eucalyptus species *Eucalyptus polybractea* and *Eucalyptus cladocalyx* accumulated various TE such as Cd or Zn to high levels. Oaks (*Quercus* spp.) are defined by a high tolerance to TE but low uptake of TE (Migeon et al. 2009; Evangelou et al. 2012, 2013) (Table 9.4). But due to this slow growth, they are not very attractive for phytomanagement purposes, although their wood is valuable. Maple (*Acer* spp.) shows a low propensity to take up Zn and Cd, while it does not differ from willow (*Salix* spp.), poplar (*Populus* spp.), or birch (*Betula* spp.) in the accumulation of Pb and Cr (Migeon et al. 2009).

9.3.2 Agricultural Crop Plants

Crops used for the production of bioethanol are wheat (*Triticum* spp.), corn (*Zea mays* L.), sweet and grain sorghum (*Sorghum bicolor* (L.) Moench), and sugar beet (*Beta vulgaris* L.), as these plants accumulate large amounts of starch or sugars in plant storage organs, which can be fermented. For biodiesel, annual plants with high seed oil content are used, such as sunflower (*Helianthus annuus* L.), rapeseed (*Brassica napus* L. var. oleifera D.C.), soybean (*Glicine max* L.), and tobacco (*Nicotiana tabacum*). Besides plant parts rich in starch and sugar and oils, also stover and straw can be used to produce bioenergy. In contrast to the use of tree species or perennial herbaceous crops, annual plants require efforts for the management of harvest transport and processing. Using crops for the phytomanagement of contaminated soils that are also utilized for food or feed production, if grown on uncontaminated soil, requires particular attention, as there is an increased risk that the products of such crops could by mistake (or deliberate action) contaminate human food (Table 9.2 and 9.3).

A low TE concentration in the seeds of plants grown for the production of biodiesel is desirable as it would reduce the costs of removing TEs from the oil, which could be hazardous for human health. Soybean (*Glycine max*), wheat (*Triticum aestivum* L.), and corn (*Zea mays*) seeds accumulate significantly more Zn (up to six times more in soybean) than stems, while there are no significant differences for Cd and Pb (Lavado et al. 2001; Salazar et al. 2012). The production of methane or ethanol through anaerobic digestion (fermentation) requires low TE concentration, as they can negatively affect the enzymes responsible for the breakdown of biomass, as well as face issues concerning the fate of the digestate, such as its application on soils. Baig et al. (2011) reported the As accumulation in crop plants decreased in the order wheat (*Triticum aestivum* L.) > corn (*Zea mays* L.) ≈ sorghum (*Sorghum bicolor* L.). Sugarcane (*Saccharum* spp.) accumulates approximately 50 % less TE in the stalks than in the leaves, with the exception of Cd where the opposite occurs (Noguiera et al. 2013). Also Xia et al. (2009) found that sugarcane (*Saccharum officinarum*) has a high ability to tolerate and accumulate Cd.

The uptake of metals into the shoots may also entail potential ecological risks. Tobacco (*Nicotiana tabacum*) accumulates Cd to relatively high levels compared to other species (Kayser et al. 2000; Keller et al. 2003; Wenger et al. 2002). Concentrations of Cd in field-grown tobacco leaves were found to range from <0.5 to 5 mg Cd kg^{-1} (Lugon-Moulin et al. 2004). Corn (*Zea mays*) can accumulate Zn in the shoots (Keller et al. 2003; Luo et al. 2005), with Zn concentrations reaching >1,000 mg kg^{-1} without significant decrease in biomass (Wenger et al. 2002). In comparison soybean (*Glycine max*) reaches even higher Zn shoot concentrations (Murakami and Ae 2009). Rapeseed (*Brassica napus*) was found to accumulate more Pb than wheat (*Triticum* spp.), corn (*Zea mays* L.), and sorghum {*Sorghum bicolor* (L.)} (Tangahu et al. 2011).

Concerning potential risks deriving from biomass produced on contaminated soils, we can conclude from the available literature that tobacco (*Nicotiana tabacum*) and sugarcane (*Saccharum* spp.) would be unsuitable for Cd-contaminated soils, soybean (*Glycine max*) for Zn-contaminated soils, and wheat (*Triticum* spp.) for As-contaminated soil. Rapeseed (*Brassica napus*) is in general unsuitable for TE-contaminated sites, as it belongs to the family of the *Brassicaceae* (e.g., *Brassica species*: *B. nigra* (*L.*) Koch; *B. carinata A.* Braun; *B. oleracea* L.; *B. campestris* L.; *B. juncea* (L.) Czern.; *B. napus* L.), which includes many hyperaccumulators (Vamerali et al. 2010) (Table 9.4).

9.3.3 Herbaceous Perennial Crops

Perennial grasses have been widely used for centuries as fodder crops, often contributing significantly to energy supply on farms being used to feed draft animals. For example, as late as 1920 in the United States, 27 M animals fuelled by some 35–40 M hectares of grasslands provided traction power on farms and in cities, (Lewandowski et al. 2003). In the twenty-first century, perennial grasses may be set for a comeback, as they have a great potential to contribute to the production of bioenergy. Candidates are in particular switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.), reed canary grass (*Phalaris arundinacea*), vetiver grass (*Vetiveria zizanioides* L.), elephant grass (*Pennisetum purpureum* Schumach), and giant reed (*Arundo donax*).

Table 9.2 Bioaccumulation factors of potential agricultural crops for phytomanagement

Species	Plant part	TE	Bioaccumulation factor	References
Soybean (*Glycine max*)	Shoot	As		Murakami et al. (2007, 2009); Zhuang et al. (2013)
		Cd	0.5–1.44–3.7	
		Cr	0.02	
		Pb	0.01–0.03	
		Zn	0.15–0.66	
	Grain	As		Salazar et al. (2012); Zhuang et al. (2013)
		Cd	0.57	
		Cr	0.02	
		Pb	0.01–0.13	
		Zn	0.54–4.95	
Tobacco (*Nicotiana tabacum*)	Shoot	As		Mench et al. (1989); Kayser et al. (2000); Keller et al. (2003); Evangelou et al. (2004, 2006, 2007); Fässler et al. (2010)
		Cd	0.66–2.6	
		Cr		
		Pb	0.03	
		Zn	0.1–0.22	
	Grain	As		
		Cd		
		Cr		
		Pb		
		Zn		
Rapeseed (*Brassica napus)*	Shoot	As		Solhi et al. (2005)
		Cd		
		Cr		
		Pb	0.03	
		Zn	0.16	
	Grain	As		Angelova et al. (2004)
		Cd	0.06–0.08	
		Cr		
		Pb	0.01–0.03	
		Zn	0.1–1.1	
Wheat (*Triticum aestivum* L.)	Shoot	As	0.04–0.11	Chen et al. (2004); Bermudez et al. (2011)
		Cd		
		Cr	0.5–1.3	
		Pb	0.02	
		Zn	0.2	
	Grain	As		Jamali et al. (2009). Bermudez et al. (2011)
		Cd	0.241–0.42	
		Cr	0.01	
		Pb	0.01–0.68	
		Zn	0.19–0.60	
Sunflower (*Helianthus annuus*)	Shoot	As		Kayser et al. (2000); Nehnevajova et al. (2009); Solhi et al. (2005); Marchiol et al. (2007); Sabudak et al. (2007); Fässler et al. (2010)
		Cd	0.2–2.7	
		Cr	0.2	
		Pb	0.01–0.07	
		Zn	0.1–0.7	
	Grain	As	8* 10^{-6}, & 5* 10^{-7}–0.009	Murillo et al. (1999); Angelova et al. (2004); Sabudak et al. (2007)
		Cd	0.01–0.05	
		Cr		
		Pb	5*10–5–0.009	
		Zn	0.01–0.7	

(continued)

Table 9.2 (continued)

Species	Plant part	TE	Bioaccumulation factor	References
Corn (*Zea mays*)	Shoot	As	0.03	Kayser et al. (2000) ; Chen et al. (2004); Chiu et al. (2005); Luo et al. (2005); Murakami et al. (2006, 2009)
		Cd	0.1–1.88	
		Cr		
		Pb	0.05–1.13	
		Zn	0.2–3.7	
	Fruit	As	0.045	Chiu et al. (2005)
		Cd		
		Cr		
		Pb		
		Zn	0.3	
Sorghum (*Sorghum bicolor* (L.) Moench),	Shoot	As	0.02–0.03	Murillo et al. (1999); Chen et al. (2004); Marchiol et al. (2007)
		Cd	0.05–0.1	
		Cr		
		Pb	0.01–0.02	
		Zn	0.09–0.15	
	Fruit	As		
		Cd		
		Cr		
		Pb		
		Zn		

Table 9.3 Bioaccumulation factors of potential perennial grasses for phytomanagement

Species	Plant part	TE	Bioaccumulation factor	References
Vetiver grass (*Vetiveria zizanioides* L.)	Shoot	As	0.04	Lai and Chen (2004); Chiu et al. (2005); Rotkittikhun et al. (2007)
		Cd	1.25	
		Cr		
		Pb	0.004–0.07	
		Zn	0.03–0.8	
Elephant grass (*Pennisetum purpureum* Schumach)	Shoot	As	0.5	Amonoo-Neizer et al. (1996)
		Cd		
		Cr		
		Pb		
		Zn		
Smilo grass (*Piptatherum miliaceum*)	Shoot	As	0.09	Marchiol et al. (2013)
		Cd	0.07	
		Cr		
		Pb	0.003	
		Zn	0.02	
Giant reed (*Arundo donax*)	Shoot	As	0.012	Boularbah et al. (2006); Guo and Miao (2010)
		Cd	0.04	
		Cr		
		Pb	0.007–0.0005	
		Zn	0.04–0.008	

Unlike trees or agricultural crops, research on the potential of perennial grasses to accumulate TE has not been so extensive (Table 9.3). Thus, a conclusion, about which perennial grasses are to be preferred for phytomanagement of TE-contaminated sites, cannot be drawn. Nevertheless, Hou et al. (2012) preferred hybrid *Pennisetum* followed by giant reed (*Arundo donax*), silver reed (*Thamnochortus cinereus*), and switchgrass (*Panicum virgatum*) for the phytoextraction of an As-, Hg-, Cu-, Cr-, Pb-, and Cd-contaminated soil. The ability of *Pennisetum* to accumulate Cd is supported by a study of Xia (2004), where *Pennisetum* reached higher Cd concentrations than vetiver grass (*Vetiveria zizanioides* L.).

Table 9.4 Suitability, positive (+) or negative (–), of various potential phytomanagement plants depending on their TE accumulation and soil degradation

	Trace elements		
	Cd	Zn	Pb
Trees			
Birch (*Betula pendula*)	+	+	– –
Eucalyptus (*Eucalyptus* spp.)	–	–	+
Oak (*Quercus* spp.)	++	++	+
Poplar (*Populus* spp.)	– –	– –	+
Maple (*Acer* spp.)	+	+	+
Scots pine (*Pinus sylvestris*)	++	++	+
Willow (*Salix* spp.)	––	––	+
Agricultural crops			
Corn (*Zea mays*)	+	–	+
Rapeseed (*Brassica napus*)	–	–	–
Sorghum (*Sorghum bicolor* (L.) Moench)			+
Soybean (*Glycine max*)		––	
Sugarcane (*Saccharum* spp.)	––		
Sunflower (*Helianthus annuus*)	–	––	
Tobacco (*Nicotiana tabaum*)	––		
Wheat (*Triticum aestivum* L.)			+
Perennial grasses			
Elephant grass (*Pennisetum purpureum*)	–	–	–
Giant reed (*Arundo donax*)	+/–	+/–	+/–
Switchgrass (*Panicum virgatum*)	+	+	+
Vetiver grass (*Vetiveria zizanioides* L.)	+	+	+

Giant reed (*Arundo donax*) is a tolerant plant species for Cd and Ni, which can accumulate high levels of these TE (Papazoglou 2007, 2009) (Table 9.4).

Owing to years of experience in the production of agricultural crops and timber, the optimal climatic and soil conditions for numerous species and varieties are well known. Perennial grasses are fairly new energy crops, and some, like miscanthus (*Miscanthus* spp.) and giant reed (*Arundo donax*), still retain wild-type characteristics such as high seed dormancy levels and insufficient winter rest ability. Breeding work for the development of varieties adapted to the different ecological/climatic zones, such as in the case of willow (*Salix* spp.) and poplar (*Populus* spp.), is still just beginning and has great potential to develop promising bioenergy varieties (Lewandowski et al. 2003).

9.4 Potential Products: Economic Revenue

9.4.1 Bioenergy

Bioenergy refers to renewable energy from biological sources, such as biomass that can be used for heat, electricity, and fuel, and their coproducts. The biomass may be used directly as heat (plants, wood, straw, and other plants) or processed into gases (from organic waste, landfill waste) or liquids, such as ethanol and biodiesel (derived from crops such as maize (*Zea mays*), sugarcane (*Saccharum officinarum*), wheat (*Triticum* spp.), rapeseed (*Brassica napus*), and soy (*Glycine max*) or from lignocellulosic material). Biomass has the great advantage over other renewable energy forms; it is currently the only renewable source of fixed carbon and thus is the only source in the long term for the production of transport fuels. Approximately 57.7 % of the worldwide oil consumption is used for transportation activities (IEA 2006), and the global primary demand for oil (excluding biofuels) will rise by 1 % per year on average, from 85 million barrels per day in 2007 to 106 mb day^{-1} in 2030 (IEA 2008). Thus, the market for biofuels will become very big, particularly owing to China's rapid expansion.

Biofuels have a potential but their economic viability is highly dependent on both the oil price and on governmental subsidies, the price of oil in the world market being of crucial importance. The starting point, from which the production of biofuels becomes profitable, is known as break-even point (balance point). In the European Union the break-even point for different biofuels can be reached from US$75–80 barrel^{-1} of oil in relation to colza oil, US$90 barrel^{-1} in relation to bioethanol, US$100 barrel^{-1} to biodiesel, and US$155–160 barrel^{-1} to fuels attained by second-generation technologies. In the United States the break-even point for bioethanol is currently reached when the oil price exceeds US$40–50 barrel^{-1}. This means that bioethanol production is not economic at oil prices below US$40 barrel^{-1}. In the case of producing ethanol in Brazil, the break-even point oscillates between US$30 and 35 barrel^{-1}. For biofuels derived from vegetal oils, a technology in its incipient stage, the indicator is estimated to be about US$60 barrel^{-1} (Evangelou et al. 2012, 2013). The break-even point for bioenergy produced, through combustion or fermentation from TE-enriched biomass, would probably be higher. Because filters would have to be installed to retain the volatile TE and the risk originating from the TE contained in the digestate after biogas or ethanol production would have to be mitigated.

9.4.2 Wood

Production and consumption of key wood products (roundwood, sawn-softwood, sawn-hardwood, panels, pulp, paper, and secondary products) are expected to continue past trends of 1–2 % annual increase until 2030. The global demand for wood products is driven by population increase and economic growth in particular in Asia (FAO 2009). Contemporaneously, more forests will be excluded from wood production due to new environmental policies and regulations. Additionally, there is less and less old-growth forest left for logging due to our exploitation and forest destruction. Worldwide there are >89 M ha of plantation forests (FAO 2001) with their area increasing rapidly. But also the land available for forest plantation is limited and under pressure by the demand for agricultural land. Again, areas with elevated TE concentrations (>33 M ha) could offer a viable alternative. They could be used for wood production, thus reducing the necessity to use natural forests.

Unlike biofuels, growing trees for timber does not produce rapid economic revenue. Plantation forests, depending on the tree species used and the intended product, will need 10–50 years before they become harvestable. Eucalyptus plantations, for example, intended for pulp production can be harvested after approximately 6 years (Clay 2004), while pine saw timber may need 30–50 years before it reaches economic maturity (Roth 1989). The long-term harvesting cycles are an advantage for phytomanagement, because the less the costs are for management (e.g., for harvesting, fertilizers etc.), the larger the revenue is for a given return. Furthermore, with longer duration between harvests (>25 years), the proportion of the TE rich bark can be reduced, thus reducing the overall TE concentration of the tree trunk (Evangelou et al. 2012, 2013).

The TE concentrations of wood produced on contaminated land should not exceed regulatory values. Swiss legislation and EPF industry standards require that wood panels intended for the market must not exceed concentrations of 50 mg kg^{-1} Cd, 90 mg kg^{-1} Pb, 25 mg kg^{-1} As, and 40 mg kg^{-1} Cu (ChemRRV 2005; EPF 2000). Packaging materials must not exceed the cumulative concentration limit of 100 mg kg^{-1} for Pb, Cd, Hg, and Cr as described in the EU Packaging and Packaging Waste Directive (94/62/EG) (European Parliament 1994). Thus, every product derived from phytomanagement should be monitored to ensure that it complies with the aforementioned as well as with other product related thresholds.

9.4.3 Biochar

Biochar is produced by pyrolysis (heat-induced carbonization in oxygen-poor atmosphere) of organic material. It is distinguished from charcoal by its main purposes, which are (1) to amend agricultural soils and thereby (2) to sequester carbon from organic matter and avoid its mineralization and release as carbon dioxide into the atmosphere (Lehmann and Joseph 2009). As the pyrolysis process can be used in the same time (3) to produce energy, biochar production has more than in one way the potential to make valuable use of organic residues and thus (4) offers an attractive alternative to other ways of organic wastes disposal.

Compared to biomass production for bioenergy, biochar production is still small. However, if biochar production were subsidized to a greater extent, it may result in similar challenges and problems as bioenergy production. As with bioenergy production, competition with food production should be avoided, thus feedstock sources should not reduce the availability and quality of cropland. Biomass originating from contaminated land could be such a source. A concern in the application of biochar originating from TE-contaminated soil could be the elevated concentrations of potentially toxic TE in the biochar. An important factor in this respect is the production temperature. Van Zwieten et al. (2010) found that the concentrations of Cu, Pb, Zn, Mg, Mn, Ni, and Ca were higher in biochar produced at 350 °C than in the feedstock but lower than biochar produced at 550 °C. Mercury and Cd are volatile when heated, even at 400 °C; thus, a low risk originates from these two particularly toxic TE when higher production temperatures are used. While the volatilization of toxic elements is positive for the subsequent use of biochar as soil amendment, it must be made sure that after volatilization, these contaminants are not released into the environment, but retained in the production facilities as in the case of burning biomass for energy. Trace element-enriched biomass should be converted into biochar only in production facilities, equipped with appropriate filter technology, which means that it will in general not be possible to produce biochar in small-scale biochar production facilities unless they are equally equipped, which will increase their production costs.

Even when the concentrations of toxic TE in biochar produced from plant biomass can be kept low with a suitable choice of the feedstock plants and biochar production temperature, the application is still not without risk. It is not yet sufficiently well known how the mobility and bioavailability of biochar bound TE will change with time, due to microbial activity, pH changes, organic matter interaction with biochar, etc. Trace element plant uptake and toxicological and mobility studies have to be performed to minimize the risk originating from TE-enriched biochar.

9.4.4 Biofortified Products

Deficiencies of the mineral micronutrients Fe, Zn, Se, and I affect more than half of humanity (Graham 2008). Other mineral elements, such as Ca, Mg, and Cu, can also be deficient in the diets of some populations (Zhao and McGrath 2009). One strategy for combating micronutrient malnutrition is to “biofortify” plant-based food through increased accumulation of critical elements in the edible parts of crop plants (Bouis 1996; Frossard et al. 2000; Welch 2002; Welch and Graham 1999). For this to be the case, the soil must be sufficiently rich in the elements targeted for biofortification such as Fe, Zn, Se, I, Ca, Mg, or Cu and sufficiently poor in undesired TE such as Cd, Pb, Hg, Sb, or As, depending on the capability of the plants used for selective uptake and exclusion of these elements. Hyperaccumulating plants are of particular interest in this respect, as most of them hyperaccumulate only one particular element (Assunção et al. 2003). To date >500 plant species have been classified as hyperaccumulators, with the majority (approximately 90 %) being Ni hyperaccumulators. There are also 32 Cu, 20 Se, 12 Zn, and 12 Mn hyperaccumulators that could be potentially used for the production of biofortified products (Ent et al. 2013).

It is rather rare that a soil is enriched in only one TE or metalloid. Nevertheless, these soils may require remediation or risk control, if that metal or metalloid is posing a threat to human health such as in the case of Se enrichment in seleniferous soils. Despite many anthropogenic Se sources, such as fossil fuel combustion, metal processing, applications of fertilizers, lime and manure, and disposal of sewage sludge, the Se content of most soils is primarily of geogenic origin. While most soils contain only 0.01–2.0 mg Se kg^{-1}, mean 0.4 mg Se kg^{-1}, the Se concentration of seleniferous soils can reach up to 1,200 mg Se kg^{-1}. Seleniferous soils are widespread in the Great Plains of the United States, Canada, South America, China, and Russia (White et al. 2007). Phytoremediation, i.e., cleansing of these soils using Se hyperaccumulators or *Brassica* sp. and barley (*Hordeum vulgare*), was found to be not feasible (Banuelos et al. 1997; Banuelos and Mayland 2000). However, if the aim is not cleansing but only control, then combining phytomanagement with the production of biofortified products can create a win-win situation. In the western part of the Central Valley, where soil are rich in Se concentrations, Banuelos and Mayland (2000) produced Se-enriched canola (*Brassica napus*) and utilized it as Se-biofortified forage to feed marginally Se-deficient lambs and cows. Similarly, plants such as rapeseed (*Brassica napus*), raya (*Brassica juncea*), sunflower (*Helianthus annuus*), cowpea (*Vigna sinensis*), guar (*Cyamopsis tetragonoloba*), wheat (*Triticum aestivum*), spearmint (*Mentha viridis*), sugarcane (*Saccharum officinarum*), barley (*Hordeum vulgare*), and bajra (*Pennisetum typhoides*) were used on various seleniferous soils to produce Se-enriched food, fodder, or fertilizer in India (Banuelos and Dhillon 2011). In Enshi, China, the so-called World Capital of Selenium, Yuan et al. (2012) used plants such as clover (*Trifolium repens*) and alfalfa (*Medicago sativa*) to produce Se-biofortified fodder.

The consumption of Se-enriched food or fodder is not without risks as Se readily becomes toxic at elevated concentration. Selenium concentrations of food and feed products have to be determined and controlled and Se-enriched biomass should be used only with great care. Some plants can easily accumulate Se to concentrations that are above the safety threshold for human or animal consumption. In search for alternative uses, Dhillon et al. (2007) incorporated Se-rich plant materials (up to 20 t ha^{-1}) into a non-seleniferous agricultural soil and produced wheat (*Triticum aestivum* L.) grains and straw with increased but safe levels of Se supplement in the diets of animals and humans living in Se-deficient areas. Using Se-rich plant material as an organic Se fertilizer for growing other crops could thus be a safe alternative for utilizing plant material that otherwise is too dangerous as direct Se source in animals and humans nutrition.

9.5 The Effect of Plants on the Mobility of Contaminants: Potential and Risks

9.5.1 Potential for Risk Mitigation

Plants can mitigate environmental and health risks arising from TE-contaminated soils by (a) preventing erosion through vegetation cover, (b) reducing leaching, and (c) immobilizing the contaminants. Protection against wind and water erosion is particularly important in cases where mineral and organic particles at the soil surface are loaded with high amounts of pollutants. Dense vegetation protects soil against wind and water erosion. The vegetation cover shields the soil surface against the impact of rainfall and wind. The root systems form a net that holds the soil together. Their exudates help to clog soil particles into larger aggregates and promote the activity of soil organisms which in turn promote the development of an aggregated soil structure and thus its mechanical stability. The extraction of soil water for transpiration promotes the formation of pores that are easily drained and facilitates soil aeration. As a result, the capacity of the soil to store and drain infiltrating water increases reducing the occurrence of surface runoff and thus water erosion. During dry periods vegetation also protects the soil surface against desiccation, so that the soil surface is stabilized by capillary cohesion of the soil particles against wind erosion.

Soil water consumption for transpiration also reduces contaminant leaching (Pilon-Smits 2005; Robinson et al. 2003b; Vose et al. 2003). How effective vegetation is in controlling leaching also depends on climate. The capacity of the atmosphere to take up water vapor sets upper limit on evapotranspiration. Actual evapotranspiration is due to limitations in water transfer from soil into plants often much lower than this limit. In dryer climates, evapotranspiration is usually greater from deep-rooted species because shallow-rooted species have less access to water during periods of drought and are, therefore, more likely to suffer from dieback or reduced transpiration and growth (Robinson et al. 2009). Robinson et al. (2003b, 2007) found that hybrid poplars (*Populus deltoides* × *nigra* ("Argyle" and "Selwin"), *Populus deltoides* × *yunnanensis* ("Kawa"), *Populus euramericana* × *yunnanensis* ("Toa"), *Populus alba* × *glandulosa* ("Yeogi"), *Populus nigra* × *manimowic* ("Shinsei")) reduced B leaching from a wood-waste landfill due to enhanced evapotranspiration but did not completely prevent substantial leaching driven by heavy rainfall events. Thus, collection and treatment of the discharge from the landfill would still be necessary.

Another way in which plants can immobilize pollutants in soil is binding them through their roots. Unlike in phytoextraction, not cellular uptake is necessary for this. It is sufficient that the contaminant is bound to the root cell walls. In the apoplast, the intercellular space including the cell walls separated by the intracellular space by the cell membranes, significant amounts of various substances can be bound owing to the high sorption capacity of the cell walls. Plant roots can immobilize contaminants also by modifying the chemical environment in the rhizosphere. For example, an increase in pH can reduce the solubility of metal cations. Root exudates promote the formation of soil organic matter and thus increase the sorption capacity of the soil. Also the transition from anaerobic to aerobic conditions in a soil can increase the TE sorption capacity in soils by inducing the oxidation of dissolved Fe(II) and Mn(II) to Fe(III) and Mn(IV), which then precipitate as oxides and hydroxides.

9.5.2 Possible Emerging Risks

Although deep roots seem generally more favorable than shallow roots, one must not forget that deep roots may create macropores which facilitate the preferential transport of contaminants to groundwater (Roulier et al. 2008). In a study by Knechtenhofer et al. (2003), it was shown that soil preferential flow paths below 20 cm, which are associated with roots surrounded by relatively wide root channels (Kretzschmar et al. 1999), played a significant role in the spatial distribution of Pb. In this large macropores, Pb may be transported as aqueous ions or bound by colloidal particles (Kretzschmar et al. 1999). A pH increase can decrease the mobility of metals, but it can also result in the solubilization of humic substances and facilitate the downward mobility of metals via preferential flow pathways.

Trace element-contaminated litter or harvest residues might be dispersed via wind or water erosion, thus potentially contaminating adjacent environments (Perronnet et al. 2000). Such litter and harvest residues decompose slower than non-contaminated plant material (Boucher et al. 2005; Cotrufo et al. 1995), as can be observed by the accumulation of litter on the forest floor near smelters (Berg et al. 1991; Freedman and Hutchinson 1980; Strojan 1978), resulting in the long-term availability of plant material in a form that can be dispersed. Thus, prevention measures should be taken to control plant material (e.g., leaves) dispersion, especially in situations where wind and water can be expected (Van Nevel et al. 2007). With the decomposition of contaminated litter, the contaminants may be released into the soil. Scheid et al. (2009) observed that the sorbed TE (Cu, Cd, Pb, Zn) were strongly bound in the litter even after 2 years of decomposition. However, if the contaminants become associated with dissolved organic matter, they will in fact be more mobile than contaminants adsorbed on mineral particles (Van Nevel et al. 2007).

9.6 Sustainability Aspects

9.6.1 Ecological Sustainability

The management of contaminated soils has to consider not only the established ecosystems on the site but also surrounding ecosystems. Trace element-contaminated land may be valuable as sites of specific floras and faunas such as Galmei-Vegetation in Germany (Engelen and Holtz 2000). The flora growing in metalliferous soils is a source of genetic material for research (Brady et al. 2005; Whiting et al. 2004). There is a trend to protect biodiversity that can accompany the agricultural/industrial development in these kinds of soils (Dickinson et al. 2009). Vidic et al. (2009) showed that the genome size of the species was related to their survival in TE-contaminated soils. Tolerant species had small genomes in comparison with non-tolerant ones. Such surrounding ecosystems can be affected by over extensive use or the use of not suitable plant species on phytomanaged sites. Cormish (1989) showed that when radiata pine (*Pinus radiata*) was planted in Australia to reduce soil erosion and increase slope stability, it reduced stream flow so effectively that naturally perennial streams were turned into ephemeral streams. There was thus a possibility that habitats of several fauna and flora species of surrounding ecosystems requiring perennial stream flow would be endangered.

An important debate relates to the use of non-endemic species for the production of biomass on contaminated land. Although most food, fiber, and landscape plants are nonnative, relatively few have proven invasive. However, some of those that are invasive have caused substantial socioeconomic and environmental impacts. Economic losses caused by invasive plants and costs for their control are estimated to be $34 bn annually in the United States (Ditomaso et al. 2010) and $10 bn annually in Europe (Hulme et al. 2009). The introduction and planting of invasive species/neophytes in various regions of the world, such as eucalyptus in Southern Europe or the giant reed in the Unites States, are caused by government actions. Johnson grass (*Sorghum halepense*) was originally grown as a forage grass but since has become a weed that greatly depresses yields of corn (*Zea mays*), soybeans (*Glycine max*), and other crops. It has invaded now in 16 states of the United States and incurs annual losses of more than $30 M in just three of them (Simberloff 2008). Another fast-growing perennial grass that has become invasive is miscanthus (*Miscanthus* spp.). It is primarily used for biofuel production and has been described as "Johnson grass on steroids" (Raghu et al. 2006). Therefore, the plant species used for phytomanagement should ideally be endemic. Growing noninvasive species might initially be economically less attractive. However, in the long term they would be more advantageous as no clearing costs would be involved, and public opinion would be less hostile.

9.6.2 Soil Sustainability

The production of biomass for biofuels, food, timber, etc., has often caused soil degradation/soil loss due to inadequate soil management practices. The main forms of soil degradation are water (56 %) and wind erosion (28 %). Other forms including chemical degradation and physical degradation sum up to 16 %. In total soil degradation affects about 2,000 M ha of land, which is equivalent to 15 % of the Earth's land surface (an area larger than the United States and Mexico combined). The causes of soil degradation include overgrazing (35 %), deforestation (30 %), agricultural activities (27 %), overexploitation of vegetation (7 %), and industrial activities (1 %) (UNEP 2002). Increased biofuel production has shown that soil degradation could become more severe in the near future. In Indonesia, for instance, two-thirds of oil palm expansion has occurred by converting large rainforest areas. In the United States, 1.3 M ha of lands in the Conservation Reserve Program designed to help check surpluses, maintain price levels, and promote an ecological balance were called back into production (UNEP 2012). Soil degradation can be influenced greatly by the user with an appropriate choice of plants, as well as management, which have to be adjusted accordingly to the soil type, the climate, and the geomorphology.

The choice of plant species is very important as they can either increase or decrease soil erosion as well as soil organic carbon (SOC) content. Sullivan (2004) found that traditional annual crops such as corn (*Zea mays*) and soybean (*Glycine max*) caused 50 times more soil erosion than sod crops. In general, soil is more exposed to the impacts of weather in row crops than in the latter. Also trees such as willow (*Salix* spp.) or poplar (*Populus* spp.) usually provide better protection against erosion than row crops. Pimentel and Krummel (1987) showed that under short-rotation woody crops (SRWC), the average erosion rate was 2 Mg ha^{-1} year^{-1} on a 5 % slope, whereas corn (*Zea mays*) grown on a 4 % slope resulted in a soil loss of 21.8 Mg ha^{-1} year^{-1}. Nevertheless, erosion can still be high under SRWC, if there is no herbaceous cover beneath the trees, especially when there is a high throughfall of rain (Kort et al. 1998). Perennial grasses are also effective in reducing erosion (Kemper et al. 1992) due to their dense network of fibrous roots close to the soil surface. The choice of the plant species for phytomanagement influences the SOC content. McLaughlin and Walsh (1998) reported that carbon sequestration rates under switchgrass (*Panicum virgatum*) may exceed those of annual crops by as much as 20–30 times, owing to carbon storage in the soil. Cultivation of temperate-zone perennial grasses such as miscanthus (*Miscanthus × giganteus*), switchgrass (*Panicum virgatum*), and others can increase SOC by 0.1–1 Mg ha^{-1} year^{-1} (Anderson-Teixeira et al. 2009). Short-rotation wood coppice, with willows (*Salix* spp.), may be even more effective in storing SOC than switchgrass (*Panicum virgatum*). Zan et al. (2001) found that relatively fertile soils in Canada beneath willows (*Salix* spp.) stored more SOC than under corn (*Zea mays*) or switchgrass (*Panicum virgatum*) 4 years after establishment.

Other agricultural management factors that have a major influence on soil erosion and soil carbon sequestration are whether crop residues are left on the field and incorporated into the soil as well as tillage practices. While there can be important differences between different tillage techniques, tillage in general increases the risk of soil erosion and SOC loss (Anderson-Teixeira et al. 2009; Williams et al. 2009), whereas crop residues that are left on the land protect the soil against erosion and SOC loss. If residues are completely removed, no-tillage soils can be as even more vulnerable to wind erosion than plowed soils during drought periods (Blanco-Canqui 2010). Blanco-Canqui and Lal (2009) considered a partial removal of 25 % of stover as the maximum rate that can be tolerated in no-tillage soils. This might be enough to control wind erosion, but it might still be too much to maintain optimal levels of SOC. Wilhelm et al. (2007) found that the amounts of corn (*Zea mays*) stover needed to maintain SOC at such a level by far exceed the amounts needed to control water and wind erosion.

9.7 Decision Support Systems

The success of phytomanagement crucially depends on the choice of the right plants and cultivation methods. The cultivation of candidate plants must be practical, economically attractive, and safe under the conditions of the given site and land use conditions. In practice, it is not possible to perform experimental trials in each specific case. However, the results of many pot and field studies in which plants have been grown on polluted soils have been integrated into model-based decision support systems (DSS), such as REC-Phyto-DSS (Onwubuya et al. 2009), Phyto-DSS (Robinson et al. 2003a), and Phyto-3 (Bardos et al. 2011).These can be of great help in the evaluation, design, and operation of site-adapted phytoremediation schemes. All the mentioned DSS either use a multi-criteria analysis or life cycle analysis or both. Phyto-3 is designed for US conditions. It provides guidance for regulators and practitioners, evaluating options of remedial phytotechnology available for the treatment of contaminated sites, with a strong focus on groundwater protection against organic contaminants. REC-Phyto-DSS is a European DSS specifically focusing on "gentle" site remediation techniques and in particular on phytoextraction and phytostabilization. It is implemented in the Dutch REC (Risk reduction, Environmental merits, and Cost) framework. Phyto-DSS is a generic tool designed to predict the efficiency of metal phytoextraction and evaluate its economic feasibility. It is based on a mechanistic model taking account of plant water use, soil metal solubility, and root distribution but used a lumped parameter to determine the ratio between metal concentrations in the xylem of the remediation plants and the soil solution. While the existing DSSs provide a good basis for the assessment of contaminated sites, as shown in Cano-Reséndiz et al. (2011), none of them however have yet a sufficient focus on the economic revenue. Thus, to encourage efficiency and increase the monetary output and keep possible risks derived from phytomanagement sites at a minimum, a DSS developed for phytomanagement is needed.

9.8 Conclusions

Starting with phytoextraction as a novel, low-tech, promising tool for soil cleaning around two decades ago, phytoremediation of contaminated soils will forever just remain a promising tool if it is not linked to profitable production of biomass, in the form of phytomanagement. Successful phytomanagement requires a multidisciplinary approach combining the design of appropriate crop management schemes, control of contaminant fluxes, assessment of associated risk, and optimization of economic revenues. Once accepted by regulators and decision makers in charge, phytomanagement could become a viable solution to use and even restore polluted soils. The phytomanagement of contaminated sites could offer an alternative income to people living nearby such areas and who lost their livelihood because of the contamination.

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10

Phytoremediation of Soils Contaminated with Heavy Metals: Techniques and Strategies

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10.1 Introduction

There is no standard definition of the term "heavy metal," but it generally includes elements (both metals and metalloids) which are toxic and have an atomic density greater than 6 g cm^{-3}. This group includes both biologically essential elements such as cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn), and zinc (Zn) and nonessential elements such as cadmium (Cd), lead (Pb), and mercury (Hg). Arsenic (As), boron (B), and selenium (Se) are usually included in this group although they are lighter elements. The essential elements (for plants, microorganisms, or animals) are required in low concentrations and hence are known as "trace elements" or "micronutrients." The nonessential metal(loid)s are phytotoxic and/or zootoxic and are widely known as "toxic elements" (Verkleij and Prast 1990; Ashraf et al. 2010). Both groups are toxic to plants, animals, and/or humans when present at high concentrations (Adriano 2001; Park et al. 2011).

Environmental pollution by heavy metals has become a severe problem worldwide. Soils became increasingly contaminated with heavy metals due to large-scale urbanization and industrialization, and this poses a threat to land ecosystems, surface and groundwater, as well as food safety and human health (Chen et al. 2004; Lorestani et al. 2012).

Metals are released into the environment by natural events such as weathering of rocks and volcanic eruptions but increasingly due to anthropogenic activities as mining, electroplating, metal processing, textile industries, battery manufacturing, tanneries, petroleum refining, paint and pigment manufacture, pesticides, printing, and photographic industries, among others (Olguín and Sánchez-Galván 2012).

Unlike organic substances, heavy metals are essentially nonbiodegradable and therefore tend to accumulate in the environment and in the body tissues of living organisms (bioaccumulation). Their concentration may increase as they pass from lower to higher trophic levels, a phenomenon known as biomagnification (Ali et al. 2013).

The total concentration of heavy metals in soils is commonly used to assess soil contamination (Sheppard et al. 1992; Dai et al. 2004). However, this does not provide an adequate indicator of their capacity to be absorbed by plants or soil organisms nor does it predict the risk of their transfer along the food chain (Morel 1997; Dai et al. 2004). In fact, the bioavailability of heavy metals in soils depends on many factors such as pH, organic matter content, cation exchange capacity, and the speciation of the metal. It has been shown that soils with larger concentrations of trace elements may be less toxic than those with smaller concentrations (Alvarenga et al. 2009, 2012). Therefore, suitable indicators of soil pollution are still needed.

High levels of metals in soil usually result in greater uptake by plants. Van Nevel et al. (2007) identified three main risks associated with the accumulation of metals in aboveground plant parts: (1) metals entering the food chain through herbivores, (2) dispersion of contaminated plant material to adjacent environments, and (3) accumulation of metals in the topsoil. The topsoil is particularly vulnerable as

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it is the part with most biological activity, and this has been shown to be highly sensitive to metal pollution (Van Nevel et al. 2011).

Remediation of soils contaminated with heavy metals remains a difficult challenge. The decision to remediate a soil depends on many factors, including the level of contamination, the risk posed by the soil, the present and future value of the soil, and ultimately on political decisions which are influenced by the population and depend on the perception of the risk and willingness to pay the cost of remediation. Excavation and burial of contaminated soil as a hazardous waste is the most commonly used technology, but the use of plants to remove or immobilize toxic metals has arisen as a very promising alternative (Al-Farraj and Al-Wabel 2007; Lorestani et al. 2012).

The uptake of metals is mainly influenced by their bioavailable fraction (Vamerali et al. 2010; Bhargava et al. 2012). Though abundant in nature, the actual bioavailability of some metals is limited because of low solubility of metals in oxygenated matrices and sorption to soil particles. Metal availability and mobility in the rhizosphere is also influenced by rhizospheric microorganisms as well as by root exudates. Acidification of the rhizosphere, exudation of organic acids, and mechanisms assisting in the acquisition of phosphorus or micronutrients may also enhance the bioavailability of certain heavy metals (Chaney et al. 2007).

Along the years, a number of related technologies were developed, which enabled the practical application of higher plants to decontaminate soils and waters, and the term "phytoremediation" started to be used in the scientific literature in 1993 (Cunningham and Berti 1993; Raskin et al. 1994; Salt et al. 1995; Maestri and Marmiroli 2011). The definition later evolved into the concept of "phytotechnologies" (ITRC 2001), which include a wide range of technologies that can be applied to remediate pollutants through (1) stabilization, (2) volatilization, (3) metabolism, including degradation in the rhizosphere, and (4) accumulation and sequestration (Maestri and Marmiroli 2011). The use of plants to remediate soils derived from the observation of wild species found in specific environments (e.g., endemic hyperaccumulators) evolved to the use of some fast-growing crops and later on led to the development of genetically modified genotypes.

The ideal plant to be used in the remediation of heavy metal-contaminated soils would be a fast-growing species with a large biomass, capable of growing in many different climatic conditions and easily cultivated, and which would allow the removal of considerable amounts of contaminants. For this purpose, researchers have selected plant species with properties required for phytoremediation, such as deep root systems, ability to grow on nutrient-poor soils, a fast growth rate, and tolerance to contaminants. In addition, genetically modified plants were produced to confer specific properties for resistance, accumulation, or detoxification (Ashraf et al. 2010; Rascio and Navari-Izzo 2011; Bhargava et al. 2012).

10.2 Toxicity of Heavy Metals to Plants

Any heavy metal may be considered a contaminant if it occurs where it is unwanted or in a form or concentration that causes a detrimental effect to humans or the environment (Verkleij and Prast 1990; Yang et al. 2005).

Heavy metals enter the environment from natural and anthropogenic sources. The most significant natural sources are weathering of minerals, erosion, and volcanic activity while anthropogenic sources include mining; smelting; electroplating; use of pesticides, phosphatic fertilizers, and biosolids in agriculture; sludge dumping; industrial discharges; emissions from municipal waste incinerators; car exhausts; atmospheric deposition; etc. (Pendias 2001; Modaihsh et al. 2004; Yang et al. 2005 ; Chehregani and Malayeri 2007; Fulekar et al. 2009; Sabiha-Javied et al. 2009; Wuana and Okieimen 2011; Ali et al. 2013).

Most of the metals are easily absorbed by plants and bioaccumulate in different organs (Wang et al. 2003; Ashraf et al. 2010) where they can impair cell metabolism if present in sufficiently large amounts. Physiological mechanisms that may be affected include enzymatic activity, protein structure, water balance, respiration and ATP content, photosynthesis, plant division, and morphogenesis (Foyer and Noctor 2005; Shao et al. 2008; Sharma and Dietz 2009; Ahmad et al. 2010; Guerra et al. 2011; Kadukova and Kavuličova 2011).

A rather common consequence of heavy metal accumulation is the enhanced production of reactive oxygen species (ROS) due to interference of the metals with electron transport (Pagliano et al. 2006; Rascio and Navari-Izzo 2011). This increase in ROS exposes cells to oxidative stress leading to lipid peroxidation, deterioration of biological macromolecules, dismantling of membranes, ion leakage, and cleavage of DNA strands (Navari-Izzo et al. 1998, 1999; Rascio and Navari-Izzo 2011).

Plants resort to a series of defense mechanisms to control uptake, accumulation, and translocation of these elements and to detoxify them by excluding the free ionic forms from the cytoplasm. One commonly employed strategy relies on the entrapment of heavy metals in the apoplasm by binding them to exuded organic acids (Watanabe and Osaki 2002; Rascio and Navari-Izzo 2011) or to anionic groups present in cell walls, thus preventing uptake into root cells (Dalla Vecchia et al. 2005; Rascio et al. 2008; Rascio and Navari-Izzo 2011). Most of the heavy metals that do enter the plant are kept in root cells where they are detoxified by complexation with amino acids, organic acids, or metal-binding peptides and/or by sequestration in vacuoles (Hall 2002; Rascio and Navari-Izzo 2011). This greatly restricts translocation to the aboveground organs, thus protecting the metabolically active photosynthetic cells from damage. A further defense mechanism generally adopted by exposed plants is the enhancement of cell antioxidant systems which counteract oxidative stress (Navari-Izzo et al. 1998; Sgherri et al. 2003; Rascio and Navari-Izzo 2011). Numerous studies suggest an important role of ROS in plant defense responses to abiotic stress, as they may act as signals for the activation of stress response and defense pathways (Knight and Knight 2001; Mittler 2002; Foyer and Noctor 2005; Shao et al. 2008; Sharma and Dietz 2009; Ahmad et al. 2010; Guerra et al. 2011; Kadukova and Kavuličova 2011).

These ROS include the superoxide radical ($O_2^{\bullet -}$), the hydroxyl radical ($OH^{\bullet -}$), and hydrogen peroxide (H_2O_2) that are produced as by-products during membrane-linked electron transport in photosynthesis and mitochondrial respiration, as well as by a number of metabolic pathways (Briat and Lebrun 1999; Mittler 2002; Shao et al. 2008; Kadukova and Kavuličova 2011). In addition, pathogens and wounding or environmental stresses (e.g., drought or osmotic stress) have been shown to trigger the active production of ROS by NADPH oxidases (Cazalé et al. 1999; Pei et al. 2000; Mittler 2002).

Under abiotic and biotic stresses, the increased generation of ROS initiates signaling responses that include enzyme activation, programmed cell death, and cellular damage (Mittler 2002; Neil et al. 2002; Pitzschke and Hirt 2006; Kadukova and Kavuličova 2011).

Although the steady-state level of ROS can be used by plants to monitor their intracellular level of stress, this level has to be kept under tight control because overaccumulation of ROS can result in cell death (Asada 1999; Dat et al. 2000; Mittler 2002) due to damage of biomolecules (e.g., membrane lipids, proteins, chloroplast pigments, enzymes, nucleic acids) and disturbances in signaling processes. In the case of stress induced by a variety of environmental stressors such as soil salinity, drought, extremes of temperature, and heavy metals, the equilibrium between ROS production and scavenging may be impaired (Rodrígez-López et al. 2000; Møller 2001; Mittler 2002; Rios-Gonzalez et al. 2002; Rhoads et al. 2006; Kadukova and Kavuličova 2011).

ROS levels are controlled by a complex and flexible network of antioxidant systems that maintain a balance between their negative and beneficial functions (Pinto et al. 2009). Cells have evolved intricate defense systems including enzymatic superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductases (GR), monodehydroascorbate reductases (MSHAR), dehydroascorbate reductases (DHAR), glutathione peroxidase (GPX), guaiacol peroxidase (GOPX), and glutathione *S*-transferase (GST) and nonenzymatic systems involving ascorbic acid (ASH), glutathione (GSH), phenolic compounds, alkaloids, nonprotein amino acids, and α-tocopherol, which can scavenge the indigenously generated ROS (Foyer and Noctor 2005; Shao et al. 2008; Sharma and Dietz 2009; Ahmad et al. 2010; Guerra et al. 2011; Kadukova and Kavuličova 2011). Ascorbate peroxidase and GSH are important components of the ascorbate–glutathione pathway responsible for the removal of H_2O_2 in different cellular compartments. GSH is oxidized by ROS forming oxidized glutathione (GSSG); ascorbate is oxidized to monodehydroascorbate (MDA) and dehydroascorbate (DHA). Through the ascorbate–glutathione cycle, GSSG, MDA, and DHA can be reduced back to GSH and ascorbate (Zhang and Kirkham 1996; Yadav et al. 2009; Kadukova and Kavuličova 2011).

One of the most deleterious effects induced by heavy metal exposure is lipid peroxidation, which can directly cause deterioration of biomembranes. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids present in membranes, is regarded as a reliable indicator of oxidative stress (Demiral and Türkan 2005; Yadav 2010).

Plants can produce low-molecular-weight thiols that show high affinity for toxic metals (Bricker et al. 2001; Yadav 2010). The most important are glutathione (GSH) and cysteine. GSH is a sulfur-containing tripeptide with the formula γ-glutamate-cysteine-glycine. Its synthesis is catalyzed by two ATP-dependent enzymes: γ-glutamylcysteine synthetase (GSH1) and glutathione synthetase (GSH2). Glutathione occurs in plants mainly as reduced GSH (95 % of the total). GSH metabolism is connected with cysteine and sulfur metabolism in plants, and GSH is a substrate for the synthesis of phytochelatins (Huang et al. 1998; Arisi et al. 2000; Pulford and Watson 2003; Freeman et al. 2004; Prasad 2006; Yadav 2010).

Phytochelatins (PCs) are a set of small, heavy metal-binding, cysteine-rich peptides with the general structure $(\gamma\text{-Glu-Cys})_n\text{Gly}$ ($n = 2$–11). They were first isolated from cell suspension cultures of a higher plant after exposure to Cd (Grill et al. 1985; Yadav 2010). Since then, PCs have been found in several eukaryotes, including higher plants, fungi, and other organisms (Grill et al. 1985, 1988; Gekeler et al. 1988; Piechalak et al. 2002; Yadav 2010). PCs are synthesized from GSH following exposure to heavy metals such as Cd, Hg, Cu, Zn, Pb, Ag, Bi, Au, and Ni, with the involvement of α-glutamylcysteine transferase (EC 2.3.2.15), which is also called phytochelatin synthase (PCS) (Zenk 1996; Mittler 2002; Vatamaniuk et al. 2000; Hirata et al. 2005; Prasad 2006; Yadav 2010).

PCs form complexes with toxic metal ions in the cytosol and are subsequently transported into the vacuole by an ATP-dependent pathway (Salt and Rauser 1995). Thus, toxic metals are swept away from cytosol protecting plants from the deleterious effects they would cause otherwise (Heldt 1997; Cobbett and Goldsbrough 2002; Kadukova and Kavuličova 2011). In fact, the vacuole is generally considered to be the main storage site for metals in plant cells, especially Cd and Zn, and compartmentalization of metals in the vacuole is an important part of the tolerance mechanism of some metal hyperaccumulator plants. For example, the Ni hyperaccumulator *Thlaspi goesingense* Halacsy enhances its Ni tolerance by compartmentalizing most of the intracellular leaf Ni into the vacuole (Krämer et al. 2000; Bhargava et al. 2012). A high level of the Ni transporter TgMTP1 in *T. goesingense* is considered to be the main factor responsible for the enhanced ability to accumulate metal ions within shoot vacuoles (Persans et al. 2001; Bhargava et al. 2012). Furthermore, intact vacuoles isolated from *Nicotiana tabacum* L. and *Hordeum vulgare* L. exposed to Zn have been shown to accumulate this metal (Bhargava et al. 2012). In conclusion, the detoxifying/sequestering mechanisms in plants consist mainly in heavy metal complexation with ligands and/or in their removal from the metabolically active cytoplasm by moving them into inactive compartments, mainly vacuoles and cell walls.

Comparative transcriptome analyses showed that the sequestration trait relies, at least in part, on constitutive overexpression of genes that encode proteins operating in the transfer of metals across the tonoplast and/or plasma membrane and responsible for their exclusion from the cytoplasm. The cation diffusion facilitator (CDF) family members, also named metal transporter proteins (MTPs), which mediate the efflux of bivalent cations from the cytosol, are important candidates for these proteins. MTP1, a gene encoding a protein localized at the tonoplast, is highly overexpressed in leaves of Zn/Ni hyperaccumulators (Dräger et al. 2004; Kim et al. 2004; Hammond et al. 2006; Gustin et al. 2009; Rascio and Navari-Izzo 2011). It has been suggested that MTP1 may also play a role in enhancing Zn accumulation. Transport of Zn into the vacuole, in fact, may initiate a systemic Zn deficiency response that includes the enhancement of metal uptake and translocation via the increased expression of ZIP transporters in hyperaccumulator plants (Gustin et al. 2009; Rascio and Navari-Izzo 2011). MTP members also mediate the vacuolar storage of Ni in *T. goesingense* shoots (Persans et al. 2001). Moreover, the finding that MTP1 is localized at both vacuolar and plasma membranes suggests that it can also operate in Zn and Ni efflux from cytoplasm to cell walls (Kim et al. 2004).

10.3 Phytoremediation: A Competitive and Sustainable Solution for Heavy Metal Contamination

Because of the potential toxicity and persistence of heavy metals, the cleanup of contaminated soils is one of the most difficult tasks for environmental engineering (Wu et al. 2007). A number of ex situ and in situ techniques have been developed to remove or reduce the load of heavy metals in contaminated soils. Phytoremediation, the use of plants and associated soil microorganisms to remove or reduce contaminants in different environmental matrices (air, soil, and water), is an environment-friendly technology that can be used to extract or immobilize metals, metalloids, and radionuclides as well as organic xenobiotics (McGrath et al. 2002; Wu et al. 2007, 2012; Jadia and Fulekar 2009; Ali et al. 2013). It represents an emerging and sustainable technology for the remediation of slightly to moderately contaminated sites and contributes to the improvement of the physical (erosion control), chemical (nutrient levels and organic matter content), and biological (biodiversity and enhanced microbial biomass and activity) quality of the soil (Miller 1996; Maegher 2000; Willscher et al. 2013). Low costs of phytoremediation are an advantage compared to the conventional technologies together with the lower environmental impact. Thus, phytoremediation has a high potential, because it is a novel, cost-effective, efficient, environment- and eco-friendly, and solar-driven remediation strategy (Clemens 2001; Chehregani and Malayeri 2007; Kawahigashi 2009; Willscher et al. 2009, 2013; Saier and Trevors 2010; Kalve et al. 2011; Sarma 2011; Singh and Prasad 2011; Vithanage et al. 2012; Ali et al. 2013).

Phytoremediation utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize contaminants within air, soil, and water (McCutcheon and Schnoor 2003; Alvarez and Illman 2006). Hydraulic control, uptake, transformation, volatilization, and rhizodegradation are important processes involved in phytoremediation (Alvarez and Illman 2006).

It is seen as having great promise compared to conventional, civil engineering methods, and several recent comprehensive reviews summarizing the most important aspects of soil–metal phytoremediation are available (Chaney et al. 1997; Navari-Izzo and Quartacci 2001; McGrath and Zhao 2003; Pilon-Smits 2005; Kidd et al. 2009; Schwitzguébel et al. 2009; Kadukova and Kavuličova 2011; Rascio and Navari-Izzo 2011).

The term "phytoremediation" encompasses several technological subsets, i.e., phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, and phytodesalination (Fulekar et al. 2009; Marques et al. 2009; Bhargava et al. 2012; Ali et al. 2013), involving various physiological and biochemical mechanisms (Huang et al. 1997; Wu et al. 2007).

As plants do not have the possibility to leave contaminated sites, they were forced to develop specific and relevant biological mechanisms to adapt to metal stress. In general, two main outcomes can be distinguished: metal sensitivity and metal resistance. Sensitivity to metals results in injury or death of plants. Resistance means that in spite of metal toxicity, plants are able to react in a way that allows them to survive high concentration of metals and to produce the next generation of plants. Resistance includes avoidance, which describes mechanisms to avoid metal uptake or enhance efflux, and tolerance in which the plant is able to survive large internal concentrations of metals (Orcutt and Nilsen 2000; Kadukova and Kavuličova 2011).

Some plants are even able to accumulate large amounts of metals in their tissues—they are called hyperaccumulators. Content of specific metals in these plants exceeds levels that are actually required for normal growth and development. The term "hyperaccumulator" was used for the first time by Brooks and his colleagues in 1977 (Brooks et al. 1977) to describe plants able to accumulate high amounts of Ni. Hyperaccumulators belong to distantly related families but share the ability to grow on metalliferous soils and accumulate extraordinarily high amounts of heavy metals in the above-ground organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects (Rascio and Navari-Izzo 2011). Three basic hallmarks distinguish hyperaccumulators from related non-hyperaccumulating taxa: a strongly enhanced rate of heavy metal uptake, a faster root-to-shoot translocation, and a greater ability to detoxify and sequester heavy metals in leaves (Rascio and Navari-Izzo 2011).

An interesting breakthrough that came about from comparative physiological and molecular analyses of hyperaccumulators and related non-hyperaccumulators is that most key steps in hyperaccumulation do not rely on specific genes but rather on different regulation and expression of genes found in both kinds of plants. In particular, it seems that a determinant role in the uptake, translocation to leaves, and sequestration in vacuoles or cell walls of great amounts of heavy metals is played in hyperaccumulators by constitutive overexpression of genes encoding transmembrane transporters, such as members of ZIP, HMA, MATE, YSL, and MTP families (Rascio and Navari-Izzo 2011).

About 450 angiosperm species have been identified so far as heavy metal (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn,) hyperaccumulators, accounting for less than 0.2 % of all known species (Rascio and Navari-Izzo 2011). However, new members of this group will continue to arise, meaning that many yet unidentified hyperaccumulators may occur in nature.

Ni is hyperaccumulated by the greatest number of taxa (more than 75 %), while a low number of hyperaccumulators has been found for Cd, which is one of the most toxic heavy metals (Rascio and Navari-Izzo 2011). Ni is also the metal that has been shown to reach the highest concentration in a living tissue. The plant *Sebertia acuminata* Pierre ex. Baillon (Sapotaceae), a tree endemic to a serpentine soil from New Caledonia, accumulates up to 26 % Ni (dry mass) in its latex (Sagner et al. 1998; Rascio and Navari-Izzo 2011).

About 25 % of discovered hyperaccumulators belong to the Brassicaceae family and, in particular, to genera *Thlaspi* and *Alyssum* (Raskin et al. 1994; Rascio and Navari-Izzo 2011). These also include the highest number of Ni-hyperaccumulating taxa (Brooks 1998; Rascio and Navari-Izzo 2011). Zn hyperaccumulators are less numerous and include *Arabidopsis halleri* L., species of *Thlaspi* among the Brassicaceae (Baker and Brooks 1989), and *Sedum alfredii* Hance (Crassulaceae) (Yang et al. 2004). *A. halleri*, *S. alfredii*, and *Corydalis pterygopetala* Handel-Mazzetti, together with *Thlaspi caerulescens* J.&C. Presl. and *T. praecox* Wulfen, are recognized species that hyperaccumulate Cd as well as Zn (Yanqun et al. 2005; Sun et al. 2007; Rascio and Navari-Izzo 2011). *Solanum nigrum* L. (Solanaceae) was reported as being the fifth Cd hyperaccumulator (Sun et al. 2006).

Species hyperaccumulating Se are distributed in genera of different families, including the Fabaceae, Asteraceae, Rubiaceae, Brassicaceae, Scrophulariaceae, and Chenopodiaceae (Reeves and Baker 2000). Other than some angiosperms, such as the Brassicaceae *Isatis cappadocica* Desv. and *Hesperis persica* Boiss. (Karimi et al. 2009, 2010), a number of brake ferns belonging to the genus *Pteris* have also been found to hyperaccumulate As (Ma et al. 2001; Zhang et al. 2004; Wang et al. 2007).

Almost all metal-hyperaccumulating species known today were discovered on metalliferous soils, either natural or man-made, often growing together with metal excluders. Actually, almost all metal-hyperaccumulating plants are endemic to these soils, suggesting that hyperaccumulation is an important ecophysiological adaptation to heavy metal stress and one of the manifestations of heavy metal resistance (Raskin et al. 1994; Sun et al. 2007; Maestri et al. 2010).

Plants can be used to treat most classes of contaminants—toxic metals, radionuclides, and recalcitrant organic pollutants, like chlorinated pesticides, organophosphate insecticides, petroleum hydrocarbons (BTEX), polynuclear aromatic hydrocarbons (PAHs), sulfonated aromatics, phenolics, nitroaromatics and explosives, polychlorinated biphenyls (PCBs), and chlorinated solvents (TCE, PCE). This method is often complementary to traditional bioremediation techniques based exclusively on the use of microorganisms (Garbisu and Alkorta 2001; Ouyang 2002; Schwitzguébel 2002; Abhilash et al. 2009).

Plants have been successfully used not only for soil remediation but also for treatment of municipal and industrial wastewaters (Newman et al. 2000; Dunne et al. 2005; Chavan et al. 2007; Khan et al. 2009; Zurita et al. 2009), and even some information is available on their application to treat air (Liu et al. 2007; Kadukova and Kavuličova 2011).

Phytoremediation may be divided into several remediation technologies, the most important of which is described in more detail below.

10.3.1 Phytoextraction

Phytoextraction, also known as phytoaccumulation, phytoabsorption or phytosequestration, is the uptake of contaminants from soil, water, or sediments by plant roots and their translocation and accumulation in aboveground biomass, i.e., shoots or any other harvestable plant parts (Sekara et al. 2005; Rafati et al. 2011; Bhargava et al. 2012; Ali et al. 2013). For this purpose, plants capable of accumulating heavy metals are grown on contaminated sites, and the metal-rich aboveground biomass is harvested, resulting in the removal of a fraction of the soil contaminant.

Metal translocation to shoots is a crucial physiological process as it is much simpler to harvest shoots than roots (Zacchini et al. 2009; Tangahu et al. 2011; Ali et al. 2013).

Phytoextraction is the main and most useful phytoremediation technique for removal of heavy metals and metalloids from polluted soils (Cherian and Oliveira 2005; Milic et al. 2012; Ali et al. 2013). It is also the most promising for commercial application (Ali et al. 2013).

The success of phytoextraction as a potential environmental cleanup technology depends on many factors like bioavailability of heavy metals, soil properties, speciation of the heavy metals, as well as the plant's ability to absorb and accumulate metals in its aboveground parts (Bhargava et al. 2012; Ali et al. 2013).

10.3.1.1 Hyperaccumulators and Phytoextraction

Many studies focus on the genetics and biochemistry of metal uptake, transport, and storage in hyperaccumulator plants so that transgenic plants with improved phytoremediation capability can be obtained. Many plant species are being investigated to determine their usefulness for phytoextraction, especially fast-growing crops capable of accumulating a large biomass (Bhargava et al. 2012). Nonetheless, the use of crops for phytoextraction of heavy metals also presents some disadvantages, in particular the risk of contamination of the food chain. In no case should field crops grown for phytoremediation purposes be used as animal feed or for direct human consumption (Vamerali et al. 2010; Ali et al. 2013). The harvested biomass is usually incinerated or composted and rarely recycled for reuse (Prasad and Freitas 2003; Bhargava et al. 2012).

The ideal plant to be used in phytoextraction should possess multiple traits like ability to grow in many climatic and edaphic conditions, a fast growth and large biomass, and capacity to accumulate a range of heavy metals in its harvestable parts (Seth 2011; Bhargava et al. 2012).

No plant is known that fulfills all these criteria. However, a fast-growing non-accumulator plant could be modified and/or engineered so that it achieves most of the abovementioned attributes (Bhargava et al. 2012). There has been significant progress in determining the biochemical and molecular basis for metal accumulation, which will provide a strong scientific basis to outline strategies for achieving this goal.

Significant progress in understanding the mechanisms governing metal hyperaccumulation has been made in the last decade through comparative physiological, genomic, and proteomic studies of hyperaccumulators and related non-hyperaccumulator plants. A great number of studies were carried out with *T. caerulescens* and *A. halleri*, which became model plants for these studies (Milner and Kochian 2008; Krämer 2010; Rascio and Navari-Izzo 2011).

A metal hyperaccumulator is a plant which, when grown in metal-enriched habitats, can accumulate 100–1,000-fold the level of metals than normal plants can. This corresponds to concentrations in aboveground tissues of >10 mg g^{-1} (1 %) for Mn or Zn; >1 mg g^{-1} (0.1 %) for As, Co, Cr, Cu, Ni, Pb, Sb, Se, or Tl; and >0.1 mg g^{-1} (0.01 %) for Cd (Baker and Brooks 1989; Reeves and Baker 2000; Verbruggen et al. 2009; Rascio and Navari-Izzo 2011; Bhargava et al. 2012).

Two different approaches have been tested for phytoextraction of heavy metals: (1) the use of hyperaccumulators, which produce comparatively less aboveground biomass but accumulate target heavy metals to a greater extent and (2) the application of other plants, such as *Brassica juncea* (L.) Czern. (Indian mustard), which accumulate target heavy metals to a lesser extent but produce more aboveground biomass so that overall accumulation is comparable to that of hyperaccumulators (Robinson et al. 1998; Tlustoš et al. 2006; Ali et al. 2013). For example, *B. juncea*, although with only one-third the concentration of Zn in its tissues compared with *T. caerulescens* (a known hyperaccumulator of Zn), is considered to be more effective at removing Zn from soils (Ebbs et al. 1997; Bhargava et al. 2012). This advantage is primarily due to the fact that *B. juncea* produces ten times more biomass than *T. caerulescens*.

Fast-growing trees, like *Populus* spp. and *Salix* spp., are ideal candidates for phytoextraction due to their extensive root systems, high rates of water uptake and transpiration, rapid growth, and large biomass. They can be harvested with subsequent resprouting without disturbing the site (Pulford and Watson 2003; Meers et al. 2007; Brunner et al. 2008; Domínguez et al. 2008; Bhargava et al. 2012). However, the use of trees requires excavation and disposal of roots at the end of the process (Mench et al. 2010; Bhargava et al. 2012).

Populus spp. can be grown in a wide range of climatic conditions and are used with increasing frequency in "short-rotation forestry" systems for pulp and paper production (Bhargava et al. 2012). This raises the possibility of using plantations across several multiyear cycles to remove heavy metals from contaminated soils. Importantly, it is unlikely that heavy metals will enter the human food chain or end up in feedstock for animals. Likewise, several species of *Salix* (*S. dasyclados* Wimm., *S. smithiana* Willd. and *S. caprea* L.) display good accumulation capabilities and remediation effectiveness, similar to herbaceous hyperaccumulators like *Arabidopsis halleri* L. and *T. caerulescens*, compensating lower metal content in shoots with higher biomass production (Fischerová et al. 2006; Keller et al. 2006; Meers et al. 2007; Bhargava et al. 2012).

The transgenic plants approach seems promising, although only a few studies have been performed under field conditions (Rascio and Navari-Izzo 2011). However, public concern about the introduction of transgenic plants may be a strong obstacle to such approach. Instead, conventional plant breeding with the emphasis on existing hyperaccumulators may be a feasible option (Baker and Whiting 2002; Chaney et al. 2007; Rahman et al. 2011a, b). Moreover, tolerance and accumulation of heavy metals, and thus the phytoextraction potential, are controlled by many genes so that genetic manipulations to improve these traits is not an easy task, requiring extensive changes in the expression of a number of genes that have to be previously identified. This means that functions and regulations of genes involved in metal uptake, translocation, and detoxification/sequestration mechanisms need to be fully understood (Rascio and Navari-Izzo 2011).

10.3.1.2 Soil Factors Affecting Phytoextraction by Plants

Several edaphic factors affect the fraction of metals that is available for plant uptake such as total heavy metal content, sorptive capacity of soil, cation exchange capacity, soil pH, and organic matter content (Cheng 2003; Chaney et al. 2007; Bhargava et al. 2012). Soil temperature also influences metal accumulation by crops (Chang et al. 1987; Shah et al. 2010). Generally, only a small fraction of the total content is readily available (bioavailable), as most of the metals are commonly found as insoluble compounds or are strongly bound to the soil matrix (Lasat 2002; Bhargava et al. 2012).

The bioavailability of metals may be increased in soils in several manners, including natural processes such as the secretion of phytosiderophores into the rhizosphere to chelate and solubilize metals (Kinnersely 1993; Shah et al. 2010) or the acidification of the rhizosphere due to proton pumps and exudation of organic acids. Heavy metals move in the soil by two main processes: (1) diffusion along the concentration gradient that is formed due to uptake of elements and the consequent formation of a depletion zone in the vicinity of roots and (2) mass flow in the water that moves toward roots due to the water potential gradient. Secondarily, roots also intercept metals as they grow and displace soil particles (Marschner 1995; Shah et al. 2010).

The increased availability of metals at low pH has led phytoextraction researchers to study the incorporation of acidifiers (NH_4-containing fertilizers, organic and inorganic acids, and elemental S) into metal-contaminated soils to improve the success of phytoextraction (Bhargava et al. 2012). However, in spite of the promise of some acidifying agents, little research has been carried out on this subject, and this needs further investigation (Bhargava et al. 2012).

The organic content of the soil has a strong bearing on the extent of phytoextraction of heavy metals. Organic matter usually accumulates in the surface and subsurface layers of the soil profile present as either bulk materials or coating on particulate matter. In a study on metal adsorption in mineral–humic mixtures, it was shown that the amount of bound metals was greater when humic acids were present compared to the metal binding on the mineral alone (Kirkham 2006). The addition of peat and manure was reported to increase Cu, Zn, and Ni accumulation in wheat (Narwal and Singh 1998; Bhargava et al. 2012). Peat and manure are heterogeneous substances that can concurrently exert mobilizing and stabilizing effects (Schmidt 2003; Bhargava et al. 2012). Acidic peat reduces soil pH, which increases the concentration of soluble metals in the soil. Correspondingly, metal desorption from binding sites and release of metal ions into solution are usually facilitated by a low pH (Lasat 2000; Fitter and Hay 2002; Ali-Zade et al. 2010).

The oxidation state of a metal contaminant also determines its solubility and relative availability for uptake by plant systems. In general, the oxidized forms of most common metal contaminants are less soluble and consequently less available for plant uptake, chromium being the exception (Bhargava et al. 2012).

In addition, due to plant–soil interactions, the presence of microorganisms and release of root exudates may alter the availability of metals, particularly when soil pH changes significantly.

10.3.1.3 Chelate-Assisted or Induced Phytoextraction

Natural phytoremediation has some common drawbacks, such as low biomass and slow growth rate of the hyperaccumulators, and also the immobility or insolubility of the target metals in soil. Therefore, the most feasible strategy to develop a low-cost, environment-friendly remediation process involves the addition of substances to enhance metal availability and uptake by existing high-yielding crops. In the pursue of this strategy, chelant-induced phytoextraction, also called induced phytoextraction, has been proposed to overcome the low phytoavailability of heavy metals (Luo et al. 2005; Rahman et al. 2011a, b).

In induced phytoextraction, common crops with a large biomass are used and the availability of metals such as Pb, Cd, and Cr increased by the application of chelants to the contaminated soil (Shen et al. 2002; Leštan et al. 2008; Rahman et al. 2011a, b).

The properties of the soil in question and the nature of the chelant applied determine the proportion of bioavailable metal (Kos and Leštan 2004; Luo et al. 2005; Rahman et al. 2011a, b). The stability constant of the chelant–metal complex is the decisive feature to take into account when selecting a chelant or ranking different chelants to be used in assisted phytoremediation. The chemical characteristics of the chelant itself and the metal speciation in the soil matrix also influence the effectiveness of the process (Elliott and Brown 1989; Huang et al. 1997; Rahman et al. 2011a, b).

Several synthetic chelates are available such as ethylenediaminetetraacetic acid (EDTA), *S,S-ethylenediaminedisuccinic* acid (EDDS), trisodium nitrilotriacetate (Na_3NTA), *N-hydroxyethyl-ethylenediamine*-triacetic acid (HEDTA), ethylenediamine di-(*o*-hydroxyphenylacetic acid) (EDDHA), trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA), ethylene glycol-bis(β-aminoethyl ether), N,N,N',N'-tetraacetic acid (EGTA), and diethylenetriaminepentaacetic acid (DTPA). They all have been studied for the ability to mobilize metals and increase metal uptake in different plant species (Blaylock et al. 1997; Kayser et al. 2000; Kos and Leštan 2003; Ashraf et al. 2010; Rahman et al. 2011a, b).

Moreover, low-molecular-weight organic acids (LMWOAs) such as citric, oxalic, and malic acids have also been tested for their effectiveness in chelate-induced phytoextraction of metals (Wu et al. 2007).

Chelants, when added, form soluble complexes with metals via desorption and dissolution of precipitated compounds. The formation of these complexes prevents re-precipitation and resorption and enhances the bioavailability of the metals, overcoming the bioavailability barrier, thus rendering phytoremediation more feasible (Blaylock et al. 1997; Kayser et al. 2000; Kos and Leštan 2003; Wu et al. 2007; Ashraf et al. 2010; Rahman et al. 2011a, b).

In the past decade, chelant-enhanced phytoremediation received much attention from the scientific community. The most promising, effective, and, therefore, commonly used chelating ligand is EDTA (Nowack and VanBriesen 2005). EDTA has been most widely used because of its relatively low cost and toxicity and effectiveness at enhancing translocation of different metals (e.g., Pb) from roots to shoots (Lim et al. 2004; Wu et al. 2007). The addition of chelates to a Pb-contaminated soil (total soil Pb of 2,500 mg/kg) increased shoot Pb concentrations in *Zea mays* L. and *Pisum sativum* L. from less than 500 mg/kg to more than 10,000 mg/kg (Huang et al. 1997; Wu et al. 2007). The increase in Pb accumulation in these plants was associated with an increase in the level of Pb in soil solution resulting from the addition of chelates. A concentration of 1.5 % Pb in the shoots of *Brassica juncea* (L.) V.M. Czernajew was reported when this was grown in a soil containing 600 mg of Pb/kg and amended with EDTA (Blaylock et al. 1997). Enhancement of phytoremediation by EDTA addition has been reported for other heavy metals such as Cd (Bricker et al. 2001; Jiang et al. 2003), Cr (Shahandeh and Hossner 2000), Cu (Wenzel et al. 2003a, b; Wu et al. 2003), and Zn (Ebbs et al. 1997). Other aminopolycarboxylic acids have also been tested, but they were all less efficient than EDTA (Huang et al. 1997; Cooper et al. 1999; Chen and Cutright 2001; Wu et al. 2003; Wu et al. 2007).

LMWOAs such as citric, malic, oxalic, acetic, and malonic acids and histidine have also been studied as amendments for phytoremediation. For example, the addition of citric acid and its salts selectively increased uranium mobility in soil and subsequent plant uptake (Ebbs et al. 1997; Huang et al. 1997; Wu et al. 2007). Nigam et al. (2001) found that Cd accumulation in *Z. mays* corn was enhanced after applying malic and citric acids to a Cd-spiked soil.

The efficiency of the phytoextraction process depends also in large part on how the chelant is applied to the contaminated soil, either as a single dose after appropriate growth of the accumulator crop or in small multiple doses during the growth cycle (Wenzel et al. 2003b). The efficiency of the extraction is reportedly improved by the combined application of different chelants to the contaminated soil. A summary of the different combinations of chelants is available from Leštan et al. (2008).

Despite the success of this technology, the enhanced mobility of metals in soil following chelant application and their potential risk of leaching to groundwater remains an important concern (Kari and Giger 1995). The persistence of metal-EDTA complexes in contaminated soils is also a major drawback of this technology.

The success of phytoextraction relies on soil–metal–chelate–plant interactions, which will be affected by a variety of factors such as soil properties (e.g., cation exchange capacity, pH, and buffer capacity, among others), contaminant (e.g., metal species, distribution), chelate used (e.g., type, rate, and mode of application), plant (species, growth stage), and even the weather conditions when remediation takes place. To adjust these factors to ensure that phytoremediation is an effective technology in every occasion is a very difficult task. There are always compromises to be made between maximal effectiveness, maximal environmental merits, lowest risks, and lowest costs. Therefore, it is rather difficult to generalize on the prospects of chelate-enhanced phytoextraction. More fundamental research is needed to investigate the mechanisms of metal solubilization by selected chelants, the biogeochemistry of metal–ligand complexes, the mechanisms of plant uptake of chelated metals, and the effects of agronomic practices and to establish counter measures to reduce negative effects of chelates.

10.3.2 Phytofiltration

The availability and quality of aquatic resources is one of the most important challenges for humanity in the twenty-first century. Water contamination with diverse pollutants such as metals, metalloids, pesticides, pharmaceuticals, and their metabolites and other persistent organic pollutants has become a major public concern throughout the world (Schwarzenbach et al. 2010; Olguín and Sánchez-Galván 2012). The presence of metals in aquatic ecosystems, namely, ponds, rivers, and lakes, represents a potential risk for ecosystems and human health because of their toxicity, bioaccumulation, biomagnification, non-biological degradation, and persistence in the environment (Olguín and Sánchez-Galván 2012).

Some metal(loid)s that do not play any physiological role (i.e., Pb, Cd, As, Hg, among others) may have adverse effects on human health because they are risk factors for the development of neurodegenerative disorders, arthritis, and cancer (Barbosa et al. 2010; Olguín and Sánchez-Galván 2012).

Within phytoremediation processes, phytofiltration is gaining interest due to multiple advantages including its low cost (Olguín and Sánchez-Galván 2010; Lu et al. 2011; Olguín and Sánchez-Galván 2012). Phytofiltration involves the removal of pollutants from aqueous streams (water or wastewater) by young plant seedlings (blastofiltration) or roots of aquatic or terrestrial plants and their associated rhizospheric microorganisms (rhizofiltration) (Raskin et al. 1997; Dushenkov and Kapulnik 2000; Fulekar et al. 2009; Akpor and Muchie 2010; Bose et al. 2011; Bhargava et al. 2012; Olguín and Sánchez-Galván 2012; Ali et al. 2013). In phytofiltration, the contaminants are adsorbed or absorbed, i.e., plant roots precipitate and concentrate toxic metals from polluted effluents. The use of plant roots to absorb, concentrate, and precipitate heavy metals may provide a cost-effective method to treat water with concentrations of heavy metals that are too low for efficient removal by conventional methods while too high to allow discharge to the environment (Dushenkov et al. 1995; Salt et al. 1995; Lee and Yang 2010). Indeed, the biologically active, high surface area biofilter that plant roots represent can be extremely effective in sorbing pollutants from water.

Terrestrial rather than aquatic plants may also be used because the former have extensive fibrous root systems covered with root hairs with extremely large surface areas. Roots of many hydroponically grown terrestrial plants such as *B. juncea*, *Helianthus annuus* L., and various grasses have been used to remove toxic metals such as Hg, Cu, Cd, Cr, Ni, Pb, Zn, and uranium from aqueous solutions (Dushenkov et al. 1995; Dushenkov and Kapulnik 2000; Raskin and Ensley 2000; Moreno et al. 2008; Lee and Yang 2010). For example, roots of *B. juncea* removed up to 95 % of Hg when grown hydroponically by both volatilization and plant accumulation (Moreno et al. 2008).

A considerable number of publications have described the different aspects of biogeochemistry, mechanisms, and uptake of toxic elements by a large number of aquatic macrophytes to develop an effective phytoremediation technology. In aquatic phytoremediation systems, aquatic plants can be either floating on the water surface or submerged. Floating aquatic plants absorb or accumulate contaminants by their roots while submerged plants accumulate metals by all organs.

Many aquatic plants have the ability to remove heavy metals from water, including species such as *Lemna gibba* L., *Hydrilla verticillata* (L.f.) Royle, *Schoenoplectus californicus* (C.A.Mey.) Palla, *Typha angustifolia* L., *Typha domingensis* Pers., *Ipomea aquatica* Forsk, *Marsilea quadrifolia* L., *Hygroryza aristata* (Retz.) Nees., *Potamogeton oxyphyllus* Miq., *Baumea juncea* (R.Br.) Palla, *Baumea articulata* (R.Br.) S.T. Blake, *Schoenoplectus validus* (Vahl) A. Love & D. Love, *Juncus subsecundus* N.A. Wakef., *Eichhornia crassipes* (C.F.P. Mart.) Solms-Laub., *Pistia stratiotes* L., *Limnocharis flava* L., *Marsilea quadrifolia* L., *Hygroryza aristata* (Retz) Nees., *Potamogeton oxyphyllus* Miq., *Halimione portulacoides* L., *Juncus maritimus* Lam., and *Salvinia minima* Baker. These species have the ability to concentrate metal(loid)s from a liquid phase, such as Al, Zn, Cu, Cr, Pb, Cd, Hg, As, Cr, Co, Ni, and Mn (Sundberg-Jones and Hassan 2007; Sánchez-Galván et al. 2008; Wang et al. 2008; Abhilash et al. 2009; Khellaf and Zerdaoui 2009; Ghosh 2010; Xue et al. 2010; Zhang et al. 2010; Ahmad et al. 2011; Anjum et al. 2011; Giri and Patel 2011; Hegazy et al. 2011; Nguyen et al. 2011; Veselý et al. 2011; Xue and Yan 2011).

Other studies showed that *Microspora* spp. and *Lemna minor* L. were efficient plant species for Pb and Ni remediation (Axtell et al. 2003). *L. minor* was also reported to accumulate Cu and Cd from contaminated wastewater (Kara 2004; Hou et al. 2007; Rahman and Hasegawa 2011).

Myriophyllum aquaticum (Vell.) Verd., *Ludwigia palustris* (L.) Ell., and *Mentha aquatica* L. have also been reported to remove Fe, Zn, Cu, and Hg from contaminated waters (Kamal et al. 2004; Rahman and Hasegawa 2011). The submerged aquatic plant *Myriophyllum spicatum* L. was shown to be efficient in the removal of several metals from wastewaters (Lesage et al. 2007; Rahman and Hasegawa 2011), and *Rorippa nasturtium-aquaticum* L. and *Mentha* spp. could accumulate As from contaminated freshwaters (Robinson et al. 2006; Rahman and Hasegawa 2011).

In the process of attempting to improve rhizofiltration, it was discovered that young plant seedlings grown in aerated water (aquaculture) were often more effective than roots in removing heavy metals (Raskin et al. 1997), and the term blastofiltration (blasto from “seedling” in Greek) was applied to this technology (Raskin et al. 1997). Blastofiltration may represent the second generation of plant-based water treatment technologies. It takes advantage of the dramatic increase in the surface to volume ratio that occurs after germination and the fact that some germinating seedlings also absorb/adsorb large quantities of toxic metal ions. This property makes seedlings uniquely suitable for water remediation. Seedling cultures used for blastofiltration can be produced in light or in darkness, and seeds, water, and air are the only components required (Raskin et al. 1997).

The term phycoremediation is applied when microalgae, macroalgae, and cyanobacteria are used for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air. In both types of technologies, it is important to assess the fate of pollutants (i.e., metals) within the various compartments of the treatment system: roots, stems, fronds or leaves, microbial biomass, and water column (Olguín and Sánchez-Galván 2012).

10.3.3 Phytostabilization

Unlike phytoextraction, phytostabilization or phytoimmobilization aims not the removal but the stabilization of contaminants through the establishment of a plant cover on contaminated sites. This reduces the mobility of contaminants within the vadose zone, thereby reducing off-site contamination by wind erosion or leaching (Bolan et al. 2011).

In phytostabilization, plants and/or soil amendments are used to reduce the solubility or toxicity of contaminants, primarily through modification of the physicochemical conditions to reduce the soil labile metal pool, through accumulation by roots or immobilization within the rhizosphere (Berti and Cunningham 2000; Hooda 2007; Ashraf et al. 2010).

Plants immobilize metals through sorption by roots, precipitation, complexation, or changes in metal valence (Barcelo and Poschenrieder 2003; Ghosh and Singh 2005; Wuana and Okieimen 2011; Ali et al. 2013). Metals of different valences vary in toxicity; by excreting redox enzymes, plants skillfully convert hazardous metals to less toxic states. For example, reduction of Cr(VI) to Cr(III) has been widely reported, the latter being both less mobile and less toxic (Wu et al. 2010; Ali et al. 2013).

Phytostabilization has proved useful for the treatment of Pb, As, Cd, Cr, Cu, and Zn (Etim 2012) and has been successful in addressing metals and other inorganic contaminants in soil and sediments (USEPA 2001).

A typical scenario in which phytostabilization can be considered is represented by metalliferous sites (e.g., abandoned mining sites, smelter sites) where the presence of wastes and mine tailings results in severe pollution and poses aesthetic impacts on the local environment (Wong 2003; Marchiol et al. 2011). Thus, phytostabilization is usually applied on highly polluted areas where the removal of metals by phytoextraction would be too slow (Dickinson et al. 2009; Marchiol et al. 2011). Phytostabilization is also advantageous when decontamination strategies are impractical because of the extent of the contaminated area or the lack of adequate funding (Santibáñez et al. 2008).

Phytostabilization requires plant species with different characteristics than does phytoextraction. Although also tolerant to contaminants in the soil and with dense root systems, they should be poor translocators of metals to aboveground tissues. Fast-growing plants have the advantage of providing a good soil cover, but grasses and other herbaceous plants, shrubs, and trees have all been tested (González Chávez et al. 2006; Alvarenga et al. 2012; Santos et al. 2013).

There is a general agreement about the potential of native metallophytes for phytostabilization. Metalliferous soils are very restrictive habitats for plants due to the phytotoxicity which results in severe selection pressures. Moreover, those sites usually have low fertility and other characteristics that limit growth of cultivated crops. Therefore, in metal-polluted soils that did not undergo prior chemical stabilization, successful phytostabilization requires tolerant plants (Córdova et al. 2011).

Phytostabilization of metal-polluted sites can rely on spontaneous revegetation of native plants from the soil seed bank (Conesa et al. 2007; Córdova et al. 2011) or it can include seeding and/or transplanting of resistant plant species (Alvarenga et al. 2008; Córdova et al. 2011). Although the former is less costly, the latter offers quicker and more effective revegetation. Spontaneous revegetation would depend on the abundance and quality of the seeds present in the topsoil and the capability of local plant species to tolerate the conditions of the site. In particular, in metal-contaminated acidic soils, the addition of soil amendments may be required to partially immobilize metal(loid)s so that toxicity thresholds are not reached (Bolan et al. 2011).

Although this technology is effective in the containment of metal(loid)s, the site still requires regular monitoring to ensure that the stabilizing conditions are maintained. Soil amendments used to enhance immobilization may need to be periodically reapplied to maintain their effectiveness (Bolan et al. 2011).

Stabilization of inorganic contaminants by processes of adsorption, binding, or coprecipitation by amendments (Karami et al. 2011) has been widely reported in the last decade (Clemente and Bernal 2006; Pérez-de-Mora et al. 2006; Madejón et al. 2008; Karami et al. 2011). Of the numerous amendments used for in situ stabilization of contaminants, organic materials such as biosolids, manures, and composts, rich in organic matter, have proved successful at reducing the mobility of contaminants in multi-metal-polluted soils (Adriano et al. 2004; Clemente et al. 2006; Karami et al. 2011; Alvarenga et al. 2012; Santos et al. 2013).

Amendments decrease leaching and bioavailability of elements by promoting adsorption to mineral surfaces, formation of stable complexes with organic ligands, surface precipitation, and ion exchange. Precipitation of salts and coprecipitation can also contribute to reduce contaminant mobility. The techniques can be used in situ and ex situ to reclaim soils and/or revegetate degraded soils and mine spoils to improve soil quality and to reduce contaminant mobility (Kumpiene et al. 2008).

The most extensively studied amendments for As immobilization are Fe-containing materials. The immobilization of As occurs through adsorption of Fe oxides by replacing the surface hydroxyl groups with the As ions, as well as by the formation of amorphous Fe(III) arsenates and/or insoluble secondary oxidation minerals (Kumpiene et al. 2008). Cr stabilization involves mostly Cr reduction from its toxic and mobile hexavalent form Cr(VI) to the more stable and less mobile Cr(III). The reduction is accelerated in soils by the presence of organic matter and Fe(II).

Clays, carbonates, phosphates, and Fe oxides are some common amendments tested for Cu immobilization (Kumpiene et al. 2008). The suggested mechanisms of Cu retention are precipitation of Cu carbonates and oxyhydroxides, ion exchange, and formation of ternary cation–anion complexes on the surface of Fe and Al oxyhydroxides (Kumpiene et al. 2008). Most of the studies on Pb stabilization were performed using various phosphorus-containing amendments, which reduce the Pb mobility by ionic exchange and precipitation of pyromorphite-type minerals. Zn can be successfully immobilized in soil by phosphorus amendments and clays (Kumpiene et al. 2008).

The successful stabilization of multi-element contaminated sites depends on the combination of critical elements in the soil and on the choice of amendments (Kumpiene et al. 2008). Clays and Fe oxides seems to be efficient for all heavy metals (Kumpiene et al. 2008). However, clay-type materials can sometimes be alkaline, e.g., fly ashes, and have a reverse effect on the retention of As, while Fe added to soil in the form of sulfides can lower soil pH and have a reverse effect on the leaching of cationic elements (Kumpiene et al. 2008).

Organic matter presents the most variable impact on element mobility as the retention depends on several factors, such as soil pH and degree of humification, i.e., dominance of compounds rich in high-molecular-weight organic acids versus highly soluble low-molecular-weight acids (Kumpiene et al. 2008). For example, fresh biosolids usually have a low degree of humification and therefore contribute to mobilize rather than retain elements. Soil constituents, e.g., clay, can increase the retention capacity of organic matter by forming ternary clay–metal–organic matter complexes (Kumpiene et al. 2008).

Three elements, Cu, Zn, and Pb (all forming cations), have similar responses to phosphorus, iron, or clay materials (Kumpiene et al. 2008). However, when adding phosphoric acid or iron sulfate to soils, additional pH-controlling measures (e.g., liming) are necessary. Leaching of Cu, Zn, and Pb is strongly pH dependent, with the lowest mobility at neutral to slightly alkaline conditions. Therefore, particular attention should be paid to changes in soil pH induced by the amendments (Kumpiene et al. 2008). Some similarities are also seen in the response of As and Cr (which form oxyanions) to amendments. Alkaline materials clearly should be avoided as the mobility of these elements usually increases in this pH range. This confirms that pH is a very important factor with a strong impact on the mobility of elements in soils. Other factors, like the redox potential, can greatly modify element mobility, especially for redox-sensitive elements like As and Cr (Kumpiene et al. 2008).

Ideally, soil amendments should be easy to handle and to apply, harmless for workers handling them, nontoxic to plants, easy to produce, and inexpensive soil amendments that have little to no economic value (Berti and Cunningham 2000; Marques et al. 2009).

A range of organic and inorganic compounds (Adriano et al. 2004), such as lime, phosphate, and organic materials with little value like biosolids, litter, compost, and manure, can be used. Liming has been considered as an important management tool in reducing the toxicity of metals in soils (Gray et al. 2006; Pérez-de-Mora et al. 2006; Madejón et al. 2008; Marques et al. 2009). There is conclusive evidence for the mitigating value of both water-soluble (e.g., diammonium phosphate) and water-insoluble (e.g., apatite) phosphate to immobilize some metals in soils, thereby reducing their bioavailability for plant uptake (Brown et al. 1995; Marques et al. 2009). Phosphate enhances the immobilization of metals in soils through various processes, including direct metal adsorption, phosphate anion-induced metal adsorption, and precipitation of metals with soluble phosphate as metal phosphates (Adriano et al. 2004; Marques et al. 2009). For example, various phosphate compounds have been found to be very effective in the immobilization of Pb in soils (Arnich et al. 2003; Chen et al. 2003; Cao et al. 2009), and USEPA recommends this technique for the remediation of Pb-contaminated sites (Park et al. 2011; USEPA 2001). In fact, Bolan et al. (2003) reported that the sole application of lime or phosphate was effective in reducing Cd availability in contaminated soils. The use of organic amendments, such as manure (Clemente et al. 2006; Marques et al. 2009), compost (Cao and Ma 2004; Clemente et al. 2006; Marques et al. 2009), and other bio-wastes (Karaca 2004; Madejón et al. 2008), is a standing practice used for the restoration of contaminated sites. As examples, Walker et al. (2004) reported lower Zn tissue concentration in *Chenopodium album* L. when plants were grown in soils amended with compost or manure; Marques et al. (2009) showed that the sole application of manure or compost led to a significant reduction in the amount of Zn leached through the contaminated soil; Ye et al. (1999) observed that *Trifolium repens* L. tended to accumulate less Pb in the shoots when manure was added. In fact, organic matter amendments are among the most promising additives, especially due to their low commercial cost and the consequent added value of their application for soil remediation purposes. They provide organic matter to improve soil physical properties, water infiltration, and water holding capacity. They also contain essential nutrients for plant growth. Immobilization of metals by such amendments is achieved through adsorption, complexation, and redox reactions (Adriano et al. 2004). The addition of organic amendments has often been shown to increase the cationic exchange capacity (CEC) of soils due to the dissociation of H^+ from functional groups in organic matter (Zhu et al. 1991). The presence of phosphates, Al compounds, and other inorganic minerals in some organic amendments is also believed to be responsible for the retention of metals (Adriano et al. 2004; Marques et al. 2009). Additionally, amendments with organic matter and its resulting mineralization may change the soil pH and thereby indirectly affect the bioavailability of metals (Yoo and James 2002; Karaca 2004; Marques et al. 2009).

Research on soil remediation has included other types of amendments, such as cyclonic ashes (Ruttens et al. 2006), zeolites (Chlopecka and Adriano 1996), steel shots (Ruttens et al. 2006), beringite (Mench et al. 1994), red mud (also known as bauxite residue) (Gray et al. 2006), leonardite (Pérez-de-Mora et al. 2006; Madejón et al. 2008), and polyacrylate polymers (Qu and Varennes 2010; Qu et al. 2010; Varennes et al. 2011), with positive effects on the decrease of soluble forms of heavy metals.

Phytostabilization of mine tailings has been proposed as an alternative cost-effective remediation strategy to stabilize the metal contaminants and prevent human and animal exposure resulting from particle suspension (dust) in the atmosphere or dissolution in surface or groundwater (Santibáñez et al. 2008; Mench et al. 2010). The goal of phytostabilization is, in this case, to establish a self-sustaining plant community on the tailings (Santibáñez et al. 2008), to stabilize the surface, and to minimize wind and water erosion. The plants chosen should develop an extensive root system and produce a large amount of biomass in the presence of high concentrations of heavy metals while keeping the translocation of metals from roots to shoots as low as possible (Rizzi et al. 2004; Santibáñez et al. 2008) and thus prevent contamination of the food chain. Common chemical and physical limitations to plant growth on mine tailings include low pH, high salt content, lack of required nutrients, metal toxicities, high bulk density, lack of soil structure, slow water infiltration, low water retention, and low air permeability (Santibáñez et al. 2008). The mine-degraded soils usually have low concentrations of important nutrients like K, P, and N (Prasad et al. 2006), and toxic metals can also adversely affect the number, diversity, and activity of soil organisms, inhibiting soil organic matter decomposition and N mineralization processes.

To overcome these limitations, organic amendments may be added. Stabilized organic matter may contribute to increase metal complexation and provide adsorption sites, decreasing metal bioavailability.

The bioavailability of metal(loid)s is minimized by allowing them to react with the soil for a longer period (aging) or by adding soil amendments. A number of studies have documented the effect of aging on the immobilization of metal(loid)s in soils (Lothenbach et al. 1999; Park et al. 2011) and also the potential value of various organic and inorganic soil amendments to reduce the bioavailability of metal(loid)s (Ownby et al. 2005; Pérez-de-Mora et al. 2006; Paulose et al. 2007; Park et al. 2011).

Research has since shown that the use of biosolids and fertilizers is a key factor in phytostabilization of mine tailings to facilitate plant establishment and growth (Marchiol et al. 2011). The expected effect of soil amendment with biosolids is an increase of organic matter and water content of the soil and a rise of the pH (Chaney et al. 2007; Nwachukwu and Pulford 2008). Farrell et al. (2009) demonstrated that the addition of composted wastes to an acidic, heavy metal-contaminated soil greatly increased soil pH and plant growth while reducing the accumulation of available pollutants in the soil.

Plants are increasingly used, with the associated microbial community, to stabilize metal(loid)s in contaminated soils (i.e., phytostabilization) (Park et al. 2011). Recent studies have indicated that plant growth-promoting bacteria (PGPB) can improve revegetation and enhance plant establishment of arid mine tailings and metal-contaminated soils (Grandlic et al. 2008; Grandlic et al. 2009). PGPB may have the potential to cause changes in the microbial community structure that can be correlated with enhanced plant growth in metal-contaminated soils (Grandlic et al. 2009).

Trees are expected to be suitable for extensive and long-term phytoremediation or phytostabilization due to their extensive root systems and high transpiration rates (Pulford and Watson 2003; Van Nevel et al. 2011). In addition to the direct stabilization of the soil by root systems, the vegetation cover decreases the risk of soil loss by wind and water erosion. Leaf fall adds significant amounts of organic matter to the surface layers, promoting nutrient cycling and soil aggregation and increasing the water holding capacity, although it may increase the risk of spreading the heavy metals in the environment. The large amount of water removed from the soil by the transpiration stream decreases the downward flow through the soil, helping to reduce the amounts of heavy metals that are transferred to surface and groundwater (Pulford and Watson 2003; Mertens et al. 2004).

The risk of environmental hazard will be reduced by choosing tree species that do not accumulate heavy metals, because access to the contaminating metals will be reduced (Pulford and Watson 2003; Mertens et al. 2004). Selecting appropriate tree species is thus crucial for achieving a successful phytostabilization.

On the other hand, tree growth may enhance metal leaching due to soil acidification and production of dissolved organic matter. Hence, it is important to select tree species that do not acidify the soil and keep the metals mostly in their root systems with little translocation (Mertens et al. 2004).

In conclusion, some of the advantages associated with this technology are that the disposal of hazardous materials is not required, and it is very effective when rapid immobilization is needed to preserve surface and groundwater. The presence of plants reduces soil erosion and decreases the amount of water available in the system and limits the accumulation of heavy metals in the biota. However, this cleanup technology has major disadvantages: the contaminants remain in the soil, application of fertilizers and amendments is often needed, and mandatory monitoring is required (Etim 2012). This technique can be adapted to a variety of sites and situations, with different conditions (e.g., soil pH, salinity, soil texture, metal levels, and contaminant types) through the careful selection not only of the appropriate plant species but also of the amendments (Berti and Cunningham 2000; Marques et al. 2009). There are thus two major components in the phytostabilization process: the plant itself and the amendments added to the system (Marques et al. 2009). Some plants have a natural ability to immobilize pollutants by providing a region around the roots where these pollutants can be precipitated and stabilized, i.e., alter the speciation of soil metals. Therefore, plants reduce the mobility and bioavailability of pollutants in the environment either by immobilization or by prevention of migration, thus rendering them harmless and less mobile and bioavailable (Raskin et al. 1997; Prasad et al. 2006; Ashraf et al. 2010; Yao et al. 2012). For instance, species tolerant to low pH such as *Cynodon dactylon* (L.) Pers., *Juncus usitatus* L.A.S. Johnson, and *Lomandra longifolia* Labill. were identified as having the potential to be used in phytostabilization programs (Liu et al. 2011). Metal-tolerant species, e.g., *Carduus pycnocephalus* L., *Dasypyrum villosum* L. P. Candargy, *Ferula communis* L., *Silybum marianum* (L.) Gaertn., *Sinapis arvensis* L., and *Stipa austroitalica* Martinovsky also promote metal stabilization and soil conservation as a result of their excluder behavior (Brunetti et al. 2009). Moreover, pioneer plants can be ideal species for the phytostabilization of mine tailings (Liu et al. 2011), e.g., *Atriplex halimus* L. *subsp. schweinfurthii* has the potential for phytostabilization of Cd-contaminated saline soils (Nedjimi and Daoud 2009).

Jatropha curcas L. is another candidate to cover and reclaim metalloid and metal-contaminated soils (Yadav et al. 2009), and *Alnus rugosa* (Du Roi) Spreng. was used in tailings and to reestablish forest ecosystems (Mehta 2005).

Recently, Zou et al. (2011) reported that *Athyrium wardii* (Hook.) H. Christ has the potential to stabilize Pb-contaminated soils.

For phytostabilization of metals, a combination of trees and grasses may work best. Fast-transpiring trees maintain an upward flow to prevent downward leaching, while grasses with their dense root systems prevent wind erosion and lateral runoff. In addition, grasses do not accumulate metals in their shoots to such an extent as do dicots, thus minimizing exposure of wildlife to toxic elements (Pilon-Smits 2005; Hooda 2007).

10.3.4 Phytovolatilization

While elements like Cd, Cr, Co, Cu, Ni, Pb, and Zn can be treated by phytostabilization or phytoextraction, As, Hg, and Se can be removed by phytovolatilization because they can form volatile chemical species through reduction and methylation reactions (Nabais et al. 2007; Wu et al. 2007).

Phytovolatilization involves the uptake of contaminants from soil or water and their transformation into volatile compounds and transfer into the atmosphere (Ashraf et al. 2010). This technique is less useful for removal of heavy metals as these must (1) be taken up by plants through roots, (2) pass through the xylem to the leaves (3), be converted into volatile compounds, and (4) volatilize to the atmosphere. Despite these limitations, this technique has been reported to be useful for the removal of Hg from polluted soils. Mercury is regarded as one of the "priority hazardous substances" by the Agency for Toxic Substances and Disease Registry (ATSDR) because of its toxicity, mobility, and long residence time in the atmosphere (Wang et al. 2012). Once Hg is released into the atmosphere, it can be retained for between 6 and 24 months and be transported over tens of thousands of kilometers before eventual redeposition on the Earth's surface (Dastoor and Larocque 2004). Both natural and anthropogenic activities emit Hg to the atmosphere (Selin 2009).

Leonard et al. (1998) studied the exchange flux of Hg between aerial plant parts and the atmosphere. Four plant species (*Lepidium latifolium* L., *Caulanthus* sp., *Fragaria vesca* L., *Eucalyptus globulus* Labill.) were grown in a soil contaminated with Hg (concentration ranging between 450 and 1,605 mg kg^{-1}). *Caulanthus* sp. had the highest rate of Hg emission during the day (92.6 ng m^{-2} h^{-1}) compared with the other plant species. Emissions in the dark were an order of magnitude lower than during the day for all plant species.

Another alternative for phytovolatilization is the use of genetic engineering to integrate genes from other organisms to enhance the phytovolatilization capabilities of plants (Wang et al. 2012). Transgenic *N. tabacum* carrying the bacterial detoxification genes *merA* and *merB* was able to remove Hg from polluted soils (Rugh et al. 1996, 1998; Bizily et al. 1999, 2000; Ashraf et al. 2010). The gene *merA* encodes mercuric ion reductase that reduces ionic mercury (Hg^+) using NADPH. In this process, Hg^+ is transformed into methylmercury (CH_3Hg^+) and phenylmercuric acetate (PMA) and finally to metallic elemental mercury ($Hg^{(0)}$) that is volatile at room temperature (Ashraf et al. 2010). The gene has been successfully used in the genetic engineering of plants for the remediation of Hg (Ruiz and Daniell 2009), and several studies have shown that both dicotyledon and monocotyledon plants with the *merA* gene, such as *Arabidopsis thaliana* (L.) Heynh., *Liriodendron tulipifera* L., *Arachis hypogaea* L., *Populus deltoids* W. Bartram ex Marshall, *Oryza sativa* L., *Spartina alterniflora* Loisel, and *Chlorophyta* spp., have been shown to be resistant to levels of up to 0.04–100 mM $HgCl_2$ (Rugh et al. 1996, 1998; Heaton et al. 2003; Czako et al. 2006; Huang et al. 2006). Unfortunately, the *merA* gene alone fails to protect against the more toxic and environmentally relevant organic Hg (Ruiz and Daniell 2009; Wang et al. 2012). Both the *merA* and the *mer*B genes are needed to protect cells from organic Hg (Ruiz and Daniell 2009; Wang et al. 2012). *A. Thaliana* carrying both genes resisted up to 5 mM of phenylmercury acetate (PMA) and 10 mM CH_3Hg^+ (Bizily et al. 2000). Hussein et al. (2007) reported that transgenic *N. tabacum* carrying the *merA* and *mer*B genes was able to absorb more Hg than its wild-type counterpart.

Nevertheless, phytovolatilization of Hg may cause secondary contamination of the environment with Hg^0. For this reason, another alternative to promote a higher efficiency of phytoextraction could be the expression of other *mer* genes in plants so that they could accumulate Hg without releasing Hg^0 into the atmosphere (Wang et al. 2012). Besides the genes *merA* and *mer*B, genes *mer*C, *mer*F, and *mer*T are known as membrane transporter genes involved in the uptake of Hg^{2+} (Morby et al. 1995; Liebert et al. 2000; Wilson et al. 2000). Plants transformed with these genes have a greater capacity to accumulate Hg in their tissues than do wild-type plants (Wang et al. 2012). For example, *A. thaliana* and *N. tabacum* carrying the *mer*C gene accumulate approximately twice as much Hg^{2+} ion as the wild-type plants (Sasaki et al. 2006).

The volatilization of Se by plants and soil microbes is also an important component of phytoremediation. Plants take up Se mostly as selenate, selenite, and/or organic Se. The uptake of selenate and organic Se is driven metabolically, whereas the uptake of selenite may have a passive component (Zayed et al. 2000). Selenate is highly bioavailable, and it is the soluble form of Se commonly found in soils and subsurface drainage waters.

Volatile Se compounds (mainly dimethyl selenide and dimethyl diselenide) form through biomethylation, a biological process in which microorganisms and/or plants convert inorganic Se into methylated volatile Se compounds (Bañuelos and Lin 2007). The final methylated selenide compounds are subject to volatilization into the atmosphere.

Plant species differ substantially in their ability to take up and volatilize Se. Plant species from the Brassicaceae family were particularly effective Se volatilizers (of the top six species, only rice was not a member of this family) (Zayed et al. 2000). It seems that the ability to volatilize Se is associated with the ability to accumulate Se in plant tissues—the rate of Se volatilization by different plant species was strongly correlated with the plant tissue Se concentration (Terry and Gary 2000; Zayed et al. 2000).

Terry et al. (1992) reported that members of the Brassicaceae are capable of releasing up to 40 g Se ha^{-1} per day as various gaseous compounds. Some aquatic plants, such as *Typha latifolia* L., also seem to have potential for Se phytoremediation (Pilon-Smits 2005). Moreover, recent genetic and genomic studies have identified new genes involved in Se tolerance. Such key genes could be the ultimate candidates for overexpression, producing the complete Se hyperaccumulator profile in high-biomass crop species (Pilon-Smits and LeDuc 2009).

Various technologies are available to remediate soils contaminated with As, including phytovolatilization. Plants absorb As through the phosphate uptake pathway, i.e., active apoplastic or symplastic mechanisms, and translocate to the aboveground parts (shoots and leaves). The amount of arsenic translocated from roots to shoots indicates the phytoremediation efficiency of that plant, but most plants store more than 90 % of the As in their roots. Few plants have the ability to translocate high amounts of As (Rahman et al. 2011a, b).

Pteris vittata L. has the highest ability to accumulate and translocate As (Ma et al. 2001), and it is considered an efficient As hyperaccumulator also capable of volatilizing As.

In conclusion, phytovolatilization uses plants that remove volatile contaminants (e.g., Hg, As, and Se) from terrestrial or aqueous systems and facilitate their conversion to volatile forms for release to the atmosphere, redistributing the pollutant in a much larger land area where its concentration does not comprise risk. However, phytovolatilization should be avoided in sites near population centers and in places with unique meteorological conditions that promote the rapid deposition of volatile compounds. Hence, the consequences of releasing the metals to the atmosphere need to be considered carefully before adopting this method as a remediation tool.

10.4 Conclusions and Future Perspectives

The contamination of soils and waters with toxic metals has become one of the most important environmental problems throughout the world. Metal toxicity affects crop yields, microbial biomass, and soil fertility. Soils polluted with heavy metals pose a serious health hazard to humans as well as plants and animals and often require soil remediation practices. Physical and chemical methods for cleanup and restoration of heavy metal-contaminated soils have serious limitations like high cost, irreversible changes in soil properties, destruction of native soil microflora, and creation of secondary pollution problems. In contrast, phytoremediation is a competitive and sustainable solution, with good public acceptance, that offers a cost-effective means for cleaning metal-contaminated soils. Since the last decade, phytoremediation gained acceptance and has been acknowledged as an important area of research. Phytoremediation's basic processes require further basic and applied research to optimize field performance. In order to exploit its full potential, a comprehensive understanding is needed on soil chemistry, plant molecular biology, plant biochemistry, plant physiology, ecology, and soil microbiology as well as environmental engineering. Information collected from basic research at physiological, biochemical, and genetic levels in plants will be helpful to understand the processes of passive adsorption, active uptake, translocation, accumulation, and chelation mechanisms. Research aimed at understanding the interactive roles among plants roots and soil microorganisms will help scientists to utilize their integrative capacity for soil decontamination. Genetic evaluation of hyperaccumulators growing in metal-contaminated soils and associated microorganisms may reveal key control genes that trigger the cascade of responses that provide tolerance and accumulation. Such genes could be the ultimate candidates for overexpression, producing the complete hyperaccumulator profile in high-biomass crops.

The discovery of metal-related genes with the aid of genome sequencing will open up new avenues for the creation of transgenic plants having desired properties that would help in establishing phytoremediation technologies for environmental cleanup.

Additional considerations for the use of transgenic plants for phytoremediation are the same as those involved with growing transgenic crops for other purposes and should also be evaluated and weighed against the risks of alternative remediation methods.

In view of the current trends of integration of scientific knowledge worldwide, it is hoped that many challenging questions about commercial application of phytoremediation will be also answered in the future.

To improve phytoremediation, a number of improvements in agronomic practices are also promising, ranging from traditional crop management techniques (use of pesticides, soil amendments, fertilizers, etc.) to more specific approaches such as improving metal solubility in soils through the use of chelators.

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Part III

Phytoremediation Applications for Metal Contaminated Soils Using Terrestrial Plants

Phytoremediation of Agricultural Soils: Using Plants to Clean Metal-Contaminated Arable Land

11

Sarah Neilson and Nishanta Rajakaruna

11.1 Introduction

Industrial pursuits such as mining and manufacturing produce large amounts of heavy metal pollution worldwide (Anderson et al. 2005; Sánchez 2008; Wuana and Okieimen 2011). Heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) and metalloids such as arsenic (As) and selenium (Se) not only pollute soils in the immediate vicinity within which they are produced but can be easily dispersed via air and water, leading to contamination of soils far from the source of the pollutants (Neilson and Rajakaruna 2012). Heavy metal contamination poses a significant threat to arable land around the world (Efremova and Izosimova 2012a, b; Liu 2006; Lin et al. 2012; Lone et al. 2008; Mico et al. 2006), reducing the availability of land suitable for producing the global food supply. Unsound agricultural practices that intensify contamination of soils by heavy metals further exacerbate this problem, making it challenging to produce food safe for human or animal consumption. As the amount of land available for agriculture decreases (Lone et al. 2008), the need for more land, or at least better use of currently available arable land, increases. According to Tang et al. (2012), one fifth of agricultural land in China is already contaminated despite such land being exploited for food production. Therefore, it is critical to consider what measures can be taken to grow food safe for consumption, even on arable land that may have varying levels of heavy metal contamination (Adefemi et al. 2012; Nicholson et al. 2003).

In this chapter, we explore methods for utilizing heavy metal-contaminated soils for agricultural production. Such methods include phytoextraction, or remediating contaminated soils using plants that hyperaccumulate heavy metals (van der Ent et al. 2012; Gall and Rajakaruna 2013); phytomining (Chaney et al. 2007), where metal-hyperaccumulating plants are harvested for disposal or extraction of the metal from processed tissues; phytostabilization (Mendez and Maier 2008), in which plant roots immobilize or take up metals to reduce or eliminate the threat of leaching; sound agricultural practices including the use of soil amendments such as lime to increase pH levels to make heavy metals less bioavailable; and non-remediation options such as growing fuel or fiber crops rather than food crops on metal-contaminated land, growing food plants which translocate little or no contaminants to edible tissues, or using raised beds so that the plant roots are not in immediate contact with contaminated soil. First, we consider the potential dangers posed to plants, humans, livestock, and ecosystems by heavy metal contamination. We then explore some of the sources of heavy metals commonly found in arable lands and methods for assessing heavy metal concentrations and bioavailability in soils and the potential for uptake of heavy metals into the tissues of crop plants. We also discuss potential methods for utilizing heavy metal-contaminated soils for agricultural production, including both remediation- and non-remediation-based approaches. We finish with a brief consideration of the potential for biotechnology to offer solutions to the problem of growing crops on arable lands contaminated by heavy metals.

11.2 Dangers of Heavy Metal Contamination

The presence and bioavailability of heavy metals may vary from site to site, depending upon the chemical form of the individual metal and the chemical and physical attributes of the soil (Rajakaruna and Boyd 2008). High concentrations of heavy metals in soil can negatively affect plant growth, as these

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metals interfere with physiological and biochemical processes, including photosynthesis and respiration, contributing to the degeneration of organelles and cells, and even plant death (Nagajyoti et al. 2010; Gupta and Sandallo 2011; Shaw 1990). Heavy metal contamination in soils may also cause changes in the composition of the soil microbial community, potentially adversely affecting soil characteristics (Giller et al. 1998; Kozdrój and van Elsas 2001) and thereby also potentially impacting plant growth and crop productivity. Consumption of plants with high heavy metal concentrations may lead to toxicity in humans and other animals and can cause acute or chronic illnesses and even death (Appenroth 2010; Bánfalvi 2011).

The specific dangers of heavy metal contamination in plants, animals, humans, and ecosystems can take many forms (Boyd and Rajakaruna 2013). Cadmium, for example, is one of the most toxic heavy metals that can enter the food chain when accumulated in edible plant tissue (Singh et al. 2011). Cadmium is also the most mobile heavy metal, with significantly increased mobility at even slightly acidic pH levels (<6.5) (Felix-Henningsen et al. 2010). Cadmium, along with Zn, can lead to acute gastrointestinal and respiratory problems and can contribute to acute heart, brain, and kidney damage (Anderson et al. 2005). People who have nutritional deficiencies—especially people with low levels of calcium (Ca), iron (Fe), and Zn—are more prone to Cd-related disorders such as kidney failure or bone demineralization and fracturing (Singh et al. 2011).

Heavy metals can be transferred through soil-plant-animal food chains and accumulate in plant and animal tissues, a process called bioaccumulation (Gall and Rajakaruna 2013). For example, Nica et al. (2012) found that Cd, Cu, Pb, and Zn transferred from contaminated soil to *Urtica dioica* (nettle; Urticaceae) leaves to *Helix pomatia* (Roman snail; Helicidae), affecting shell height and whorl number, while also accumulating in the muscular foot. Miranda et al. (2009) found that cattle raised on Ni-enriched serpentine soils in Spain accumulated toxic levels of Ni in their kidneys and of Cu in their livers. The authors found a direct correlation between concentrations of Ni in the kidneys of cattle and both total and extractable levels of Ni found in the soil and forage. There is much evidence that toxic amounts of heavy metals can enter the food chain through contaminated soils (Gall and Rajakaruna 2013), necessitating research into ways in which heavy metal-contaminated soils can be safely used for agricultural production.

11.3 Sources of Contamination and Common Contaminants

Contamination of agricultural soils by both natural and anthropogenic sources of heavy metals is a global problem (Arao et al. 2010; Efremova and Izosimova 2012a; Kien et al. 2010; Li et al. 2008; Lone et al. 2008; Rahman et al. 2012). Agricultural soils can become contaminated via atmospheric deposition of heavy metals from factories, vehicular exhaust, and other sources; application of chemical and organic fertilizers (pig slurries, manures, sewage sludge) and pesticides; and weathering of rocks containing high levels of heavy metals (Singh et al. 2011; Qishlaqi and Moore 2007; Rajakaruna and Boyd 2008). France, Belgium, and the Netherlands all suffer from heavy metal contamination of agricultural soils due to smelter emissions. In China, 13,330 ha of farmland are contaminated by Cd (Singh et al. 2011). In Western Europe, approximately 1,400,000 sites have been contaminated with heavy metals (Wei et al. 2005). In the country of Georgia, irrigation water has been contaminated with heavy metal-laden mining waste for several decades (Felix-Henningsen et al. 2010). In the United States, there are 600,000 brownfield sites contaminated with heavy metals and need reclamation (McKeehan 2000).

The dangers posed by each heavy metal are variable. For example, Cu, Molybdenum (Mo), and Zn are essential, in small quantities, for humans, animals, and plants. However, As, Cd, Hg, and Pb are not essential and can cause toxicity even at very low concentrations (Singh et al. 2011). Cadmium, Cr, Cu, Ni, Pb, and Zn are all known to be harmful to human and ecosystem health when they enter the food chain (Singh et al. 2011; Felix-Henningsen et al. 2010; Qishlaqi and Moore 2007). Lead, for instance, can remain in the soil for thousands of years (Kumar et al. 1995) and is known to cause cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, and renal impairment in humans (Flora et al. 2012; Patrick 2006).

11.4 Assessing Soil Conditions and Potential for Crop Contamination

The risks of heavy metals being transferred into the food chain are dependent on the mobility of the heavy metal species in question and its bioavailability in soil (Richards et al. 2000; Rajakaruna and Boyd 2008). The processes of uptake and accumulation of heavy metals by plants are influenced by several soil factors, including pH, redox potential, clay content, soil organic matter (SOM) content, cation exchange capacity (CEC), nutrient balance, concentrations of other trace elements in soil, soil moisture (and aeration), and soil temperature (Neilson and Rajakaruna 2012; Gall and Rajakaruna 2013; Qishlaqi and Moore 2007; Singh et al. 2011; Tang et al. 2012). Two of the most important factors affecting the bioavailability of heavy metals are pH and SOM (Felix-Henningsen et al. 2010; Guo et al. 2011; Puschenreiter et al. 2005). Soil pH is important because most heavy metals, including Cd, Cr, Cu, Ni, Pb, and Zn, become more bioavailable under acidic (low pH) soil conditions (McLaughlin 2002; Qishlaqi and Moore 2007; Rajakaruna and Boyd 2008).

Acidic soils have a higher amount of hydrogen ions (H^+) compared to alkaline soils, and cationic metals become more bioavailable under acidic conditions because they are displaced from negatively charged binding sites of soil particles such as clay and SOM by the abundant H^+ in the soil solution. In some cases, however, a decrease in soil pH may not necessarily result in an increase in metal bioavailability. Molybdenum in soil, which is in the form MoO_4^{2-}, is less soluble under low pH levels (Kabata-Pendias and Pendias 2001); similarly, anionic forms of some heavy metals may become more bioavailable under increased pH levels.

Soil organic matter, including humic compounds, bears negatively charged sites on carboxyl and phenol groups, allowing for metal complexation (Brady and Weil 2007). Thus, the presence of high amounts of SOM is often negatively correlated with plant metal uptake. Cation exchange capacity, a function of clay and organic matter content in soil, also controls the bioavailability of heavy metals. In general, an increase in CEC causes a decrease in the uptake of heavy metals by plants. Bioavailability of heavy metals in soil is also directly correlated with the redox potential; under similar pH values, heavy metal bioavailability generally increases as redox potential decreases (Yaron et al. 1996). Heavy metals are also more available in sandy soils than in clayey soils, as sand particles have a much lower surface area and contain fewer cation exchange sites relative to clay particles. Soil drainage is another critical factor affecting the bioavailability of heavy metals. Drainage improves soil aeration and allows for metal oxidation, often causing heavy metals to be less soluble and therefore less bioavailable. However, some heavy metals, such as Cr, can be more available in oxidized forms (Chattopadhyay et al. 2010). Finally, absorption of heavy metals by roots is controlled by the concentration of other elements in the soil solution (Taiz and Zeiger 2010). Such relationships may be positively or inversely correlated, with the uptake of a heavy metal being enhanced or suppressed by the concentration of other elements in the soil.

Heavy metals frequently interact strongly with the soil matrix, and soil conditions can greatly influence heavy metal availability. Assessments of the suitability of soils for agricultural production should take into account any potentially available heavy metals (i.e., heavy metals that are strongly bound to free Fe- or Mn-oxides or to organic substances or carbonates) as this fraction of the heavy metal pool is most likely to be mobilized with changes in soil factors that influence adsorption of heavy metals onto soil particles (i.e., pH, SOM, CEC, redox potential, aeration, clay content, etc.; Felix-Henningsen et al. 2010). Adding alkaline materials such as lime, tillage and rotation practices, and fertilizer management all contribute to changing physicochemical factors that could directly impact the bioavailability of heavy metals in agricultural soils (Lasat 2000; Singh et al. 2011).

The chemical behavior of heavy metals in agricultural soils is not only influenced by factors such as pH, SOM, CEC, redox potential, clay, and other elemental interactions but also by biotic factors such as morphological and physiological characteristics of crop species and the microbial community within the crop rhizosphere (Singh et al. 2011). Hence, when assessing the potential for agricultural production in heavy metal-contaminated soils, it is important to make decisions based on both the abiotic and biotic factors affecting heavy metal bioavailability. For example, one could plant a heavy metal hyperaccumulator to remove contaminants, choose crops which exclude heavy metals or do not translocate them in toxic amounts to edible parts, grow crops for fiber and fuel instead of food, or pursue a combination of such options. These approaches will be further explored in the following section.

11.5 Remediation Options

The use of metal-hyperaccumulating plants should be explored as a viable option to remediate agricultural soils moderately contaminated with heavy metals. Of the approximately 582 species of known metal hyperaccumulators from more than 50 families of vascular plants worldwide, approximately 25 % belong to the Brassicaceae (Gall and Rajakaruna 2013), a family containing many food crop species. There are well over 400 hyperaccumulators of Ni globally, as well as two dozen or so for Cu; at least a dozen each for Co, manganese (Mn), and Zn; and a few for As, Cd, Cr, Pb, Se, thallium (Tl), and uranium (U) (Cutright et al. 2010; Islam et al. 2007; Li et al. 2011; van der Ent et al. 2012). Although metal hyperaccumulators have the ability to detoxify and accumulate metals in their leaf tissues, they do have limits to this extraordinary capacity to deal with metals, and the threshold for hyperaccumulation depends on the metal under consideration. The threshold concentration for hyperaccumulation is generally two or three orders of magnitude greater than that of most species growing on "normal" soils and at least one order of magnitude greater than the usual range found in other plants from metal-enriched soils. Hyperaccumulators of Cd, Se, and Tl accumulate >100 $\mu g\ g^{-1}$ in their dry leaf tissue; hyperaccumulators of cobalt (Co), Cr, and Cu accumulate >300 $\mu g\ g^{-1}$ in their dry leaf tissue; and hyperaccumulators of As, antimony (At), Ni, and Pb accumulate >1,000 $\mu g\ g^{-1}$ in their dry leaf tissue. Hyperaccumulators of Zn accumulate >3,000 $\mu g\ g^{-1}$, whereas those of Mn accumulate >10,000 $\mu g\ g^{-1}$ in their dry leaf tissue (Reeves and Baker 2000; van der Ent et al. 2012). For recent reviews of metal hyperaccumulation, see Krämer (2010) and van der Ent et al. (2012).

Metal-hyperaccumulating species such as *Noccaea caerulescens* and *N. rotundifolia* (Brassicaceae), *Haumaniastrum robertii* (Lamiaceae), *Ipomoea alpina* (Convolvulaceae), *Virotia* (formerly, *Macadami*a) *neurophylla* (Proteaceae), and *Psychotria douarrei* (Rubiaceae) are good candidates for removing metals from agricultural soils (Islam et al. 2007). A common condiment crop in North America and Europe, *Brassica juncea* (Indian mustard; Brassicaceae), is also a popular choice for phytoremediation (Lim et al. 2004; Neilson and Rajakaruna 2012). Although not a hyperaccumulator, with the ability to accumulate Cd, Pb, Se, and Zn and a biomass at least tenfold greater than that of *N. caerulescens*, *B. juncea* has been used with success in several phytoremediation studies and field trials (Bhargava et al. 2012; Szczyglowska et al. 2011; Warwick 2011). Although Pb hyperaccumulation is rare, several species, including *Apocynum cannabinum* (hemp dogbane; Apocynaceae), *Ambrosia artemisiifolia* (common ragweed; Asteraceae), *Carduus nutans* (nodding thistle; Asteraceae), and *Commelina communis* (Asiatic dayflower; Commelinaceae), appear to accumulate high levels of Pb in their leaf tissues (Lasat 2000) and could be used for phytoremediation in agricultural settings. Recent experiments have shown that some populations of *N. caerulescens* from southern France can accumulate Pb at >1,000 μg g^{-1} in leaf dry matter in the field and under hydroponic conditions (Mohtadi et al. 2011). Similarly, Pb accumulation was documented in 26 taxa collected from a Pb mine in Thailand (Rotkittikhun et al. 2006). High foliar concentrations of Pb can be achieved with the use of Pb-complexing and mobilizing agents such as EDTA and EDDS ("induced phytoextraction" sensu Salt et al. 1998), as documented for *Brassica carinata* and *B. juncea* grown in hydroponic solution or in EDTA-treated soil (Kumar et al. 1995; Vassil et al. 1998).

Hyperaccumulators can selectively accumulate heavy metals in their aerial parts at 10 to 500 times the level of heavy metals found in edible crops (Islam et al. 2007), making them model species for remediation of contaminated agricultural soils (but see Vamerali et al. 2010). However, not all regions of the world have native species that hyperaccumulate the various heavy metals of concern, making it unsafe or unsuitable to utilize non-native hyperaccumulators for agricultural purposes (see Neilson and Rajakaruna 2012). Hyperaccumulators are not edible and must be considered toxic waste unless the metals are to be extracted via phytomining (Wilson-Corral et al. 2012) or tissue can be used as fertilizer to treat micronutrient deficiencies in crop plants (Wood et al. 2006). Thus, other remediation options, such as those that can be combined with agricultural production, may be more appealing in agricultural settings.

Many options are available for crop production on soils contaminated with heavy metals. Appropriate techniques for growing crops on heavy metal-contaminated soils depend primarily on the concentration of metals in the soil. The approach to remediation of heavy metal contamination should be risk-based in the sense that if metals are tightly bound to soil particles and are not bioavailable, as determined with bioassays and other toxicity assays, it may not be necessary to remove heavy metals from the soil entirely. Singh et al. (2011) describe "gentle" and "harsh" remediation techniques which are further categorized into soil-specific and plant-specific approaches. Gentle techniques are mainly geared toward stabilizing heavy metals, and "harsh" techniques are mainly geared toward removal of heavy metals from the soil. One of the most common gentle soil-specific approaches is the addition of alkaline soil amendments such as lime (i.e., liming). In situ stabilization using lime, organic matter, phosphates, and mineral oxides is aimed at reducing the bioavailability of heavy metals and thereby reducing transfer of these metals into food crops. However, such approaches are dependent on the crop species or cultivar used, soil conditions, and agricultural practices (Singh et al. 2011). Liming can reduce mobility and the soluble fraction of Cd, Ni, Zn, and other heavy metals, but results are variable and over-liming can reduce the availability of essential nutrients such as Fe and Zn (Singh et al. 2011). Liming can also influence microbial activity in the soil leading to increases or decreases in metal availability (Weyman-Kaczmarkowa and Pedziwilk 2000). Adsorption agents with high CEC, such as clay, can also increase the metal-binding capacity of soil (Singh et al. 2011). Other soil amendments that can decrease solubility or precipitate metals include SOM and phosphate- or silicon-based amendments (Tang et al. 2012). Silicon has been shown to decrease toxic effects of heavy metals on plants and can be applied as a foliar spray. Phosphate can immobilize Pb by binding or precipitation due to its negative charge, although care should be taken when using phosphate fertilizers as they can contain up to 300 mg Cd kg^{-1} dry product and can cause Cd to accumulate in soils if applications are too frequent or their rates are too high (Grant 2011). Organic materials such as manure can have dual effects of increasing yield and decreasing metal uptake (Tang et al. 2012). Rhizospheric interactions and root activity can also reduce the solubility and transport of heavy metals. For a detailed review of rhizospheric interactions, see Neilson and Rajakaruna (2012). See Tang et al. (2012) for a review of agricultural practices that can reduce metal contamination.

A novel approach to removing heavy metals from soil described by Guo et al. (2011) consists of "washing" contaminated soil with a mixture of acidic chelators, including EDTA, citric acid, and KCl, followed by the application of lime. In this study, *Zea mays* (corn; Poaceae) was planted following initial soil washing to assess the bioavailability of heavy metals after the treatment with chelators, and after the corn was harvested, *Sedum alfredii* (Crassulaceae) was used for phytoextraction to further reduce heavy metals in the soil. This method turned out to be somewhat ineffective, as levels of Cd, Pb, and Zn still exceeded Chinese food safety limits, and liming the washed soil significantly increased heavy metal leaching and failed to reduce Pb and Cd concentrations in the corn. However, levels of Cd, Cu, Pb, and Zn were all increased in the phytoextractor *S. alfredii* after soil washing (Cd and Zn concentrations increased by almost 50 % compared to unwashed soils). Therefore, phytoextraction by *S. alfredii* after soil washing may be effective at removing the mobile Cd activated by the soil washing (Guo et al. 2011). Interestingly, they found that although the liming treatment and phytoextraction by itself successfully reduced exchangeable fractions of heavy metals in the soil, this was not the case when the two approaches were combined. Liming is most effective at decreasing metal concentrations in crops when the soil is already acidic and is only moderately contaminated (Guo et al. 2011; Islam et al. 2007); when soils are not already acidic or are heavily contaminated by heavy metals, liming may be less effective. Phytoremediation using soil amendments such as solubilizing ligands or chelators can be a time-consuming process; therefore, creating the right balance between metal bioavailability and metal uptake potential of the hyperaccumulator species is needed. Application of chelators to the rhizosphere should also be restricted to avoid leaching to groundwater (Singh et al. 2011).

Both greenhouse and field studies have reported success in reducing metal bioavailability with liming (Puschenreiter et al. 2005). When applying lime as a soil remediation technique, it is important to take into account that lime works best with acidic soils with low levels of heavy metal contamination restricted to the upper layers of the soil profile and that liming may need to be repeatedly applied over time (Guo et al. 2011; Islam et al. 2007; Puschenreiter et al. 2005). In addition, the effectiveness of liming treatments varies with the form of the metal and the crop species being grown. Liming seems to be more effective in reducing uptake of Ni and Zn than Cd, and lime-induced reductions in Cd accumulation are more pronounced, for example, in carrots (Apiaceae) and lettuce (Asteraceae) than potatoes (Solanaceae) and peanuts (Fabaceae; Puschenreiter et al. 2005). The application of Zn to soil has been shown to reduce uptake of Cd in flax (Linaceae) and durum wheat (Poaceae) by 40 and 60 %, respectively, and to reduce translocation to seed and grain, respectively, by more than 30 % (Singh et al. 2011). However, Zn amendments are only effective in soils that are already low in Zn and high in Cd so as to avoid potential Zn contamination and toxicity.

Another technique for reducing heavy metal transfer to crops is to grow plants that are heavy metal-tolerant but that are also heavy metal-excluding. Some heavy metal-tolerant crops may still accumulate relatively high levels of metals in their aerial parts, increasing the potential for re-deposition of heavy metals with leaf senescence (Wei et al. 2005). One way to avoid the accumulation of heavy metals is through phytostabilization, or the use of soil amendments to immobilize contaminants and then grow a metal-tolerant (but metal-excluding) crop. As discussed above, lime, SOM, silicon, and carbonates could serve as such amendments. An experiment carried out by Krzyzak et al. (2013) found that As-, Cd-, Pb-, and Zn-contaminated soil amended with lignite and lime reduced metal accumulation in *Festuca arundinacea* (tall fescue; Poaceae) threefold for As, Pb, and Zn and twofold for Cd.

A lesser-studied but important area in agricultural phytoremediation is the role of bacteria, arbuscular mycorrhizal (AM) fungi, and other microorganisms in the rhizosphere. Studies have shown that populations of soil microorganisms are many orders of magnitude larger in the rhizosphere than elsewhere in the soil and that these organisms release organic compounds which may facilitate root uptake of essential as well as non-essential nutrients, including heavy metals (Lasat 2000). Arbuscular mycorrhizal fungi have been implicated in the survival of plants on contaminated soils by enhancing nutrient acquisition and phytostabilization while absorbing and detoxifying metals (Leung et al. 2013). For example, a strain of the bacterium *Stenotrophomonas* (formerly, *Pseudomonas*) *maltophilia* (Xanthomonadaceae) was found to reduce the toxic and mobile Cr^{6+} to the immobile and nontoxic Cr^{3+}, in addition to reducing the mobility of other toxins like Cd^{2+}, Hg^{2+}, and Pb^{2+} (Lasat 2000). While it is widely accepted that relations between plants and microorganisms in the rhizosphere are important for plant health and crop production, more research is needed on the prevalence and importance of such relationships in heavy metal-contaminated agricultural soils.

11.6 Non-remediation Options

If the level of heavy metal contamination of soil is not excessively high, one of the easiest and simplest ways to use the land for agricultural production is to choose crops which do not translocate high amounts of heavy metals to their edible tissues and crops that are less likely to become contaminated on the surface due to soil splashing or dry/wet deposition. Levels of uptake and translocation of heavy metals can vary drastically among plant species as well as among crop cultivars (Puschenreiter et al. 2005; Tang et al. 2012; Table 11.1).

For example, leafy vegetables accumulate higher amounts of heavy metals in edible parts than in fruits or roots due to high levels of transpiration, translocation, and aerial deposition; spinach (Amaranthaceae) and lettuce have a particularly high capacity to uptake heavy metals (Puschenreiter et al. 2005; Tang et al. 2012). Tobacco (Solanaceae) and celery (Apiaceae) have also been found to accumulate relatively high Cd concentrations when grown on Cd-contaminated soil (Puschenreiter et al. 2005). Other crops that have been

Table 11.1 Crop plants known to accumulate metals above ‘normal’ concentrations in shoot and roots. Latin names for crops provided if mentioned in the source

Crop	Metal accumulation in shoot	Metal accumulation in root	Source
Amaranthaceae			
Spinach	Pb, Zn	–	Islam et al. (2007); Puschenreiter et al. (2005); Singh et al. (2011)
Apiaceae			
Carrots	Cd, Zn	Zn	Islam et al. (2007); Singh et al. (2011)
Celery (*Apium graveolens* var. *dulce*)	Cd, Cu, Zn	Cd, Cu, Zn	Islam et al. (2007); Puschenreiter et al. (2005)
Parsley	Cd, Cu, Pb, Zn	–	Islam et al. (2007); Maleki and Zarasvand (2008)
Amaryllidaceae			
Leek (*Allium ampeloprasum*)	Cr, Cu, Pb	–	Maleki and Zarasvand (2008)
Asteraceae			
Lettuce	Pb, Zn	–	Islam et al. (2007); Puschenreiter et al. (2005); Singh et al. (2011)
Tarragon (*Artemisia dracunculus*)	Cd, Cr, Cu, Pb	–	Maleki and Zarasvand (2008)
Sunflower	–	Cd, Cr, Cu, Zn	Tang et al. (2012)
Brassicaceae			
Chinese cabbage (*Brassica chinensis* L. cv. Zao-Shu 5)	Cu	Zn	Islam et al. (2007)
Garden cress (*Lepidium sativum)*	Cd, Cr, Cu, Pb	–	Maleki and Zarasvand (2008)
Pakchoi (*Brassica chinensis*)	Cd, Cu, Zn	–	Islam et al. (2007)
Radish root	Zn	–	Puschenreiter et al. (2005)
Winter greens (*Brassica rosularis* var. Tsen et Lee)	Cd	–	Islam et al. (2007)
Cucurbitaceae			
Cucumber	Cd, Cu, Pb, Zn	–	Islam et al. (2007)
Fabaceae			
Beans	–	Cd	Puschenreiter et al. (2005)
Pea	–	Cd, Zn	Puschenreiter et al. (2005)
Lamiaceae			
Mint (*Mentha piperita* and *M. arvensis*)	–	Cd, Cu, Mn, Pb, Zn	Zheljazkov and Nielsen (1996)
Sweet basil (*Ocimum basilicum*)	Cd, Cr, Cu, Pb	–	Maleki and Zarasvand (2008)
Poaceae			
Durum wheat	Cd	–	Singh et al. (2011)
Maize (*Zea mays*)	Cd, Pb	Zn	Lasat (2000); Puschenreiter et al. (2005)
Oats (*Avena* spp.)	–	Cd	Puschenreiter et al. (2005)
Rice (*Oryza sativa*)	Cd	–	Singh et al. (2011)
Wheat (*Triticum* spp.)	–	Cd	Puschenreiter et al. (2005)
Salicaceae			
Poplar (*Populus* spp.)	–	Cd, Cr, Cu, Zn	Tang et al. (2012)
Willow (*Salix* spp.)	–	Cd, Cr, Cu, Zn	Tang et al. (2012)
Solanaceae			
Potatoes	Cd, Cu, Pb, Zn	–	Islam et al. (2007)
Tobacco (*Nicotiana tabacum*)	Cd	–	Puschenreiter et al. (2005); Singh et al. (2011)
Tomatoes	Cd, Cu, Pb, Zn	–	Islam et al. (2007)

found to accumulate Cd include leafy greens, carrots, durum wheat, and rice (Poaceae; Singh et al. 2011). On the other hand, legumes (Fabaceae) and cereals (Poaceae) have low transfer factors (i.e., total metal concentration in plant relative to the total metal concentration in soil; Puschenreiter et al. 2005). Lavender and mint (Lamiaceae) can also be grown in heavy metal-contaminated soils without affecting essential oils. Although a yield reduction may occur when growing these species on heavy metal-contaminated soils, a profit can still be made on such crops (Puschenreiter et al. 2005; Zheljazkov and Nielsen 1996).

Bioenergy-producing plants like *Populus* spp. (poplar; Salicaceae), *Salix* spp. (willow; Salicaceae), and *Helianthus* (sunflower; Asteraceae) have high tolerance to Cd, Cr, Cu, and Zn and may be good alternatives to the more metal contamination-prone crops (Tang et al. 2012). Another alternative bioenergy crop is *Jatropha curcas* (Euphorbiaceae), an oil-yielding perennial in tropical/subtropical regions that can tolerate extreme growing conditions, including heavy metal toxicity, and could be used for phytostabilization (i.e., binding contaminants to roots to reduce mobility and leaching; Tang et al. 2012). Another option for a bioenergy crop includes *Eucalyptus* species (Myrtaceae), all of which are non-hyperaccumulating but can contribute toward phytoremediation while also providing agroforestry products such as fuel and timber (Rockwood et al. 2004).

Phytomining, or using hyperaccumulator plants to reclaim heavy metals from contaminated soils, has proved promising in greenhouse experiments, but field conditions, including fertilization practices, pest control methods, plant phenology, cropping calendar, temperature, and rainfall, have myriad influences on the actual success of such operations (Neilson and Rajakaruna 2012; Tang et al. 2012). For such undertakings, it can also be difficult to determine the best stage for harvesting (i.e., when metal concentrations are highest in shoots; Tang et al. 2012). Important fiber crops that are tolerant to heavy metals include *Cannabis sativa* (hemp; Cannabaceae) and *Boehmeria nivea* (ramie; Urticaceae), although these crops require the addition of soil amendments which reduce the bioavailability of heavy metals, even on moderately contaminated soils (Tang et al. 2012).

Co-cropping of hyperaccumulators with non-accumulators could have synergistic or antagonistic effects, such as accumulating more than one heavy metal or preferentially accumulating one heavy metal and leaving another for increased accumulation by the crop (Tang et al. 2012). Co-cropping of two non-accumulator crops could also alter metal availability (Tang et al. 2012), especially if one crop tends to acidify the soil. The soil matrix is a complicated system, and there are many factors influencing effective agricultural use of contaminated soils that are not yet well understood or documented. Antagonistic and synergistic effects between crop species, as well as soil microorganisms, must be taken into account when selecting potential crops or soil amendments for use in heavy metal-contaminated soils (Puschenreiter et al. 2005).

Healthy agricultural management practices can also contribute to the safety of food crops grown on contaminated soils. Heavy metal levels in edible parts of plants are influenced by agricultural practices and the genotype of the crop used (Singh et al. 2011). For example, fertilizer management, choice of suitable crops or cultivars, and the use of crop rotation methods are acceptable agricultural practices that could reduce the risk of heavy metal contamination of edible plant tissue, including the spread of heavy metals to higher trophic levels via the food chain (Islam et al. 2007; Lasat 2000; Singh et al. 2011). For example, *Lupinus* (lupine; Fabaceae) has been found to increase Cd levels in wheat grain when grown in a crop rotation before wheat as its roots release citric acid which acidifies soil, thereby increasing the mobility and availability of heavy metals (Singh et al. 2011). Crop rotation is also important because any monoculture will, over time, become more susceptible to pests and pathogenic diseases. However, studies have shown that a monoculture of hyperaccumulator plants could be effective if the contamination is moderate enough to only need around three years of phytoremediation or less (Lasat 2000). Deep tillage could also be an option as it could dilute the distribution of contaminants by blending surface and subsurface soil layers (Singh et al. 2011).

While the approaches outlined here may all be viable in theory, it is difficult to advise farmers to switch from growing edible plants to growing biofuel or fiber crops without additional incentives. There are many personal, cultural, economic, climatic, and other considerations which go into the decision of what plants to grow in agricultural settings. However, phytostabilization using fuel or fiber crops can provide added economic benefits without high input and labor costs. Further, phytoextraction and phytomining using agronomic practices would be less destructive overall than using technological or chemical methods of remediation (Neilson and Rajakaruna 2012; Tang et al. 2012) and have the potential for being more socially acceptable methods of environmental cleanup.

11.7 Potential for Genetic Modification

Hyperaccumulation often results from the over-expression of genes which code for specialized protein transporters and chelators (Chaffai and Koyama 2011; Rascio and Navari-Izzo 2011; Maestri et al. 2010; Verbruggen et al. 2009; Jabeen et al. 2009). Genetic modification has successfully increased the potential for metal accumulation in several species, including those in genera *Brassica* and *Arabidopsis* (Brassicaceae; Gall and Rajakaruna 2013). Genetic modification could also lead to metal tolerance via metal exclusion. Identification of genes that exclude heavy metals from species known to exclude heavy metals and subsequent transfer of those genes to food crops could be a way to safely grow food plants on contaminated land (Wei et al. 2005). Metal-tolerant plants often exude compounds from their roots, such as citric and malic acids, which can chelate metals in the rhizosphere much like lime or a synthetic chelator would (Lasat 2000), making the contaminants less bioavailable. This characteristic could be useful in limiting translocation of heavy metals to edible tissues. Variation in metal tolerance and accumulation among cultivars of crops (Cutright et al. 2010; Xin et al. 2010; Zhang et al. 2013a, b) could help in breeding for heavy metal tolerance or exclusion; however, this is not an easy task as cultivars must also meet standards of yield, disease resistance, nutritional quality, and commercial viability (Singh et al. 2011).

11.8 Conclusions and Future Directions

Although much progress has been made in the exploration of how best to reconcile the growing need for arable land and the simultaneous increase in heavy metal contamination in agricultural soils worldwide, much remains to be learned. More research into how bacteria, fungi, and other soil microorganisms may be exploited for reducing metal transfer into plants and the food chain is an important area of future study. Concerted efforts to establish international standards with respect to heavy metal concentrations in edible plants and other agricultural products and the proper regulation of acceptable levels of heavy metals in food are also vital. Finally, field-based research on eco-friendly soil amendments to reduce bioavailability of metals and increase crop productivity under metal stress and the potential use of crops genetically modified to be tolerant of heavy metals or crop varieties that exclude heavy metals from edible tissue will drive the future of phytoremediation of contaminated agricultural soils.

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Biomonitoring the Genotoxicity of Heavy Metals/Metalloids Present in Soil Contaminated by Fly Ash from Coal-Fired Thermal Power Plant Using *Tradescantia pallida*

12

Neelima Meravi and Santosh Kumar Prajapati

12.1 Introduction

Environmental pollution in recent years has increased manifolds due to rapid growth of industries and anthropogenic pressure worldwide. Out of the different types of environmental pollutants, the problem of heavy metal pollution is of serious concern to the environmentalists since they persist in the environment and may have serious repercussions to the biotic world. There are various sources of heavy metal coming from the environment such as mining industries; electroplating industries; thermal power plants; textile, leather and tanning industries; etc. Out of the above, fly ash coming from thermal power plants is also an important source. Huge amount of fly ash is generated in India from the coal-fired thermal power plants leading to environmental pollution (TERI 2000). The fly ash contains several heavy metals/metalloids such as As, Mo, Se, Cd and Zn (el-Mogazi et al. 1988). Presence of these metals/metalloids and other components may make the fly ash genotoxic which may have deleterious impacts on human health by inhalation of dust originating from the power plants and through the leachates from fly ash dykes. Environmentally released metals are mainly deposited in soils and are mobilised either by leaching or by uptake into plants (Prajapati 2012).

Assessment of heavy metals/metalloids for genotoxicity is a tedious process using chemical methods; however, plant bioassay provides means of assessing the toxicity of heavy metals/metalloids originating from fly ash even without knowing the chemical composition (Watanabe and Hirayama 2001). Even though animal assays are well suited for laboratory studies of pathogenic mechanisms (Saldiva and Böhm 1998), no effective animal assay for in situ monitoring is available so far. Plant bioassays are best suited for addressing the effects of air pollutants as they are more sensitive to environmental stresses than other currently available bioassay systems (Gopalan 1999).

Higher plants, such as *Tradescantia pallida* which are multicellular and eukaryotic, have been effectively used for monitoring the genotoxicity of air pollutants (Sparrow et al. 1972; Ma et al. 1982a, b; Arutyunyan et al. 1999; Batalha et al. 1999; Monarca et al. 1999; Guimaraes et al. 2000). The *Tradescantia*-micronucleus (Trad-MCN) bioassay using several clones of *Tradescantia* has been used extensively for this purpose (Ma 1981; Rodrigues et al. 1997, Grant 1998). Among the different plant assays, the *Tradescantia-micronucleus* (Trad-MCN) bioassay with pollen tetrads is the most extensively validated procedure (White and Claxton 2004). This test system has been used worldwide to evaluate the genotoxic potential of chemicals, air, soil and water (Gong et al. 2003; Monarca and Feretti 1997; Cotelle and Masfaraud 1999; Crebelli et al. 2005; Prajapati and Tripathi 2008). Although the genotoxic effects detected by *Tradescantia*-micronucleus (Trad-MCN) bioassay cannot be extrapolated directly to human populations, these bioassays are very useful tools for screening the mutagenic potential in the environment (Ma et al. 1994). In the present study, *Tradescantia*-micronucleus (Trad-MCN) bioassay was performed to assess the genotoxicity of heavy metals/metalloids present in the fly ash.

12.1.1 Genotoxicity

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. A substance that has the property of genotoxicity is known as a genotoxin. Genotoxins are mutagens; they can cause mutations which may lead to cancer. Genotoxins include both radiation and chemical genotoxins. Observations on putative metal genotoxicity are scarce, and majority of the cellular and molecular aspects of metal toxicity in plants are unknown, even though deleterious effects on crop production

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have long been recognised. At the organ level, this symptom is common to numerous metals (Punz and Sieghardt 1993). Most metallic salts are effective mitotic poisons (turbagens) at a particular concentration due to their known affinity for thiol groups and induce various types of spindle disturbances.

In plant systems, in vivo solubility of the salt in water is of primary importance. The degree of dissociation and the availability of cations affect the number of aberrations produced quantitatively. The viscosity of the plasma membrane may be changed through changes in the ionic environment and/or formation of chelated complexes, leading to spindle dysfunction.

Metal binding to the cell nucleus causes promutagenic damage including DNA base modifications, inter- and intramolecular cross-linkage of DNA and proteins, DNA strand breaks, rearrangements and de-purination. Chemical reactions driving this damage, and the resulting mutations, are characteristics of an oxidative DNA attack (Kasprzak 1995). Metal-mediated production of reactive oxygen species in the DNA vicinity generates principally the promutagenic adduct 8-0xoG (7,8-dihydro-8-oxoguanine) that could miss pair with adenine in the absence of DNA repair, resulting in C to T transversion mutations (Cunningham 1997).

Although oxidative damage explains most of the mechanisms involved in metal-mediated carcinogenicity and acute toxicity, other pathways also have to be considered. It has been shown that the metals induce chromosomal abnormalities and also decreases the rate of cell division. The genotoxic effects depend on the oxidation state of the metal, its concentration and duration of its exposure. In general, effects are more pronounced at higher concentrations and at longer duration of exposures (Bandyopadhyay 1997; Bhowmik 2000; Lerda 1992; Patra 1999). Plant species respond differently to exposure to the same metal depending on the number of diploid chromosomes, total length of the diploid complement and the number of metacentric chromosomes (Ma et al. 1995). The response was also found to vary with the method of treatment and the plant parts used for exposure (Bhowmik 2000).

12.1.2 Genotoxicity of Heavy Metal on *Tradescantia*

Assessment of environmental genotoxicity has been developed using *Tradescantia.* The *Tradescantia*-micronucleus tests were used to evaluate for genotoxicity. The micronucleus (MCN) test on tetrads of *Tradescantia* (Trad-MCN) is currently the most widely used bioassay on plants for detecting genotoxins in the environment. According to a recent paper, approximately 160 chemicals have so far been tested, and 100 articles on complex environmental mixtures have been published.

12.1.3 The Genus *Tradescantia*

Tradescantia is a genus of perennial herbaceous plants in the family Commelinaceae. It originates from the New World and comprises around 500 species with distribution going from southern Canada to northern Argentina (Watson and Dallwitz 1992). In the seventeenth century, they were introduced into Europe, where they are used for ornamentation, just as they are used in most other countries.

The height of the plants varies in most species between 30 and 60 cm, and its oval shaped leaves are distributed along the stem. The inflorescence, which may be axillary or terminal, is protected by bracts that may or may not be differentiated from the leaves. The flowers present radial symmetry and are hermaphroditic. The pedicles vary in size between the species, and the calyx and corolla are formed, respectively, by three sepals and three petals. The stamens are free and six in numbers and the anther has two thecae. The gynoecium has a trilocular sessile ovary, and each locule holds two ovules. The style is simple and the stigma is capitate. In most of the species, the fruits present in a capsule with a locus, and each locus holds two seeds.

Plants and clones of the genus *Tradescantia* have been used for environmental monitoring since the time of the first investigation of the mutagenic activity of chemical and physical compounds in relation to components of the atmosphere, soil or water. In addition to its high sensitivity to mutagen action, its ease of cultivation, short life cycle and especially its large chromosomes that are few in number are characteristics that make plants and clones of the genus *Tradescantia* valuable instruments for environmental monitoring.

Most of the studies using *Tradescantia* plants and particularly those developed in Europe have been conducted using clone 4430, which is a diploid hybrid between *T. hirsutiflora* Bush (2461C), with a blue flower, and *T. subacaulis* Bush (2411), with a pink flower (Isidori et al. 2003; Klumpp et al. 2006; Ma et al. 1996). This clone, which was developed by Sparrow et al. in 1960, is very versatile and has, since then, been widely cultivated indoors, although it requires special conditions for it to grow and flower. On the other hand, it presents great sensitivity to the action of chemical and physical mutagens in the environment. Because it is sterile, it has the advantage that its genetic uniformity is maintained. It can be used both for the micronucleus test (Trad-MCN) and for the stamen hair mutation test (Trad-SHM).

The *Tradescantia* species most frequently used for carrying out Trad-MCN is *T. pallida* (Rose) D.R. Hunt. cv. *purpurea* Boom. This is a small-sized herbaceous plant (reaching a maximum height of 25 cm) with spear-shaped succulent leaves that are native to North America and Central America (Mexico and Honduras) (Lorenzi and Souza 2008). The epidermis of the leaves presents large quantities of anthocyanin, which gives them a purple colour, particularly in very bright light (Joly 1998). Two large canoe-shaped bracts protect the inflorescence, which presents pink flowers.

Phytoremediation is the use of vegetation for in situ treatment of contaminated soils, sediments and water. Soil contaminated by fly ash is heavily polluted with heavy metals/metalloids and needs remediation. Modern technologies are quite costly, energy intensive and metal specific. Contrary to this, phytoremediation offers a promising technology for heavy metal removal. Phytoremediation involves phytoextraction (Kumar et al. 1995), rhizofiltration (Dushenkov et al. 1995), phytostabilisation (Salt et al. 1995) and phytotransformation/phytodegradation (Susarla et al. 2002). Aquatic macrophyte *Pistia stratiotes* L. has been extensively used for phytoremediation (Quian et al. 1999). *Pistia stratiotes* was used in laboratory experiments for the removal of several heavy metals (Fe, Cu, Zn, Mn, Cr and Pb) resulting from anthropogenic activity (Miretzky et al. 2004).

In the present study, an attempt has been made for biomonitoring the presence of heavy metals/metalloids using plant *Tradescantia*-micronucleus bioassay (Trad-MCN) of the soil contaminated by the fly ash generated from coal-fired thermal power plants. Simultaneously, the heavy metal/metalloid removal capacity of the *Tradescantia* has also been assessed for phytoremediation of contaminated soils.

12.2 Materials and Methods

Soil sample was taken from the vicinity of a super thermal power plant (NTPC, Sipat, Chhattisgarh, India) located at 22°07′ N and 82°16′ 43 E with a capacity of 2,980 MW. *Tradescantia pallida* (Rose) D.R. Hunt was used for the biomonitoring of heavy metals/metalloids present in the fly ash-contaminated soil and the phytoremediation of the heavy metals/metalloids present in the soil (Fig. 12.1). *Tradescantia pallida* is a species of spiderwort commonly known as purple heart or queen and is planted as an ornamental plant in the garden. The plants were planted in cemented pots for the study of biomonitoring and phytoremediation study. All the pots were kept in the open air, so as to avoid shade, and watered twice a week. Samples were closely inspected to keep them healthy. No fertiliser and pesticides were applied to or around the plants during the experiment so as to avoid their effect. Before planting the *Tradescantia pallida* in pots, analysis of soil contaminated by fly ash for heavy metals/metalloids (Fe, Zn, Pb, Cd, Mo, Cu, Cr, Co and Ni) was performed with the help of atomic absorption spectrophotometer (AAS) AA-7000 model, Shimadzu, and the standards were prepared using standard metal solution of Inorganic Ventures.

Tradescantia-micronucleus (Trad-MCN) bioassay was performed using the protocols established by Ma (1981). Young inflorescences were collected from the flowering plants. The inflorescences collected from different sampling sites were fixed overnight in a 1:3 glacial acetic acid–ethanol solution separately and then stored in 70 % ethanol. The young anthers from inflorescence were taken out, dissected and squashed on micro-slides in a solution of acetocarmine stain. Only micro-slides containing early tetrads were considered.

Fig. 12.1 *Tradescantia pallida* planted in pot containing contaminated soil

12.3 Results and Discussions

The analysis of soil contaminated by fly ash emitted from thermal power plant was analysed with the help of AAS for Fe, Zn, Pb, Cd, Mo, Cu, Cr, Co and Ni in order to assess their concentration. The heavy metal/metalloid concentration present in contaminated soil is depicted in Table 12.1. It is evident from the table that Fe was present in maximum concentration and Cd was present in minimum concentration, whereas Pb and Cu were not detected in the soil. It is also clear from the given table that fly ash is an important source of metals/metalloids that may contaminate the soil, and in due course of time, they may accumulate to a high level because of their non-biodegradable nature. This may have serious environmental consequences in the future.

In order to assess the metal/metalloid removal efficiency of *Tradescantia pallida* for phytoremediation purposes, the metals/metalloids present in different plant parts were also estimated. The metal/metalloid concentrations present in different plant parts are shown in Table 12.2. The table shows that different plant parts have varying accumulating capacity for the same metal. It is also clear from the table that plant and plant parts have different affinity for different metals.

In order to assess the differential capacity of plant parts for heavy metals/metalloids, CFPP (concentration factor for plant parts) was calculated. CFPP values for different metals/metalloids are given in Table 12.3.

Table 12.1 Heavy metal/metalloid concentration (µg g^{-1} dry wt. of soil) present in contaminated soil

Metal/metalloids	Fe	Zn	Pb	Cd	Mo	Cu	Cr	Co	Ni
Concentration	33.02	1.402	0.00	0.082	4.49	0.00	0.213	0.176	0.127

Table 12.2 Metal/metalloid concentration (µg g^{-1} dry wt. of plant parts) present in different plants

Metals/metalloids	Stem	Root	Leaf
Fe	3.517	19.819	17.297
Zn	1.142	3.822	1.990
Pb	0.354	0.310	N.d.
Cd	N.d.	0.029	N.d.
Mo	N.d.	N.d.	N.d.
Cu	N.d.	N.d.	N.d.
Cr	N.d.	N.d.	N.d.
Co	0.147	0.115	0.096
Ni	0.310	0.255	0.421

Table 12.3 CFPP values for different metals/metalloids

	CFPP values		
Metal	Stem	Root	Leaf
Fe	0.106	0.600	0.523
Zn	0.813	2.726	12.337
Pb	0.354	0.310	0.00
Cd	0.00	0.354	0.00
Mo	0.00	0.00	0.00
Cu	0.00	0.00	0.00
Cr	0.00	0.00	0.00
Co	0.835	0.635	0.545
Ni	2.440	2.007	3.314

Concentration factor for plant parts (CFPP) is calculated as follows:

$$\text{CFPP} = \frac{\text{concentration in plant part}}{\text{concentration in soil}}$$

The CFPP values for Mo, Cu, Cr and Co were nil, while it was nil for Cd only for the stem. CFPP values for different metals/metalloids are different. Maximum CFPP values are mainly for root and leaf, for example, Ni and Zn were high in leaf but for Fe it was in root. CFPP was high in case of Pb for stem.

Tradescantia-micronucleus (Trad-MCN) bioassay was also performed in the present study for the soil contaminated with fly ash so as to assess the genotoxicity of the heavy metals/metalloids present in the soil. Micronuclei (MCN) are DNA-containing extracellular bodies surrounded by a plasma membrane, which are formed as a consequence of chromosomal breakage (clastogenicity) or aneuploidy. In the present study, the phy-toremediation capability of *Tradescantia* plant has been established indicating that heavy metals/metalloids are accumulated in it. The accumulated heavy metals/metalloids were able to form micronuclei in the *Tradescantia pallida* (Fig. 12.2). Therefore, it can be assessed that this plant can be also used for biomonitoring purposes.

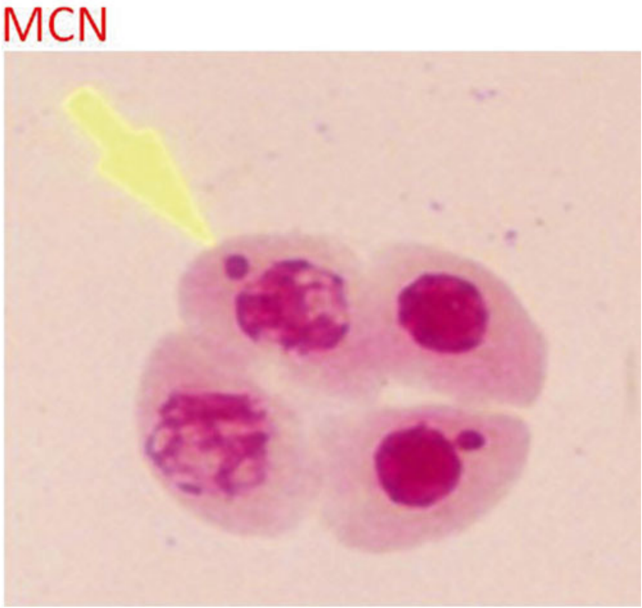

Fig. 12.2 MCN formed as a consequence of chromosomal breakage in *Tradescantia pallida* planted in soil contaminated with fly ash

12.4 Conclusions

Bioassay is a method determining the concentration, activity or effect of change to a substance by testing its effect on a living organism and comparing this with the activity of an agreed standard. Bioassays developed using plants and clones of the genus *Tradescantia* are considered to be valuable tools for assessing genotoxic effects of environmental contaminants. For this reason, since the beginning of genetic toxicology, they have been widely used in studies and biomonitoring programmes. The micronucleus test on tetrads of *Tradescantia* (Trad-MCN) is currently the most widely used bioassay on plants for detecting genotoxins in the environment (Misík et al. 2011).

Tradescantia-MCN bioassay is based on the formation of micronuclei resulting from chromosomal breakage in the meiotic pollen mother cells of *Tradescantia* ssp. Inflorescence has

Table 12.4 Induction of micronuclei by heavy metals in stem absorption assays with *Tradescantia*

Metal	Concentration range (mM)	MCN (fraq.)	Reference
As+3	0–5	2.86	Steinkellner et al. (1998)
Lead nitrate	0–12	1.65	
Cadmium chloride	0–11	0.79	
Zinc chloride	0–14	0.48	
Copper sulphate	0–2	–	

been one of the most frequently applied genotoxicity assays for detecting clastogenicity of contamination with varying amounts of heavy metal (As, Cd, Cr, Cu, Mn, Ni, Pb, Zn, etc). Trad-MCN consists of a set of procedures for exposing plants of the genus *Tradescantia* to contaminating agents, culminating in estimates of the micronucleus frequencies in mother cells of pollen grains at the tetrad phase. Micronuclei are structures that result from whole chromosomes or fragments of chromosomes that, because they do not bind to the spindle fibres, are not included in the nuclei of the daughter cells. Instead, they remain in the cytoplasm of the interphase cells, where they are observed as corpuscles resembling the nucleus, measuring 1/3 to 1/5 of the size of the nucleus. Since micronuclei results both from chromosomal fragments and whole chromosomes lagging behind in anaphase, they reveal the clastogenic/aneugenic action of a given mutagen (Fig. 12.2). Estimation of the numbers of micronuclei in tetrads of *Tradescantia* was first purposed at the end of the 1970s, in a pioneering study conducted by Ma et al. (1978). In that study, they used clone 4430 to compare the formation of micronuclei in tetrads (Trad-MCN) with stamen hair mutations in cells (Trad-SHM), after exposing the plants to 1,2-dibromoethane (DBE). The results obtained revealed that the sensitivity of Trad-MCN was approximately 30 times greater than that of Trad-SHM. The greater sensitivity of Trad-MCN in relation to Trad-SHM has been observed in several other studies, such as that of Gichner and Velemínský (1999) and Minouflet et al. (2005). The Trad-MCN bioassay was developed in 1976 and used in detecting the gaseous agent, ethylene dibromide. Later it was adapted for tests of liquid agents (Ma 1979, 1990; Ma et al. 1984). In 1984, a validation study was carried out in which more than 100 chemicals and common foods and drugs were tested (Ma et al. 1984). In addition, the Trad-MCN bioassay has been used in the monitoring of air (Ma 1990; Ma et al. 1984), waste water (Grant et al. 1992; Ruiz et al. 1992; Chen and Xiang 1982) and drinking water (Ma et al. 1985), as well as with the testing of many well-known mutagens/carcinogens (Fang 1981; Sandhu et al. 1989; Knasmüller et al. 1992; Ma et al. 1984). A summary of the results obtained with the Trad-MCN in the assessment of environmental mutagenesis is presented in Tables 12.4 and 12.5.

The present study shows that coal-fired plants are an important source of heavy metal/metalloid pollution coming out of fly ash. The speciation of fly ash showed the presence of Fe, Zn, Pb, Cd, Mo, Cr, Co and Ni. Presence of these metals/metalloids can also be biomonitored with the help of *Tradescantia pallida*, and the same can be used for phytoremediation purpose. CFPP (concentration factor for plant parts) was also calculated to show the ability of the *Tradescantia pallida* for phytoremediation of heavy metal-/metalloid-contaminated soils from fly ash. The genotoxicity assessment of heavy metal-/metalloid-contaminated soils was also performed with the help of Trad-MCN bioassay. The present study clearly indicates that heavy metals/metalloids present in the soil are genotoxic in nature and have the capability to break the chromosomes leading to chromosomal aberrations. It can be concluded from the present study that *Tradescantia pallida* can be used for biomonitoring, phytoremediation purposes as well as assessing the genotoxicity of environmental pollutants particularly heavy metals/metalloids originating from coal-fired thermal power plants.

The assessment of genotoxic effects of heavy metal compounds in the *Tradescantia* have been conducted by determining the induction of MCN. The present studies have shown that plant bioassays, in particular the Trad-MCN, are valuable tools for the detection of genotoxic carcinogens in the environment. This bioassay is highly sensitive towards compounds which cause negative or moderate effects in other widely used systems. For example, heavy metal cannot be detected in bacterial mutagenicity assays, which are also not sensitive towards ionising radiation. Furthermore, it is important to note that, due to the intensitivity of most mutagenicity assays, concentration procedures are required which may lead to loss of active compounds (Ohe et al. 2004; Stahl 1991); such procedures can be avoided in Trad-MCN experiments. Another point, which argues for the use of Trad-MCN bioassay, concerns the fact that more than 120 individual compounds and about 100 complex environmental mixtures have been tested (Ma et al. 1984, 2005), and therefore the sensitivity of the assay to different environmental pollution is well known. On the basis of the currently available data, it can be concluded that the Trad-MCN bioassay is complementary to other current methods for the detection of environmental genotoxins. Therefore, the assay should be included in test batteries that are used in studies concerning environmental pollution with radioactive materials, heavy metals and air pollution. The test system is based on the detection of micronuclei which refer to clastogenic (chromosome

Table 12.5 Genotoxic effects with special reference to the Trad-MCN bioassay of various types of pollutants

	Dose range			Result	
Pollutants	Exposure time-max	Concentration	+/–	Statistical significance	Remarks
In situ monitoring air pollution	4–6 h		–	$P<0.01$	Parking garage, Chicago, IL
	1–4.5 h		–	$P<0.01$	Parking garage, Decatur, IL
	2–6 h		+	$P<0.01$	Parking garage, Peoria, IL
	2–4 h		–	$P<0.01$	Truck and bus stop
	2–3 h		–	$P<0.01$	Truck and bus stop
	2.5–5 h			$P<0.01$	Truck and bus stop
	3 months		+	$P<0.01$	Industrial site Granite City, IL
	3.5 h		+	$P<0.01$	Industrial site, Granite City, IL
	4.5 h		–	$P<0.01$	Residential area, china
	4–6 h		+	$P<0.01$	Agrochemical industry site, China
	5 h		–	$P<0.01$	Bus station, China
	6 h		+	$P<0.01$	Bus station, China
	6 h		+	$P<0.01$	Rubber company, China
	4 h		–	$P<0.01$	Office environment, China
	3–6 h		+	$P<0.01$	*p*-Dichlorobenzene-treated herbarium, China
O_3	6 h	100 ppb	+	$P<0.01$	
HN_3	6 h	136–272	+	$P<0.01$	Single application of gas, without replenishment
Municipal incinerator	4–6 h	50–500 m from source	+	$P<0.01$	Positive result obtained with stagnant atmosphere
Soil contaminants					
Sewage sludge	24 h	4-fold dilution	+		
Tetrachloroethylene	2 h	30 ppm	+	$P<0.05$	Positive only when gaseous form
Arsenic trioxide	30 h	3.96 ppm	+	$P<0.05$	Diluted in NaOH
Lead tetraacetate	30 h	0.44 ppm	+	$P<0.05$	Diluted in DMSO
Hazardous waste-site soil	30 h	0.5 % aqueous extract	+	$P<0.05$	Over 5,000 ppm mixed PAH_S
Pesticides					
Cyanazine	3–12 h	200–600 ppm	+	$P<0.001$	Root tip mitosis
Malathion	6 h	5.5–1,650 ppm	–	$P<0.05$	Stem absorption and spray application
Malathion	6 h	4,125	–	$P<0.05$	Applied as liquid Negative Ames test
2,4-D	6 h	200 ppm	–	$P<0.05$	Negative in the Ames test
Radiation					
X-rays	8 min	75–200 rad	+		Chromosome breaks, mostly at mitosis
X-rays	~5 min	77–416 rad	+	$P<0.05$	Chromatid aberration in pollen tubes and microspores, and significance measured for coefficients in exponential fit of data
^{60}Co rays	30 min	100–400 rad	+	$P<0.01$	
Cosmic rays	In situ exposure in space satellite		+		Unusual chromosome aberrations, i.e. nonreciprocal translocations and spherical fragments

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breaking) and aneugenic effects in meiotic pollen tetrad cells. The MCN frequencies are scored in buds, which contain early tetrads and are highly synchronised. In conclusion, the studies reviewed here demonstrate that *Tradescantia* plants, particularly the Trad-MCN bioassay, provide a very sensitive, easily manipulated system for the study of genotoxicity, especially under the in situ conditions.

These simple and quick monitoring studies serve two purposes: to establish a database for environmental conditions in the various regions of the world and to demonstrate the genetic effects of pollutants by means of clear-cut evidence of chromosome damage and/or gene mutation. In addition, these assays may be used as tools for environmental education. Although mutagenesis in plants and the risk of malignancies in humans cannot be equated, Trad-MCN can be very useful as a proxy for assessing human risk. Short-term assay in field conditions may help to predict the genotoxicity.

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13

Utilization and Supplementation of Phytoextraction Potential of Some Terrestrial Plants in Metal-Contaminated Soils

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13.1 Introduction

Rapid growth of technology and population has changed our lifestyle on a global scale in the last century and dramatically increased pollution. The range of different pollutants that can contaminate land is broad and extensive, but the groups of contaminants that are of particular interest to this study are *heavy metals*. Heavy metals are ubiquitous environmental contaminants, and their content in soils has accelerated dramatically since 1900, the beginning of the industrial revolution (Nriagu 1979).

13.1.1 Heavy Metal Contamination

In the environmental context, the term *heavy metals* is often used to describe a disparate group of elements associated with pollution and potential toxicity, though this definition is somewhat imprecise. These elements are natural components of soils in trace amounts. However, when their concentration exceeds thresholds of bio-tolerance, heavy metals are toxic and have to be regarded as dangerous soil pollutants. Human activity is the main contributor to heavy metal pollution. The main man-made sources of heavy metal pollution include metal smelters, refineries, industrial wastes, military operations, mining, landfill runoffs, agricultural chemicals, and automobile emissions.

Several heavy metals such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), or molybdenum (Mo) are essential for the growth of organisms. Mostly, others are always toxic to organisms: cadmium (Cd), lead (Pb), uranium (U), thallium (Tl), chromium (Cr), silver (Ag), and mercury (Hg). Arsenic (As) and selenium (Se) are non-heavy metals. However, since they partly share toxicity features with heavy metals, they are often referred to as "metalloids" in publications.

Contamination of soil, aqueous streams, and groundwater with toxic metals poses a major environmental problem and a serious danger to human health. This still requires an effective and affordable technological solution. In most developed countries, current heavy metal pollution cases are localized and are declining due to cleaner industrial practices and conversion to non-heavy metal-based products like unleaded fuel. However, in developing regions of the world, heavy metal contamination of the environment is still widespread (Krämer 2005).

13.1.2 Heavy Metal Toxicity

Contamination of the soil by heavy metals can result in unbalanced microflora and microfauna and toxic effects on plants and mesofauna. In particular, such soils show poor plant growth and low covering of the soil surface resulting in leaching of metals into runoff water with subsequent deposition into ground and surface waters (Lasat 2000). In today's industrial society, there is no possibility to avoid exposure to toxic chemicals and metals. Exposure to high levels of these metals has been linked to adverse effects on human health and wildlife. Lead poisoning in children causes neurological damage leading to reduced intelligence, loss of short-term memory, learning disabilities, and coordination problems. The effects of arsenic include cardiovascular problems, skin cancer and other skin effects, peripheral neuropathy (WHO 1997), and kidney damage. Cadmium accumulates in the kidneys and is implicated in a range of kidney diseases

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(WHO 1997). 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 (WHO 1997). Long-term exposure to heavy metals may result in chronic physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Also, toxic heavy metals cause DNA damage, and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Baudouin et al. 2002). Heavy metal toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems of our modern society.

13.2 Remediation Methods

13.2.1 Conventional Technologies

Due to the risk of pollution to groundwater and air caused by contaminated soil, different remediation methods have been developed in the last few decades. Excavation, solidification, soil washing, isolation, and containment are some physical remediation methods. Chemical methods include extraction, reduction/oxidation processes, and soil washing. Thermal methods are used for separating volatile contaminants from soil. Foolproof and economically affordable remediation methods have not yet been developed. While these technologies continue to be improved upon, other alternative and potentially cost-saving remediation techniques are in the process of development. These "innovative treatment technologies," including *bioremediation* and *phytoremediation*, accounted for 21 % of treatment projects implemented on contaminated sites between 1982 and 2002. Since 1997, the use of innovative technologies has dramatically increased, reaching nearly half of the selected technologies in 2001 (EPA 2003).

Bioremediation or biodegradation uses microbes to remediate the subsurface of soils contaminated with hazardous chemicals. It is applied most effectively for organic pollutants (such as benzene, toluene, and phenols) where, depending on the microbe and pollutant, it can result in the complete mineralization of the pollutant. For inorganic pollutants like heavy metals, this form of remediation is less effective.

13.2.2 Phytoremediation

The word phytoremediation comes from the Greek word *phyton* or plant and Latin *remedium* to remediate. Driven by the spiraling costs associated with conventional soil remediation techniques and the desire to use a "green," sustainable process, workers have produced an impressive body of research on the uses of plants to remediate metals, pesticides, solvents, explosives, petroleum hydrocarbons, and radionuclides. Phytoremediation consists of different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. The various pathways of soil–metal–plant interaction have been elucidated in Fig. 13.1.

Phytoextraction is the most commonly recognized of all phytoremediation technologies. It involves the removal of toxins, especially heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs. Pollutants accumulated in stems and leaves are harvested and removed from the site. *Rhizofiltration* utilizes plant roots to take up and sequester metal contaminants and/or excess nutrients from aqueous growth substrates. *Phytodegradation* utilizes the rhizospheric associations between plants and soil microorganisms to degrade complex organic-metal contaminant mixtures. This approach is suitable for organic contaminants like TNT, PAHs, and petroleum hydrocarbons. *Phytostabilization* utilizes tolerant plants to inhabit heavy metal-contaminated environments where they prevent soil erosion, reduce heavy metal leaching, and minimize uptake, hence effectively containing and minimizing the spread of heavy metals. *Phytovolatilization* uses the ability of plants to absorb and subsequently volatilize the contaminant into the atmosphere. This approach is suitable for remediating metal(loid)s that may exist in gaseous form in the environment such as As, Hg, and Se.

The environmental impact of the conventional technologies discussed before can be very high. Most of these are invasive and employ heavy, noisy, large construction equipment that is costly and can potentially spread the contamination (EPA 2001). Implementing phytoremediation as a natural environment can enhance or restore the physical appearance of a site.

Phytoremediation has gained attention because it is potentially cheaper, approx. 90 % (Hinchman et al. 1998), than conventional treatments, which are primarily chemical based and pursue an energy intensive approach. Conventional excavation and disposal methods would cost US$400,000 per acre of land with a remediation depth of 50 cm compared to US$60,000–100,000 for phytoremediation (Salt et al. 1995) and, hence, suggested that phytoremediation is an economically viable technology. It is emerging as a new tool for in situ remediation to clean up contaminated soils and water. The remediation agenda of the USEPA included phytoremediation of metals and radionuclides as a thrust area up to 25 % during 2000. Furthermore, existing physicochemical technologies are intended primarily for intensive in situ or ex situ treatment of highly contaminated sites and thus are not appropriate for vast, diffusely polluted areas where contaminants occur at low concentrations (Mulligan et al. 2001).

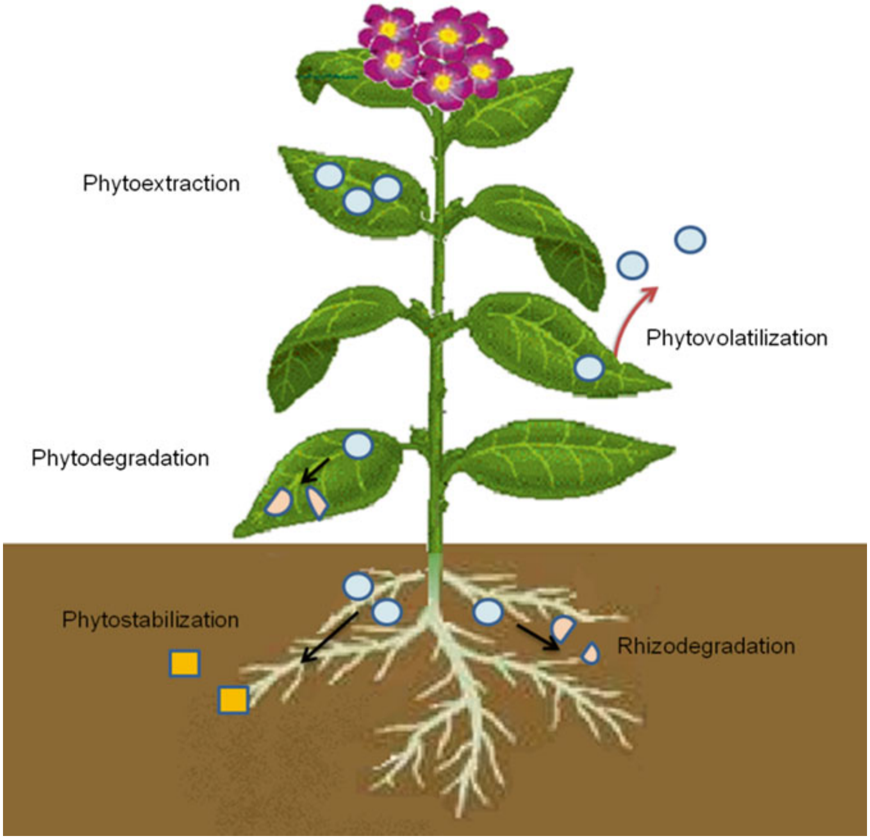

Fig. 13.1 Phytoremediation strategies

However, like all technologies, phytoremediation too has its limits. In order to implement a successful phytoremediation system, an appropriate plant species must be chosen to suit the contaminant of concern as well as the contaminated site. Additionally, the process is slow (USEPA 1998). Many years are needed in order to remediate a contaminated site, whereas other methods like "dig and dump" are completed immediately. These other methods may also require less planning and long-term maintenance. Further, there is possibility that the food chain could be affected and toxicity could be transferred to higher trophic levels including humans.

13.3 Plant Species for Phytoremediation: An Ideal Prototype

The potential for any plant species to remediate successfully heavy metal-contaminated sites depends on all of the following prerequisite factors: a) the amount of metals that can be accumulated by the candidate plant, b) the growth rate of the plant in question, and c) the planting density (Saxena et al. 1999). The growth rate of a plant in a chemically contaminated soil is important from the perspective of biomass. The rate of metal removal from the soils can be calculated if information on the abovementioned parameters is available. An ideal plant species for phytoremediation should have either one of the following characteristic combinations (USEPA 2000): a) a low biomass plant with a very high metal accumulation capacity or b) a high biomass plant with enhanced metal uptake potential. In addition to these characteristics, versatility of the candidate plant to tolerate and at the same time accumulate multiple metal contaminants and/or metal-organic mixtures would be an asset for any phytoremediation system.

13.4 Heavy Metal Hyperaccumulation

The term *hyperaccumulator* was first used by Brooks et al. (1977), to describe a plant species that could accumulate substantial amounts of a given heavy metal in aboveground tissue without deleterious effects to the plant. By definition, a plant to be called hyperaccumulator must accumulate at least 100 mg kg^{-1} (0.01 % dry wt.) Cd, As, and some other trace metals; 1,000 mg kg^{-1} (0.1 % dry wt.) Co, Cu, Cr, Ni, and Pb; and 10,000 mg kg^{-1} (1 % dry wt.) Mn, Zn, and Ni (Reeves and Baker 2000). Although some plant species are clear hyperaccumulators for Cu, Zn, or Cd, no species can store excess of all heavy metals.

More than 450 species from 45 families of metal hyperaccumulators have been identified (Prasad and Freitas 2006), 75 % are Ni hyperaccumulators (Clemens 2001). A large number of those identified belong to the Brassicaceae and Cruciferae families and, when combined, contain more than 150 hyperaccumulating species (Palmer et al. 2001). The best known hyperaccumulator may be *Thlaspi caerulescens* (alpine pennycress), while most of the plants shows toxicity symptoms at Zn accumulation of 100 mg kg^{-1}; *T. caerulescens* was shown to accumulate up to 26,000 mg kg^{-1} without any injury (Brown et al. 1995). Known Pb hyperaccumulators include *Polycarpaea synandra*, *Minuartia verna*, *Armeria maritima*, *Thlaspi alpestre*, and *T. rotundifolium* (Huang et al. 1997a).

13.5 Bioavailability of Heavy Metals

Bioavailability is largely determined by the equilibrium between heavy metals in soil solution and the solid phase. The fraction available to plants is not the same as the total concentration in the soil, much of which is locked up in the solid phase. Bioavailable metals are either in soil solution, weakly absorbed to the solid phase, or adsorbed to the solid phase but able to transfer in solution during plant growth.

Bioavailability of heavy metals is governed by physical, chemical, and biological processes within the soil environment. Both physical and chemical aspects provide the framework in which biological factors can modify heavy metal availability. Physical processes are largely dependent on soil type and include physical resistance (restricting root penetration), soil structure, texture, and low water storage capacity. Chemical aspects such as soil acidity, redox potential, and speciation may also influence the bioavailability and plant uptake of heavy metals (Ernst 1996). The role of soil pH is well documented in determining heavy metal mobilization. A decline in soil pH increases plant heavy metal uptake (Sappin-Didier et al. 2005). Chemical speciation of certain heavy metals may limit their bioavailability for plant uptake. For example, As^{V} is considered less toxic and mobile than As^{III} (Hossain 2006). Similarly, the mobility of Cr in soil depends on its oxidation state with Cr^{VI} considered more toxic and mobile than Cr^{III} (Kumpiene et al. 2008).

Biological factors including soil bacterial and fungal rhizosphere associations and higher plants may significantly modify the chemical and physical conditions which determine heavy metal bioavailability (Ernst 1996).

13.6 Agronomic Enhancement of Phytoremediation

The main bottlenecks limiting phytoextraction efficiency of plants are metal bioavailability in the soil and translocation of metals to the aboveground plant parts. Phytoavailability of metals is strongly influenced by soil characteristics such as pH, cation exchange capacity (CEC), or organic matter content, any of which may limit successful soil remediation (Kayser 2000). The effectiveness of phytoremediation efforts can be enhanced by suitable use of soil amendments and agronomic practices. Soil amendments can be chosen to increase or decrease the bioavailability of a contaminant for uptake. Also, they can increase the tilth and benefit overall plant health. Plant responses to chelates, hormones, and mycorrhizae have been investigated in order to assess plant tolerance and metal uptake and the most appropriate doses and ways of application of these means. Metals react with organic materials forming complex bonds. Thus, organic amendments such as compost shift heavy metals to a more available form. Further, composting agents are inexpensive compared to other amendments like EDTA, organic acids, hormones, etc. and require less management (Huang et al. 1997b). Arbuscular mycorrhizal fungi (AMF) symbioses occur in most habitats; mycorrhizal colonization of plant roots occurs spontaneously in almost all soils (Maier et al. 2000). Heavy metal uptake depends on plant and soil microbe interaction, especially AMF (Khan et al. 2000; Leyval et al. 2002).

Chelates solubilize metals by forming ligand-metal bonds, freeing metals from soil particles, or improving their mobility within the plant biological system. Chelates like EDTA, citrate, oxalate, etc. have been used to increase metal diffusion in soil solution and to keep the contaminants in plant available forms (Hinchman et al. 1998). These have yielded promising results. Ethylenediaminetetraacetic acid (EDTA) forms complexes with many of the metal contaminants within the natural environment. EDTA is a commonly selected chelator due to its proven effectiveness with phytoremediation applications (Madrid et al. 2003).

13.7 Case Study: North Central India

Urban soils are increasingly acting as a sink for a wide range of contaminants including heavy metals due to the rapid pace of development. Heavy metals are a threat to urban environmental quality and health of urban residents as they persist in the pedosphere for a long period of time, even after removing the pollution sources (Imperato et al. 2003). In this context, a study was made to obtain a comprehensive profile of Zn, Co, Cd, Pb, Cr, Ni, Cu, and As contamination of soils in a semi-arid urban industrial zone in India. The study area comprised two industrial centers—Agra and Firozabad. Agra (27°10′N, 78°05′E, 169 msl), on the banks of the river Yamuna, is located in Uttar Pradesh in the north central part of India. It is roughly 200 km southeast of the national capital, New Delhi. Bounded by the Thar Desert of Rajasthan on its southeast, west, and northwest peripheries, it is a semiarid area. The world renowned Mughal monument, the Taj Mahal, is situated here. Agra is world renowned for its leather industry

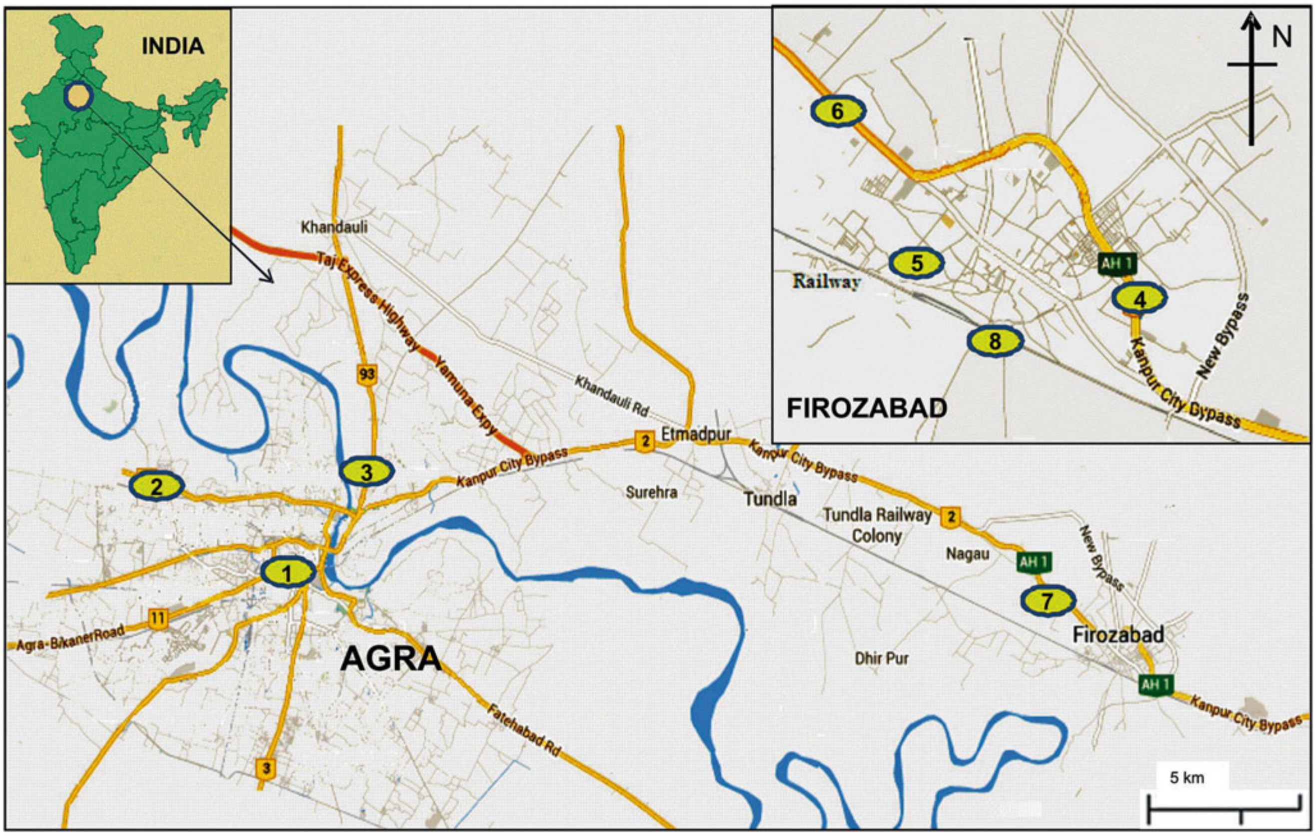

Fig. 13.2 Map of the study area

and marble handicrafts, but it also boasts a cast iron and engineering goods industry. Firozabad (27°09′N, 78°24′E, 164 msl), a town renowned for its glass industry, is about 40 km away from Agra. The Yamuna flows at a distance of 2 km. Firozabad is situated on one of the busiest national highways (NH-2) which also runs through Agra on to the capital New Delhi. The town faces a lot of traffic that includes transport vehicles that carry its glass products as well as vehicles that pass through on their way to various major cities.

Three sites at Agra (1–3) were selected and categorized based on industrial density in addition to five sites (4–8) at Firozabad evaluated earlier by the authors (Varun et al. 2012). Site 1 is a residential area near a railway station; site 2 is near national highway 2; and site 3 represents a heavily industrialized area in Agra (Fig. 13.2). Sites 4–8 present the soil-metal profile in Firozabad. Site 4, along national highway 2 in the heart of the city, is characterized by dense population of the working class with numerous small workshops in every second or third household for processing and finishing of glassware.

Site 5 represents similar demographics albeit in narrower lanes in a more backward residential area. Site 6 is the newly developed industrial estate with the biggest production units. Site 7 is another important production hub, while site 8 is a densely inhabited area along railway tracks. The area is dotted with junkyards where glass cullet is segregated for recycling and workshops. Thus, the eight sites selected characterize different urban localities, viz., roadside/industrial (sites 2, 4), industrial (sites 3, 6, 7), and residential (sites 1, 5, 8) zones. At each site, five composite samples were collected. Each composite sample was made from five random samples from a depth of 0–15 cm using a core sampler. Plant species growing wild at these sites were identified, and samples (shoot and root) were collected to compare their accumulation and tolerance of metal(s) in order to identify potential candidate species for efficient, practical phytoremediation. Control/background values were obtained from a rural farm.

13.7.1 Physicochemical Profile

Physicochemical properties of the soil collected from different sites are given in Table 13.1. The study area is a part of Indo-Gangetic alluvium of quaternary age. The topsoil is sandy loam (sand 60–80 %, silt 10–24 %, clay 8–16 %). It has high exchangeable sodium percentage (ESP) values and moderate water-retaining capacity. The soils had neutral to slightly alkaline pH (pH 6.24–8.04). Soils samples from sites 2, 3, and 8 had slightly lower pH.

Table 13.1 Physicochemical profile of soil samples

Sites	pH (1:1.25)	Electrical conductivity (dS m^{-1}) (1:2.5)	Organic matter (%)	Available phosphate (kg ha^{-1})	Available potash (kg ha^{-1})	Available nitrogen (kg ha^{-1})
1	7.41–7.73	0.44–0.53	0.5–0.82	50.0–67.2	143–191	76.3–87.8
2	7. 06–7.47	0.46–0.51	1.53–1.81	115.2–133.1	298–322	60.8–68.2
3	6.24–7.02	0.38–0.44	0.1–0.26	131.3–138.5	94–124	55.2–63.7
4	7.67–7.73	0.57–0.69	0.72–1.64	51.3–68.01	534–663	72.0–86.3
5	7.23–7.41	0.39–0.44	0.25–0.34	17.2–36.6	245–386	54.2–68.3
6	7.85–8.04	0.77–0.96	1.23–1.8	53.2–72.2	653–786	88.4–99.2
7	7.35–7.54	0.45–0.51	0.43–0.62	24.6–43.6	447–566	64.9–74.3
8	7.03–7.26	0.32–0.37	0.16–0.23	11.7–16.8	204–332	32.1–48.1
Control	7.21	0.54	1.68	70.5	393	112.9

Soils with values <25 kg ha^{-1} of available phosphate are classified as low phosphate soils whereas those in the 25–50, 50–100, and >100 kg ha^{-1} bracket as medium, high, and very high phosphate soils, respectively. Available phosphate (P_2O_5) values of the samples indicate that soils from sites 2 and 3 fall in the very high phosphate availability bracket; soils from sites 1, 4, 6, and control site fall in the high category; and sites 5 and 8 fall in the medium category. Available potash (K_2O) values for soils were found in the range of 94–786 kg ha^{-1}. Soils containing <130, 130–330, 330–600, and >600 kg ha^{-1} of available K_2O are categorized as low, medium, high, and very high potash soils, respectively. In the present study, it was noted that soils from site 6 fall under the very high potash category; those from sites 5 and 7 fall under the high potash category; those from sites 1, 2, and 8 fall under the medium potash category; and soils from site 3 are in the low potash category. Available potash in soils from site 4 ranged from medium to high. Available nitrogen content in the soil samples ranged from 32.1 to 99.2 kg ha^{-1} all below the control soil nitrogen content of 112.9 kg ha^{-1}.

13.7.2 Heavy Metal Concentrations in Soils

Individual elements displayed remarkably different patterns of accumulation in soils. Furthermore, observed differences in the magnitude of accumulation suggest that the relative contribution of the individual elements to total heavy metal contamination varies. Of the eight elements studied, Zn, Ni, and As showed a markedly greater accumulation, whereas accumulation of Pb, Cd, Cu, and Cr was high in limited samples. Samples from industrial sites exhibited higher metal content reflecting the land use pattern. Romkens and Salomons (1998) assessed the impact of both land use and soil textures on Cd, Zn, Pb, and Cu based on samples collected from the major land use/land cover pattern of Dutch forests and arable soils drawn from six different sites. Metal content in agricultural and industrial soil is found to be higher than the forest soil.

Concentrations of heavy metals in the soil samples have been summarized in Table 13.2. High metal contamination profile of almost all sites indicates the extent of damage caused in the pedosphere. Heavy metal contamination of soils ranged from 35.5 to 780 mg Pb kg^{-1}, 3.64 to 107 mg Cd kg^{-1}, 78.6 to 1,247 mg Zn kg^{-1}, 25.7 to 300 mg Cu kg^{-1}, 4.2 to 63.7 mg Co kg^{-1}, 19.1 to 158 mg Cr kg^{-1}, 23.1 to 285 mg Ni kg^{-1}, and 9.25 to 204 mg As kg^{-1}. One-way analysis of variance (ANOVA) indicated statistically significant difference ($P<0.05$) in mean metal(loid)s content of soil samples in the case of Zn, Cu, Co, Cr, Ni, and As.

Sites 4, 6, and 8 exhibited consistently higher concentrations of metals. The contamination at sites 1, 2, 3, and 7 was in general lower than the industrial thresholds mentioned in Table 13.2. However, As concentrations were above this limit at all sites. Maximum concentration of Cd, Zn, Cu, Cr, and As and second highest levels of Co and Ni were obtained from soils at site 4. Highest Pb content was recorded at site 8. This was to be expected, given the fact that the study was carried out in industrial areas with little or no monitoring of processes (including usage and disposal of hazardous chemicals), but high indices in nearly every zone are alarming because these include densely populated areas of the lower economic strata (sites 4, 5, and 8) that depend on this industry for its livelihood. The local populace is, thus, exposed to a wide range of well-established toxins and even carcinogens. In a preliminary survey carried out by the authors, it was also found that most prevalent ailments among the workers (and their families in the case of small workshops) were lung, kidney, and eye related. These are in fact the most common health issues related to metal toxicity (Vamerali et al. 2010).

When compared with the toxic levels for humans as reported in soil (SEPAC 1995), all metals especially Cd exceeded the levels prescribed, except for Ni (at three sites, i.e., 5, 7, and 8) and Cu (site 7). The other metals were also high (especially sites 4, 5, 6, and 8) in context of the thresholds for industrial soils suggested in the Canadian Environmental Quality Guidelines (2003). The situation is drastically different in the perspective of the residential thresholds as these limits are exceeded in almost all zones. This is extremely relevant since sites 4, 5, and 8 are densely populated residential zones with small, virtually unmonitored workshops in narrow lanes. Sites 6 and 7 are established industrial areas with the larger production units; hence, there is some extent of planning and monitoring involved. The extent of soil contamination was not surprising, given the fact that the study was being carried out in an area which has already been contaminated with metals, but high concentrations at nearly every site are alarming because these include heavily populated areas of the lower economic strata. The local populace is, thus, exposed to a wide range of historically well-established toxins and even carcinogens. The situation is surely compounded by vehicular pollution in some areas. Vehicular emissions are a significant source of many pollutants (Imperato et al. 2003).

It is significant to note here that in studies similar to the present one, the degree of contamination and the resulting "hazard indices" for soils may vary when different thresholds, existing in only a few countries, are considered (Modis and Komnitsas 2007). To increase the reliability of risk estimation due to contaminants, global consensus on such thresholds is urgently needed.

Several authors have pointed out the need for a better knowledge of urban soils. In the past few years, studies on urban soils in many cities have been carried out around the world. The mean heavy metal contents in soils of some cities around the world are presented in Table 13.3. The differences concerning population, living habits, industrial activities, etc. cause significant differences in the metal contamination profile. Average concentrations of Pb in nearly all cases were comparable to cities like London (UK), Naples, and Palermo (Italy). In the case of Cd, it was up to five times higher than some other Indian cities. Zn content was higher than most cities in Table 13.3. Cu, Co, Cr, and Ni patterns were not very different from other cities in most cases. The metal content reported in the literature was highest at Kattedan, Andhra Pradesh (industrial hub), of India.

13.7.3 Potential of Indigenous Species

Wild and/or native plants are generally considered better phytoremediators as compared to known metal bioaccumulators from the literature (Porębska and Ostrowska 1999). Metal hyperaccumulators like *Thlaspi caerulescens* and *Alyssum bertolonii* are able to take up and accumulate appreciable amounts of metals in their tissues; their use in the field is limited because they are slow growing with shallow root systems and are not very widespread. In most cases, their biomass yield is also quite low (Saraswat and Rai 2009; Saifullah et al. 2009). Also, the technology for their large-scale cultivation is not fully developed; therefore, their use is rather limited (Pulford and Watson 2003). If soil at contaminated sites, e.g., mines, is naturally high in a particular metal, native plants will often become adapted over time to the locally elevated levels (Pratas et al. 2013). Allowing native species to remediate soils is an attractive proposition since native wild species do not require frequent irrigation, fertilization, and pesticide treatments, while simultaneously a plant community comparable to that existing in the vicinity can be established. The choice of plant species is, thus, an important task in any phytoremediation-based technique. Many native, well-adapted plants have been investigated and even used for heavy metal bioindicatoring and phytoremedial purposes including lemongrass and other wild grasses, vetiver, *Sesbania*, *Avena*, *Crotalaria*, *Crinum asiaticum*, *Typha latifolia*, *Calotropis procera*, etc. (Yang et al. 2003; Uraguchi et al. 2006; Varun et al. 2011a, b; D'Souza et al. 2010). Keeping in mind these facts, the phytoremediation potential of wild plant species growing at the test sites was collected and analyzed for the presence of heavy metals/metalloids. Roots and shoots were analyzed separately to get a picture of accumulation patterns. 25 plant species (Table 13.4) including 20 herbs, 3 shrubs, and 2 grasses were assessed for metal concentrations. Individual elements and species displayed remarkably different trends of accumulation in shoots and roots (Table 13.5). Metal translocation patterns indicate that >55 % plants had higher concentrations of Zn, Cu, and As in their shoots. Pb, Cr, and Ni were almost equally partitioned between shoots and roots. It has been reported that As, Cr, and Pb are stored mainly in root cells (Mellem et al. 2009), whereas Zn is accumulated in green tissues like leaves (Probst et al. 2009). Co and Cd were predominantly partitioned in the roots in ~65 % of plants studied. Chaney and Giordano (1977) classified Mn, Zn, Cd, B, Mo, and Se as elements readily translocated to the plant shoots and Ni, Co, and Cu as intermediate; and Cr, Pb, and Hg were translocated to the lowest extent (Alloway 1995).

Maximum Cu accumulation was observed in *Poa annua* at 742.06 mg kg^{-1} dry weight in shoot. The species has been reported to accumulate up to 600, 460, and 350 mg kg^{-1} dry weight of Cu, Zn, and Ni, respectively, in shoot (Pedron et al. 2009). Comino et al. (2009) reported high removal efficiency in the plant with accumulation up to 290 mg kg^{-1} dry weight for As. None of the species in the present study crossed the hyperaccumulation thresholds in aboveground biomass for different metals concerned. However, the uptake ranges are encouraging when compared to reported toxic concentrations in plants (Table 13.5).

In the case of Pb, Cr, and Cu, all 25 species were able to withstand the toxic levels. *Croton bonplandianum*, *Sida longifolia*, and *Eclipta alba* accumulated up to 4.5 times the toxicity threshold of Pb; *Cannabis sativa* and *Rumex dentatus* accumulated up to 20 times the Cr threshold; and in the case of Cu, *Poa annua* accumulated above 35 times

Table 13.2 Heavy metal concentrations in soils (mg kg^{-1})

Sites		Pb	Cd	Zn	Cu	Co	Cr	Ni	As
1	Range	136.7–177	13.4–16.7	105–126	25.7–36.4	6.1–7.5	31.4–42.7	33.2–46.3	16.3–20
	Avg.	153	14.6	115	30.1	6.8	35.6	38.4	17.6
	SD	14.7	1.27	7.89	3.91	0.61	4.28	4.85	1.45
2	Range	196.4–279	14.7–20.6	70.3–116	32.3–41.2	4.2–5.71	65.4–73.4	30.1–39.6	18–23.4
	Avg.	223	17.6	91.8	36.3	4.66	72.4	34.5	20.3
	SD	29.6	2.58	16.1	3.32	0.62	3.08	3.65	1.98
3	Range	267.4–331	20.1–22.7	215–261	57.4–68.7	4.79–5.3	82.4–97.6	58.6–73.0	27.5–30.1
	Avg.	294	21.6	238	61.8	5.01	90.2	66.4	28.4
	SD	23.2	1	17.7	4.26	0.19	6.08	6.57	1.01
4[a]	Range	92.9–638	3.77–107	87–935	37.5–300	16.9–63.7	133.7–158	25.8–218	10.3–204
	Average	237.3	34.3	533	178	30.0	147	101	96.7
	SD	229	43.1	311	116	19.1	9.67	80.9	97.3
5[a]	Range	35.5–781	7.99–37.7	76.3–1,247	22.4–191	14.7–35.2	19.1–34.4	23.1–48.4	9.25–60.3
	Average	303	17.1	377	75.9	20.1	26.8	30.8	31.5
	SD	312	12.8	492	68.9	8.55	5.83	10.1	24.3
6[a]	Range	50.3–354	6.7–17.6	198–974	37.3–265	13.3–54.3	32.9–106	24.6–285	18.9–43.2
	Average	216	11.9	435	133	32.3	62.5	131	28.5
	SD	118	4.18	314	96.3	16.6	29.1	98.3	11.2
7[a]	Range	47.5–86.6	3.64–5.50	78.6–123	26.2–51.2	16.4–26.2	35–57.4	30.9–61.2	22.3–657
	Average	66.1	4.58	91.1	34.4	21.7	49.2	46.8	40.5
	SD	16.9	0.86	18	9.7	4.43	9.35	11.5	13.7
8[a]	Range	99.9–776	11.8–35.0	322–435	52.9–76.4	10.9–24.3	27.3–47.2	31.5–52.3	70.2–117
	Average	378	26.6	390	63.3	18.7	37.9	39.8	91.8
	SD	256	8.9	50.3	10.1	5.1	8.7	8.5	17.8
	F value	ns	ns	*	*	*	*	*	*
Control/background values of study area		39.4	4.41	269	19.3	18.4	25.3	22.7	5.2
Suggested thresholds in soil[b]	Industrial	600	22	360	91	–	87	50	12
	Residential	140	10	200	63	–	64	50	12
Suggested thresholds in soil[c]	Background	–	–	140	–	–	100	35	29
	Intervention	–	–	720	–	–	380	210	55
Suggested thresholds for human health[d]		250	0.3	200	50	–	150	60	30

F value, "*" statistically significant and "ns" not significant.

SD standard deviation

[a] Varun et al. (2012)

[b] Canadian Environmental Quality Guidelines 2003 proposed by The Canadian Council of Ministers of the Environment

[c] VROM (Dutch Ministry of Housing, Spatial Planning and the Environment), Ministerial Circular on Target and Intervention Values for Soil Remediation, 2000

[d] State Environmental Protection Administration of China (1995), Chinese Environmental Quality Standard for Soils (GB15618-1995)

the toxicity threshold. In the case of As, nearly all the species were able to withstand the reported toxicity threshold with *Poa annua* and *Eclipta alba* displaying nearly seven times the limit. The picture of Cd tolerance across the species evaluated was also good with four species crossing the limit in shoots and about eight species in roots. *Poa annua*, *Datura stramonium*, *Rumex dentatus*, and *Lycopersicon esculentum* were able to withstand toxic levels of Zn. Chlorophyll and free proline pattern indicate that most plants have evolved strategies to counter phytotoxicity due to accumulated Cd, Pb, and Cu at much above reported toxic levels.

Table 13.3 Average heavy metal concentrations in urban soils from different cities across the world (mg kg^{-1})

City	Pb	Cd	Zn	Cu	Co	Cr	Ni	As	Reference
London	294	–	183	73	–	–	–	–	Thornton (1991)
Madrid	161	–	210	72	–	75	14	–	De Miguel et al. (1998)
Rostock	83	–	100	35	–	48	30	–	Kahle (2000)
Sevilla	161	–	107	64.6	–	42.8	23.5	–	Madrid et al. (2004)
Belgrade	53.2	–	129	29	–	33.2	67.4	–	Crnković et al. (2006)
Palermo	253	–	151	77	–	39	19.1	–	Manta et al. (2002)
Naples	262	–	251	11	–	74	n.a.	–	Imperato et al. (2003)
Nanjing	107	–	163	66.1	–	84.7	n.a.	–	Lu et al. (2003)
Hong Kong	93.4	–	168	24.8	–	n.a.	n.a.	–	Li et al. (2004)
Kattedan	195–6,241	0.08–0.16	130–3,191	72–1,450	12–36	77–586	63–494	0.10–0.21	Sekhar et al. (2006)
Agra	204	15.5	141	39	5	59	44	20	Varun et al. (2011c)

Table 13.4 Taxonomical information of plants surveyed

S. No.	Botanical name	Common name	Family	Habit
1	*Amaranthus spinosus*	Spiny amaranth	Amaranthaceae	Herb
2	*Boerhavia diffusa*	Red spiderling	Nyctaginaceae	Herb
3	*Croton bonplandianum*	Bonpland's croton	Euphorbiaceae	Herb
4	*Cynodon dactylon*	Bermuda grass	Poaceae	Grass
5	*Eclipta alba*	False daisy	Asteraceae	Herb
6	*Leucas cephalotes*	Drona puspi	Lamiaceae	Herb
7	*Ricinus communis*	Castor bean	Euphorbiaceae	Shrub
8	*Datura stramonium*	Thorn apple	Solanaceae	Shrub
9	*Euphorbia hirta*	Asthma Weed	Euphorbiaceae	Herb
10	*Parthenium hysterophorus*	Santa Maria feverfew	Asteraceae	Herb
11	*Solanum nigrum*	Black nightshade	Solanaceae	Herb
12	*Tephrosia purpurea*	Wild Indigo	Fabaceae	Herb
13	*Withania somnifera*	Winter cherry	Solanaceae	Herb
14	*Argemone mexicana*	Mexican prickly poppy	Papaveraceae	Herb
15	*Gnaphalium luteo-album*	Jersey cudweed	Asteraceae	Herb
16	*Poa annua*	Annual bluegrass	Poaceae	Grass
17	*Sida longifolia*	Common wireweed	Malvaceae	Herb
18	*Chenopodium murale*	Goosefoot	Amaranthaceae	Herb
19	*Lycopersicon esculentum*	Tomato	Solanaceae	Herb
20	*Calotropis procera*	Milkweed	Asclepiadaceae	Shrub
21	*Physalis minima*	Native gooseberry	Solanaceae	Herb
22	*Cannabis sativa*	Hemp	Cannabaceae	Herb
23	*Heliotropium ellipticum*	Heliotropes	Boraginaceae	Herb
24	*Rumex dentatus*	Toothed dock	Polygonaceae	Herb
25	*Tridax procumbens*	Tridax daisy	Asteraceae	Herb

13.7.4 Metal Accumulation Characteristics

Phytoremediators may be metal specific, performing well for a particular metal or metals in combination. Moreover, phytoremedial potential of plants is also influenced by the mobility and availability of heavy metals in soil and plants. The following factors are utilized universally to evaluate the phytoextraction/phytostabilization potential of plant species:

$$\text{Bioabsorption coefficient}\left[\text{BAC}\right] = \frac{\text{metal content in shoot}}{\text{metal content in soil}}$$

$$\text{Bioconcentration factor}\left[\text{BCF}\right] = \frac{\text{metal content in root}}{\text{metal content in soil}}$$

$$\text{Translocation factor}\left[\text{TF}\right] = \frac{\text{metal content in shoot}}{\text{metal content in root}}$$

Table 13.5 Heavy metal concentrations in plants surveyed (mg kg^{-1})

Sites	Plants	Pb		Cd		Zn		Cu	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
1	*C. bonplandianum*	45.9 ± 5.23	38.2 ± 6.41	1.72 ± 0.32	2.26 ± 0.86	7.98 ± 2.41	8.86 ± 3.51	8.10 ± 2.51	4.62 ± 1.04
	C. sativa	18.3 ± 3.91	27.8 ± 4.07	2.92 ± 0.67	2.43 ± 1.08	22.9 ± 4.61	16.3 ± 6.28	15.1 ± 4.06	11.0 ± 3.64
	D. stramonium	22.9 ± 4.62	28.6 ± 3.6	9.34 ± 2.44	8.49 ± 3.61	34.2 ± 5.12	25.3 ± 4.82	31.1 ± 6.14	9.63 ± 4.27
	A. spinosus	21.4 ± 2.75	47.4 ± 5.17	2.92 ± 1.06	3.70 ± 1.22	14.8 ± 2.87	25.2 ± 6.22	6.30 ± 1.88	14.4 ± 2.66
	C. procera	166 ± 23.6	114 ± 16.8	17.5 ± 3.22	33.4 ± 4.7	116 ± 22.8	154 ± 28.6	38.1 ± 8.21	47.6 ± 8.3
2	*L. esculentum*	134 ± 17.6	112 ± 18.4	17.9 ± 4.6	13.8 ± 2.83	119 ± 19.6	198 ± 33.4	36.6 ± 3.87	31.3 ± 6.81
	R. dentatus	35.6 ± 4.71	47.5 ± 7.21	5.28 ± 2.4	6.04 ± 1.42	37.6 ± 5.21	28.9 ± 6.03	22.1 ± 4.61	27.0 ± 4.52
	P. annua	312 ± 28.7	86.7 ± 4.9	11.4 ± 3.21	19.4 ± 4.67	56.9 ± 7.32	81.3 ± 3.61	149 ± 27.3	15.9 ± 1.8
	S. nigrum	13.0 ± 2.43	13.1 ± 2.71	12.4 ± 4.63	9.04 ± 3.72	24.7 ± 4.62	14.0 ± 2.87	10.9 ± 4.07	5.88 ± 2.61
3	*R. communis*	67.6 ± 9.61	55.4 ± 4.6	2.8 ± 0.73	2.34 ± 1.62	47.4 ± 8.2	67.7 ± 4.62	27.8 ± 5.12	13.6 ± 3.71
	S. longifolia	97.0 ± 7.27	69.3 ± 9.04	9.07 ± 2.06	11.4 ± 4.76	43.2 ± 10.5	37.2 ± 3.76	49.4 ± 8.62	38.2 ± 7.12
	B. diffusa	26.4 ± 3.71	39.8 ± 5.71	2.64 ± 0.42	2.44 ± 1.08	30.1 ± 5.08	28.1 ± 4.21	11.7 ± 9.41	10.1 ± 6.58
	A. mexicana	37.4 ± 6.70	85.0 ± 11.6	5.61 ± 2.7	3.12 ± 1.32	42.6 ± 5.4	54.6 ± 5.61	17.0 ± 5.2	10.1 ± 2.44
4	*A. spinosus*[a]	37.0 ± 13.4	86.6 ± 5.5	8.20 ± 1.45	11.6 ± 1.46	79.8 ± 13.8	127 ± 14.2	34.1 ± 3.7	94.6 ± 7.3
	B. diffusa[a]	27.9 ± 3.14	40.3 ± 2.6	8.53 ± 1.2	6.71 ± 1.24	58.7 ± 4.05	53.6 ± 1.91	28.3 ± 4.91	25.3 ± 2.7
	C. bonplandianum[a]	89.7 ± 7.68	33.5 ± 4.5	3.53 ± 0.71	4.11 ± 0.25	45.8 ± 12.1	39.5 ± 4.35	50.8 ± 25.1	24.1 ± 3.27
	C. dactylon[a]	31.5 ± 4.05	26.6 ± 2.43	5.46 ± 0.82	7.22 ± 0.63	73.1 ± 6.7	87.1 ± 5.75	23.0 ± 3.9	16.9 ± 2.87
	E. alba[a]	40.7 ± 15.4	97.4 ± 15.6	6.54 ± 0.96	12.1 ± 1.3	79.9 ± 6.2	125 ± 15.0	23.1 ± 2.28	55.3 ± 6.4
	L. cephalotes[a]	49.1 ± 2.90	53.4 ± 5.02	2.91 ± 0.52	2.72 ± 0.58	42.8 ± 1.34	23.6 ± 2.67	124 ± 8.16	50.5 ± 18.3
	R. communis[a]	47.6 ± 20.5	27.4 ± 7.12	3.74 ± 0.67	3.03 ± 20.6	88.6 ± 15.8	116 ± 13.6	101 ± 13.3	41.7 ± 6.2
	D. stramonium	26.0 ± 5.32	30.3 ± 4.02	12.4 ± 2.31	11.4 ± 3.21	156 ± 22.6	130 ± 18.4	180 ± 17.9	58.1 ± 8.42
	C. murale	142 ± 18.2	79.0 ± 13.6	38.2 ± 4.87	35.3 ± 6.23	559 ± 37.4	349 ± 24.1	182 ± 28.3	71.7 ± 5.2
	W. somnifera	28.4 ± 4.61	79.3 ± 10.1	10.2 ± 1.52	11.4 ± 2.8	74.4 ± 13.1	94.2 ± 15.2	64.1 ± 11.8	156 ± 23.6
5	*D. stramonium*[a]	41.3 ± 19.1	44.0 ± 4.78	12.8 ± 2.1	9.18 ± 2.05	142 ± 39.1	99.4 ± 61.7	146 ± 4.66	40.4 ± 7.02
	E. hirta[a]	61.5 ± 4.01	46.9 ± 10.7	9.03 ± 0.24	13.7 ± 0.51	82.2 ± 5.9	73.4 ± 7.7	34.2 ± 2.92	17.6 ± 4.05
	P. hysterophorus[a]	36.3 ± 11.2	48.7 ± 31.9	4.27 ± 1.14	3.98 ± 0.82	52.5 ± 10.2	36.6 ± 9.06	30.0 ± 6.04	47.5 ± 39.2
	S. nigrum[a]	28.4 ± 5.21	23.8 ± 4.05	12.9 ± 1.13	9.73 ± 3.37	82.6 ± 6.01	47.8 ± 8.5	18.1 ± 5.4	9.05 ± 1.57
	T. purpurea[a]	50.7 ± 3.01	26.4 ± 7.1	11.8 ± 2.8	8.25 ± 2.5	75.2 ± 5.2	51.9 ± 6.05	56.5 ± 8.41	22.7 ± 5.6
	W. somnifera[a]	32.2 ± 11.2	94.7 ± 8.57	9.12 ± 2.4	10.4 ± 3.57	84.5 ± 8.5	123 ± 22.3	33.2 ± 9.4	106 ± 4.8
	C. bonplandianum	99.5 ± 18.1	62.1 ± 12.4	1.87 ± 0.26	2.33 ± 0.83	37.6 ± 3.76	38.2 ± 5.23	22.8 ± 7.23	13.9 ± 2.86
	E. alba[a]	45.3 ± 8.11	104 ± 18.1	3.92 ± 0.63	6.53 ± 2.4	63.9 ± 9.84	107 ± 17.2	8.36 ± 1.62	18.5 ± 1.22
6	*A. mexicana*[a]	31.0 ± 3.06	63.5 ± 7.21	3.98 ± 0.65	2.47 ± 0.15	57.8 ± 9.3	78.8 ± 4	31.0 ± 2.1	19.1 ± 0.85
	G. luteo-album[a]	25.2 ± 3.21	33.1 ± 3.5	6.04 ± 1.08	6.14 ± 2.07	78.1 ± 9	66.1 ± 5.47	27.3 ± 4.3	33.6 ± 9.4
	P. annua[a]	298 ± 14.6	78.1 ± 22.8	9.15 ± 5.01	16.7 ± 5.52	178 ± 24.6	286 ± 15.8	742 ± 32.8	66.2 ± 8.5
	S. longifolia[a]	75.1 ± 19.3	56.9 ± 6.95	5.47 ± 0.65	7.54 ± 0.38	51.7 ± 3.47	46.7 ± 3.14	92.4 ± 12.1	56.1 ± 6.6
	B. diffusa	30.2 ± 4.12	32.4 ± 5.06	2.36 ± 1.06	1.91 ± 0.41	39.2 ± 3.23	41.2 ± 5.2	17.3 ± 3.52	21.2 ± 4.22
	R. communis	41.0 ± 6.08	26.1 ± 4.22	1.60 ± 0.22	1.71 ± 0.23	65.3 ± 4.77	93.2 ± 11.7	81.8 ± 7.34	38.9 ± 2.85
	P. hysterophorus	32.4 ± 7.83	40.5 ± 2.71	2.75 ± 0.41	2.99 ± 1.1	69.6 ± 10.2	58.0 ± 7.28	41.9 ± 4.85	68.6 ± 11.4
7	*C. murale*[a]	58.8 ± 3.2	37.3 ± 9.06	9.62 ± 1.86	5.60 ± 0.47	92.8 ± 33.03	63.6 ± 9.66	36.6 ± 19.4	13.7 ± 9.6
	L. esculentum[a]	53.7 ± 8.6	49.9 ± 7.38	5.20 ± 1.21	4.25 ± 0.9	102 ± 12.6	167 ± 8.21	36.2 ± 2.7	30.4 ± 4.2
	C. procera[a]	49.7 ± 17.6	56.9 ± 8.26	6.91 ± 3.91	12.7 ± 0.67	95.6 ± 10.0	138 ± 18.1	40.7 ± 9.94	53.6 ± 6.9
	A. spinosus	11.3 ± 1.54	23.1 ± 2.1	0.50 ± 0.01	1.37 ± 0.67	10.1 ± 1.87	20.1 ± 3.16	5.50 ± 1.07	17.2 ± 3.76
	S. nigrum	9.24 ± 3.40	7.10 ± 1.6	3.66 ± 1.13	2.37 ± 1.22	26.4 ± 5.26	14.6 ± 2.73	11.0 ± 2.59	6.27 ± 2.01
	E. alba	7.90 ± 2.16	20.8 ± 3.88	0.73 ± 0.12	1.46 ± 0.86	16.3 ± 2.61	23.7 ± 4.52	3.09 ± 0.84	6.73 ± 1.08
8	*P. minima*[a]	39.7 ± 21.8	26.4 ± 2.32	3.57 ± 0.79	1.99 ± 0.32	45.6 ± 3.07	41.7 ± 2.8	19.8 ± 3.12	18.4 ± 2.4
	C. sativa[a]	30.9 ± 6.35	53.9 ± 4.9	4.49 ± 1.03	4.48 ± 0.8	63.2 ± 7.8	46.8 ± 4.75	28.2 ± 3.6	21.2 ± 2.8
	H. ellipticum[a]	35.1 ± 9.01	53.5 ± 37.1	11.2 ± 5.9	7.08 ± 2.51	59.6 ± 13.3	56.3 ± 21.8	28.8 ± 4.6	58.0 ± 7.14
	R. dentatus[a]	45.8 ± 7.27	67.1 ± 21.4	6.68 ± 3.6	8.03 ± 3.85	117 ± 14.6	115 ± 14.2	34.2 ± 11.6	46.2 ± 9.3
	T. procumbens[a]	34.7 ± 9.3	21.4 ± 4.92	7.36 ± 2.78	13.2 ± 1.12	68.1 ± 17.12	97.1 ± 13.5	28.9 ± 6.27	14.4 ± 2.1
	E. hirta	58.2 ± 9.22	42.6 ± 6.73	11.9 ± 2.4	15.2 ± 3.21	89.6 ± 9.41	76.1 ± 8.34	26.3 ± 4.23	16.4 ± 4.87
	C. murale	254 ± 28.3	133 ± 14.2	34.5 ± 5.2	31.4 ± 3.4	358 ± 18.7	156 ± 28.6	75.9 ± 10.9	32.6 ± 7.31
	C. dactylon	22.0 ± 3.82	20.6 ± 4.08	4.97 ± 1.3	3.97 ± 0.54	42.9 ± 3.53	52.8 ± 9.34	6.30 ± 2.31	5.27 ± 0.82
	Phytotoxicity levels[b]	10–20		5–10		150–200		15–20	

(continued)

Table 13.5 (continued)

		Co		Cr		Ni		As	
Sites	Plants	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
1	*C. bonplandianum*	1.08 ± 0.33	1.61 ± 0.62	3.20 ± 1.36	8.21 ± 2.63	7.20 ± 1.41	9.00 ± 2.65	4.50 ± 1.61	7.04 ± 2.41
	C. sativa	3.46 ± 1.61	2.66 ± 0.72	39.1 ± 8.23	13.6 ± 3.51	11.5 ± 3.76	8.20 ± 1.92	7.92 ± 0.84	4.32 ± 0.86
	D. stramonium	2.04 ± 0.97	2.26 ± 0.57	29.8 ± 3.7	7.44 ± 1.65	13.5 ± 2.75	11.9 ± 3.59	18.8 ± 2.14	9.32 ± 2.72
	A. spinosus	1.10 ± 0.28	1.20 ± 0.72	4.20 ± 1.86	2.90 ± 0.86	4.90 ± 1.05	13.2 ± 2.83	1.40 ± 0.28	1.28 ± 0.46
	C. procera	2.44 ± 0.53	3.22 ± 1.4	11.0 ± 3.23	9.12 ± 2.71	15.3 ± 2.62	11.8 ± 3.07	14.2 ± 1.51	11.9 ± 3.15
2	*L. esculentum*	1.86 ± 0.36	2.16 ± 1.06	26.3 ± 5.31	49.2 ± 6.19	11.0 ± 3.61	8.49 ± 1.23	26.4 ± 5.21	17.5 ± 3.84
	R. dentatus	1.67 ± 0.62	1.76 ± 0.64	80.3 ± 13.7	152 ± 17.3	12.1 ± 1.95	13.1 ± 2.56	7.71 ± 1.89	8.96 ± 1.85
	P. annua	1.39 ± 0.41	2.33 ± 0.92	36.2 ± 6.24	42.5 ± 4.77	4.14 ± 2.83	5.17 ± 1.02	44.7 ± 5.71	89.3 ± 12.7
	S. nigrum	1.86 ± 0.87	2.07 ± 0.56	7.16 ± 4.98	<1	12.0 ± 3.62	9.18 ± 3.71	6.49 ± 1.15	2.82 ± 0.62
3	*R. communis*	0.71 ± 0.12	0.88 ± 0.09	28.8 ± 5.21	9.02 ± 2.87	12.3 ± 3.1	8.20 ± 1.21	6.53 ± 1.94	3.73 ± 1.2
	S. longifolia	0.95 ± 0.18	1.18 ± 0.72	8.10 ± 2.77	16.2 ± 3.61	6.64 ± 2.83	8.30 ± 0.92	7.38 ± 2.17	8.26 ± 1.86
	B. diffusa	1.66 ± 0.63	2.21 ± 1.02	10.6 ± 4.21	<1	9.30 ± 2.04	10.3 ± 0.52	6.20 ± 2.6	4.20 ± 1.31
	A. mexicana	0.75 ± 0.08	1.47 ± 0.44	24.3 ± 8.21	32.0 ± 7.21	5.77 ± 1.76	8.12 ± 2.51	16.4 ± 3.18	20.6 ± 4.72
4	*A. spinosus*[a]	5.61 ± 1.5	5.80 ± 1.42	11.4 ± 3.9	7.83 ± 0.8	12.6 ± 0.71	28.8 ± 2.4	7.97 ± 0.9	4.64 ± 1.5
	B. diffusa[a]	7.97 ± 1.84	6.63 ± 0.67	13.1 ± 5.4	<1	17.8 ± 4.87	11.8 ± 3.37	22.9 ± 3.12	12.9 ± 1.81
	C. bonplandianum[a]	5.06 ± 0.22	7.04 ± 0.47	8.26 ± 2.27	26.2 ± 2.05	16.1 ± 3.43	18.0 ± 3.1	20.2 ± 4.08	28.2 ± 2.76
	C. dactylon[a]	5.24 ± 0.32	3.68 ± 0.17	34.6 ± 4.23	18.7 ± 2.7	14.4 ± 2.1	8.16 ± 2.2	21.5 ± 3.45	27.0 ± 3.72
	E. alba[a]	6.22 ± 0.57	11.2 ± 3.42	21.2 ± 4.63	47.9 ± 8.42	10.8 ± 2.46	19.2 ± 2.42	84.3 ± 10.2	146 ± 11.6
	L. cephalotes[a]	5.18 ± 0.9	11.5 ± 2.01	24.9 ± 1.28	16.2 ± 3.23	9.38 ± 1.02	8.13 ± 0.6	15.3 ± 2.83	26.4 ± 5.75
	R. communis[a]	6.01 ± 1.1	6.51 ± 1.24	38.8 ± 18.6	10.9 ± 6.6	17.2 ± 2.92	9.93 ± 1.64	22.7 ± 5.87	12.4 ± 2.56
	D. stramonium	7.03 ± 2.14	6.39 ± 2.1	31.4 ± 4.21	8.97 ± 1.87	16.2 ± 6.22	13.4 ± 3.6	78.2 ± 14.3	39.5 ± 6.1
	C. murale	4.18 ± 1.64	4.98 ± 1.4	17.6 ± 6.7	19.2 ± 5.2	13.4 ± 2.8	17.3 ± 4.17	58.0 ± 8.08	76.3 ± 14.2
	W. somnifera	16.2 ± 2.87	13.4 ± 3.2	13.2 ± 3.41	22.1 ± 4.6	22.4 ± 4.13	24.6 ± 4.2	28.2 ± 5.6	22.4 ± 6.71
5	*D. stramonium*[a]	5.24 ± 0.8	5.02 ± 0.6	27.3 ± 18.6	6.65 ± 2.78	13.4 ± 0.26	11.8 ± 2.65	40.0 ± 14.8	15.7 ± 12.6
	E. hirta[a]	8.42 ± 0.8	11.7 ± 1.3	17.5 ± 2.08	20.8 ± 1.9	19.8 ± 1.53	13.9 ± 0.34	38.2 ± 2.26	19.4 ± 1.54
	P. hysterophorus[a]	4.74 ± 0.75	4.38 ± 0.7	5.72 ± 2.7	4.11 ± 1.6	11.1 ± 1.33	11.2 ± 1.24	5.46 ± 1.1	4.83 ± 2.6
	S. nigrum[a]	7.59 ± 2.26	6.18 ± 1.4	1.62 ± 0.53	<1	11.5 ± 2.6	9.29 ± 2.07	9.48 ± 0.63	3.81 ± 1.08
	T. purpurea[a]	4.14 ± 0.21	3.24 ± 0.41	2.61 ± 0.75	3.90 ± 0.92	7.18 ± 1.4	11.7 ± 2.17	11.4 ± 2.15	6.38 ± 1.12
	W. somnifera[a]	10.0 ± 4.4	8.96 ± 4.3	5.12 ± 1.5	5.68 ± 0.9	14.6 ± 1.5	16.5 ± 4.17	9.65 ± 1.74	6.05 ± 1.68
	C. bonplandianum	3.80 ± 0.65	5.02 ± 1.6	2.41 ± 0.75	8.04 ± 2.7	6.10 ± 1.06	6.41 ± 2.31	8.19 ± 3.11	9.98 ± 3.1
	E. alba	3.61 ± 0.34	7.08 ± 1.34	4.28 ± 1.2	8.93 ± 3.4	4.62 ± 2.4	7.45 ± 1.87	25.2 ± 4.8	45.3 ± 7.42
6	*A. mexicana*[a]	6.27 ± 0.46	8.91 ± 0.88	18.4 ± 2.1	25.6 ± 4.05	8.56 ± 0.47	10.3 ± 0.77	18.0 ± 3.16	21.7 ± 1.9
	G. luteo-album[a]	6.07 ± 0.73	7.13 ± 0.93	13.4 ± 5.2	21.0 ± 2.92	14.2 ± 2.8	17.4 ± 1.58	18.6 ± 3.95	29.1 ± 9.0
	P. annua[a]	6.56 ± 1.71	13.5 ± 4.91	33.3 ± 7.33	41.6 ± 8.5	11.4 ± 1.61	18.6 ± 3.7	78.6 ± 17.2	147 ± 31.1
	S. longifolia[a]	4.52 ± 0.7	6.70 ± 0.93	6.45 ± 1.35	10.8 ± 2.4	8.73 ± 0.51	11.6 ± 2.71	7.60 ± 0.84	8.78 ± 2.56
	B. diffusa	7.75 ± 1.21	8.64 ± 2.4	3.77 ± 1.08	<1	16.2 ± 6.32	13.5 ± 4.8	5.68 ± 1.83	4.70 ± 1.8
	R. communis	6.79 ± 2.87	7.50 ± 1.31	18.7 ± 5.28	6.04 ± 1.76	14.2 ± 2.84	10.9 ± 2.54	5.68 ± 2.16	3.55 ± 1.62
	P. hysterophorus	6.46 ± 2.11	5.88 ± 2.6	15.3 ± 2.3	12.5 ± 4.8	26.1 ± 4.5	23.8 ± 7.4	3.98 ± 0.96	2.84 ± 0.38
7	*C. murale*[a]	5.08 ± 1.9	5.53 ± 1.09	8.09 ± 4.17	11.2 ± 3.7	9.09 ± 0.95	12.3 ± 3.09	17.4 ± 2.54	20.6 ± 5.42
	L. esculentum[a]	5.33 ± 1.4	7.29 ± 2.08	22.1 ± 4.4	45.5 ± 13.4	16.6 ± 3.67	13.6 ± 4.28	48.0 ± 11.3	26.5 ± 4.04
	C. procera[a]	7.11 ± 3.09	8.62 ± 2.5	19.5 ± 4.6	13.2 ± 3.2	17.2 ± 3.7	15.4 ± 1.88	38.6 ± 5.8	33.2 ± 4.17
	A. spinosus	3.30 ± 0.34	3.60 ± 0.72	2.90 ± 0.34	2.40 ± 0.66	4.68 ± 1.47	12.1 ± 1.87	4.05 ± 2.06	2.66 ± 0.78
	S. nigrum	7.34 ± 1.21	5.64 ± 1.3	4.92 ± 1.52	<1	14.5 ± 3.87	11.2 ± 2.6	10.6 ± 3.61	4.76 ± 1.6
	E. alba	4.98 ± 0.91	9.40 ± 2.6	8.36 ± 3.6	17.4 ± 4.51	6.08 ± 3.1	11.9 ± 2.4	36.4 ± 6.2	60.7 ± 7.34
8	*P. minima*[a]	5.17 ± 0.8	4.82 ± 0.81	10.9 ± 8.6	4.07 ± 2.8	10.3 ± 2.13	6.48 ± 0.56	18.7 ± 13.1	4.98 ± 2.07
	C. sativa[a]	8.30 ± 2.33	8.05 ± 4.05	41.2 ± 2.6	15.1 ± 8.7	14.2 ± 3.38	10.6 ± 2.32	33.1 ± 3.4	18.7 ± 3.35
	H. ellipticum[a]	5.40 ± 2.1	4.95 ± 0.6	14.5 ± 2.22	11.7 ± 4.6	14.6 ± 3.8	12.3 ± 1.82	13.2 ± 12.1	142 ± 15.4
	R. dentatus[a]	5.83 ± 2.25	6.58 ± 1.7	40.1 ± 10.5	102 ± 13.6	15.9 ± 1.64	18.1 ± 2.9	26.1 ± 6.01	28.6 ± 3.33
	T. procumbens[a]	6.42 ± 0.78	12.1 ± 1.65	18.7 ± 3.9	23.5 ± 3.5	15.8 ± 2.93	8.50 ± 1.81	25.2 ± 3.8	23.6 ± 5.61
	E. hirta	8.17 ± 3.21	10.2 ± 1.6	25.7 ± 4.4	34.8 ± 7.31	27.8 ± 6.26	18.5 ± 7.1	55.1 ± 11.6	39.3 ± 7.23
	C. murale	3.72 ± 1.7	4.13 ± 2.1	7.65 ± 1.8	10.4 ± 2.8	5.97 ± 2.7	7.40 ± 1.8	50.5 ± 8.04	66.4 ± 8.3
	C. dactylon	3.54 ± 0.66	2.90 ± 0.44	9.72 ± 3.64	6.43 ± 2.5	7.82 ± 1.87	5.11 ± 2.4	17.4 ± 4.2	24.2 ± 4.62
	Phytotoxicity levels[b]	60–170		1–2		20–30		20	

[a] Varun et al. (2012)

[b] Vamerali et al. (2010)

These ratios help to identify the suitability of plants for phytoextraction and phytostabilization by explaining the accumulation characteristics and translocation properties of metals in plants. The BAC values of the plants studied were >1 in five species for Cu; four species for As; three species each for Zn, Cd, and Cr; and two species for Pb. On evaluating BCF, four species were found to have a ratio >1 for As; three species for Cd; 2 each in the case of Zn and Cu; and 1 in the case of Cr. All studied plants had low BCF values for Co, Pb, Cr, and Ni, indicating that the species had limited capabilities of mobilizing these metals in the root zone at the concentrations present in soil at all sites. Higher soil pH values can also facilitate adsorption of metals by soil organic materials since protons may compete with metal ions for adsorption sites. Adsorbed metals generally show different behavior such as low mobility in soil. For Ni and Co, Xu and Tao (2004) observed not only similar adsorption behavior over a wide range of pH values but also similar uptake and accumulation among different plant species. Soil pH does not seem to have an effect on the uptakes as all zones presented a similar picture.

TF values indicate that most of the plants allocated a greater proportion of metal absorbed to aboveground biomass. Seventeen species recorded a TF > 1 for Cu; 15 for Zn; 14 each for Cd, Ni, and As; 13 for Pb; and 10 each for Co and Cr. Several plants were found to have a TF > 1 for multi-metal combinations, indicating the fact that they were naturalized to the contaminated matrix in which they had germinated and grown and that their metabolism could at least tolerate the multi-metal contamination. Similar studies have shown that leaves act as main sinks for metals in hyperaccumulator plants (Salt et al. 1995; Psaras and Manetas 2001). This is attributed to the efficient translocation of metals from roots to shoots and is considered an advantageous strategy as the root system is the primary target in metal toxicity (Baker et al. 1994). Accumulation of potentially toxic metals in the green parts is also thought to be a plant's defensive strategy against herbivores (Liphadzi and Kirkham 2005).

According to Fitz and Wenzel (2002), plants with BAC as well as TF value both greater than 1 are considered promising phytoextractors in general. Yoon et al. (2006) demonstrated *Phyla nodiflora* having TF values of 12 and 6.3 for Cu and Zn as a suitable phytoextractor. In this investigation, *Datura stramonium* showed such values for Cu, Cr, and As; *Chenopodium murale* for Zn, Cd, and Cu; *Lycopersicon esculentum* for Cd, Cu, and As; *Poa annua* for Pb and Cu; *Cannabis sativa* for Cr; *Calotropis procera* for Pb; and *Euphorbia hirta* for As (Table 13.6). These species exhibited potential for possible use in phytoextraction.

Plants with a high BCF and a low TF are considered potential phytostabilizers (Mendez and Maier 2008). Such species keep the translocation of metals from roots to shoots as low as possible which may be due to immobilization of metals in roots by vacuole sequestration or cell wall binding, thereby preventing interaction with high-molecular-weight compounds in the plant cell cytoplasm (Salt et al. 1995). *Gentiana pennelliana* having BCF values of 11, 22, and 2.6 for Pb, Cu, and Zn, respectively, has been categorized as a phytostabilizer (Yoon et al. 2006). High BCF and low TF values (Table 13.6) were observed in *Calotropis procera* for Zn, Cd, and Cu; in *Poa annua* for Cd and As; in *Withania somnifera* for Cu; in *Heliotropium ellipticum*, *Gnaphalium luteo-album*, and *Eclipta alba* for As; in *Lycopersicon esculentum* for Zn; and in *Rumex dentatus* for Cr, thus establishing them as good phytostabilizers for these metals and metal combinations. In another study in the same locality, *Typha latifolia* was assessed for phytoremediation of heavy metals in the sediments of industrial discharge pits (Varun et al. 2011b). The plant partitioned a major part of metals in the root itself. Along with the especially low TF values, it was found suitable as a phytostabilizer for Zn, Mn, Cr, and As with some potential for Co, Cd, and Ni also.

13.8 Evaluation of Phytoremedial Potential: Greenhouse Study

Various criteria must be considered before selecting a plant for a phytoremedial study to achieve maximal phytoextraction. Due to the frequently poor nutrient availability at contaminated sites along with the obvious toxicity issues and considering cost as an important factor, plants that require little attention (e.g., fertilizers and frequent cutting) are preferable (Harris et al. 1996). Based on their survival at the polluted sites and their promising potential, three species—*Calotropis procera*, *Chenopodium murale*, and *Poa annua*—were selected to assess their uptake/sequestration/transfer of Pb, Cd, and Cu at different concentrations under experimental conditions. Simultaneously, metal reduction in soil was determined. Pots were prepared with garden soil. Each pot could hold 4 kg of soil and was prepared with a layer of crocks and gravel of about 1.5 in. depth at the bottom. The soil used in these studies had a pH of 7.21, EC of 0.54 dS m^{-1}, and an organic carbon content of 1.68 %. The bulk density was 1.45 g cm^{-3}. Available nitrogen, phosphate, and potash were 112.9, 70.5, and 393 kg ha^{-1}, respectively.

The seeds were first allowed to germinate in a germination tray. Upon germination, seeds were transplanted to each treatment pot (1 plant/pot). The sets were allowed to mature for 3 weeks; after which, individual metals (Pb, Cd, Cu) were added separately to each pot in increasing concentrations. Each pot (treatment) was set up with nine replicates. Metals were applied to the pots in aqueous solution as $Pb(NO_3)_2$, $CdCl_2{\cdot}5H_2O$, and $CuSO_4{\cdot}5H_2O$. Control pots were set up with test plant without any metal treatment. The pots were kept in a random block design and watered as and when

Table 13.6 Species surveyed with potential for phytoremediation

	Potential phytoextractors (BAC > 1 < TF)				Potential phytostabilizers (BCF > 1 > TF)			
	Site	Plants	BAC	TF	Site	Plants	BCF	TF
Pb	2	*P. annua*	1.40	3.60				
	6	*P. annua*	1.38	3.81				
	1	*C. procera*	1.08	1.45				
Cd	7	*C. murale*	2.10	1.72	7	*C. procera*	2.78	0.54
	8	*C. murale*	1.30	1.10	1	*C. procera*	2.29	0.52
	7	*L. esculentum*	1.14	1.22	6	*P. annua*	1.39	0.55
	4	*C. murale*	1.11	1.08	2	*P. annua*	1.10	0.59
	2	*L. esculentum*	1.02	1.30				
Zn	4	*C. murale*	1.05	1.60	2	*L. esculentum*	2.16	0.6
	7	*C. murale*	1.02	1.46	7	*L. esculentum*	1.83	0.61
					7	*C. procera*	1.50	0.7
					1	*C. procera*	1.35	0.75
Cu	6	*P. annua*	5.58	11.20	1	*C. procera*	1.58	0.8
	2	*P. annua*	4.10	9.36	7	*C. procera*	1.56	0.76
	5	*D. stramonium*	1.92	3.62	5	*W. somnifera*	1.39	0.31
	8	*C. murale*	1.20	2.33				
	7	*C. murale*	1.06	2.66				
	7	*L. esculentum*	1.05	1.19				
	4	*C. murale*	1.02	2.53				
	2	*L. esculentum*	1.01	1.17				
Cr	1	*C. sativa*	1.10	2.86	8	*R. dentatus*	2.69	0.39
	8	*C. sativa*	1.09	2.74	2	*R. dentatus*	2.10	0.53
	5	*D. stramonium*	1.02	4.11				
As	2	*L. esculentum*	1.30	1.51	6	*P. annua*	5.16	0.53
	5	*D. stramonium*	1.27	2.54	2	*P. annua*	4.40	0.50
	5	*E. hirta*	1.21	1.97	8	*H. album*	1.55	0.09
	7	*L. esculentum*	1.19	1.81	4	*E. alba*	1.51	0.58
	1	*D. stramonium*	1.07	2.42	7	*E. alba*	1.50	0.60
					5	*E. alba*	1.44	0.56
					6	*G. luteo-album*	1.02	0.64

required, in such a way as to prevent loss of contaminants by leaching. A plastic tray was kept below the treatment pot to collect any leachate, which was returned to the pots at next watering. The whole experiment was conducted in greenhouse for 4 months. Any symptoms of metal toxicity exhibited by plants were visually noted during the whole experimental period. Uncontaminated, unamended plants were maintained as controls.

At 30, 60, and 120 days after treatment (DAT), three replicates of each pot were taken apart, and soil and plant samples (root/shoot) were collected and analyzed for metal content. Growth parameters, i.e., shoot/root length and biomass (dry), were also measured at 120 DAT.

The control soil was uncontaminated, and its initial total Pb, Cd, and Zn concentrations were 0.0026 ± 0.05 mg Pb kg^{-1}, 0.0011 ± 0.03 mg Cd kg^{-1}, and 0.0034 ± 0.08 mg Cu kg^{-1}, respectively. Metals were applied at three concentrations each—150, 300, and 500 mg Pb kg^{-1}; 15, 30, and 50 mg Cd kg^{-1}; and 50, 100, and 150 mg Cu kg^{-1}. After artificial spiking, the total metal concentrations of treatments were as follows: 148.3 ± 2.03 mg kg^{-1}, 297.3 ± 2.7 mg kg^{-1}, and 493.2 ± 0.8 mg kg^{-1} for Pb; 14.8 ± 0.18 mg kg^{-1}, 29.3 ± 3.2 mg kg^{-1}, and 49.4 ± 1.6 mg kg^{-1} for Cd; and 49.2 ± 0.86 mg kg^{-1}, 98.7 ± 2.14 mg kg^{-1}, and 148.6 ± 4.13 mg kg^{-1} for Cu, respectively, which were close to our target concentrations.

13.8.1 Metal Uptake in Plants

13.8.1.1 Lead (Pb)

A steady increase in Pb uptake and accumulation was observed for all treatments up to 120 DAT. High concentrations of metals may not necessarily imply their release or their availability for plant uptake (Sánchez-Martin et al. 2007). Tight binding of Pb to soils and plant material explains at least partially the low mobilization of the metal in soil and plants. It is known to be extremely insoluble in

Table 13.7 Evaluation of phytoremedial potential: metal concentrations in test plants (mg kg^{-1}) at successive days of study

			30 DAT		60 DAT		120 DAT	
			Root	Shoot	Root	Shoot	Root	Shoot
Pb	*C. procera*	150	57.3 ± 7.1	16.1 ± 3.3	70.9 ± 6.2	24.3 ± 3.9	128.2 ± 10.8	73.7 ± 5.1
		300	93.7 ± 10.2	24.3 ± 2.8	121.6 ± 11.6	39.8 ± 4.6	179 ± 17.2	93.8 ± 3.9
		500	116.6 ± 6.2	27.7 ± 2.1	157.8 ± 16.2	47.3 ± 6.1	239.4 ± 24.1	114.9 ± 6.2
	C. murale	150	103.6 ± 13.8	144.2 ± 13.2	122.4 ± 9.2	167.5 ± 11.7	149.1 ± 9.2	156.3 ± 11.9
		300	128.3 ± 11.2	297.6 ± 18.2	148.1 ± 5.2	341.2 ± 27.4	207.2 ± 23.5	261.4 ± 16.2
		500	112.7 ± 9.5	124.1 ± 10.5	127.3 ± 2.9	151.6 ± 18.3	124.7 ± 8.5	204.7 ± 11.1
	P. annua	150	48.4 ± 3.8	114.2 ± 17.3	61.7 ± 4.8	166.4 ± 9.4	83.3 ± 6.2	232.2 ± 29.1
		300	78.1 ± 8.3	288.5 ± 15.2	95.6 ± 3.2	358.2 ± 27.2	113.7 ± 9.7	441.6 ± 15.2
		500	69.6 ± 4.8	263.4 ± 11.7	93.1 ± 2.8	354.7 ± 14.1	107.8 ± 6.9	435.1 ± 8.6
Cd	*C. procera*	15	7.4 ± 1.9	8.6 ± 2.2	12.9 ± 3.1	13.7 ± 1.1	19.6 ± 1.9	17.4 ± 2.7
		30	14.1 ± 2.3	20.9 ± 4.1	27.4 ± 2.0	27.0 ± 3.1	36.1 ± 4.1	23.2 ± 1.4
		50	24.3 ± 3.6	31.2 ± 6.8	38.6 ± 3.4	46.4 ± 5.8	58.4 ± 6.6	26.5 ± 3.6
	C. murale	15	6.6 ± 0.8	11.3 ± 4.1	8.4 ± 1.4	15.7 ± 2.8	12.6 ± 2.3	23.1 ± 3.1
		30	14.2 ± 4.1	25.1 ± 2.8	19.7 ± 2.6	31.3 ± 4.1	26.7 ± 2.8	42.6 ± 5.2
		50	17.3 ± 2.4	29.6 ± 1.5	21.6 ± 3.5	35.6 ± 2.8	27.3 ± 1.6	48.4 ± 2.7
	P. annua	15	6.9 ± 1.8	1.4 ± 0.4	9.3 ± 1.6	2.0 ± 0.4	14.4 ± 1.2	5.7 ± 0.8
		30	7.3 ± 2.1	2.5 ± 0.9	10.8 ± 1.2	4.7 ± 1.1	23.2 ± 2.5	10.4 ± 1.4
		50	11.4 ± 3.0	4.2 ± 1.2	18.5 ± 3.5	10.3 ± 2.6	31.6 ± 4.1	19.6 ± 3.6
Cu	*C. procera*	50	36.5 ± 6.2	9.5 ± 3.7	54.1 ± 6.9	15.3 ± 2.9	75.2 ± 6.2	22.4 ± 3.8
		100	44.6 ± 2.4	10.7 ± 2.1	60.4 ± 3.9	15.0 ± 3.3	83.2 ± 5.1	22.6 ± 1.8
		150	29.1 ± 4.3	5.2 ± 1.3	40.8 ± 8.9	7.4 ± 2.1	48.7 ± 5.9	10.7 ± 2.7
	C. murale	50	12.4 ± 1.8	27.9 ± 2.9	14.6 ± 1.7	32.6 ± 4.8	27.4 ± 10.2	56.3 ± 4.2
		100	9.9 ± 2.3	24.3 ± 4.2	11.8 ± 2.7	28.3 ± 1.6	23.2 ± 5.9	50.2 ± 2.9
		150	5.6 ± 1.7	16.2 ± 4.8	8.5 ± 2.2	23.2 ± 3.9	15.4 ± 2.6	38.5 ± 1.6
	P. annua	50	47.2 ± 6.2	297.1 ± 26.2	68.1 ± 3.8	477.3 ± 15.2	72.5 ± 4.9	522.4 ± 27.1
		100	64.9 ± 3.7	462.6 ± 33.8	72.6 ± 4.9	541.2 ± 27.1	83.2 ± 9.8	674.3 ± 17.3
		150	68.5 ± 6.1	524.2 ± 47.1	79.0 ± 7.2	632.6 ± 31.8	92.6 ± 10.6	802.4 ± 36.5

the normal range of soil pH, and also its translocation from roots to aerial shoots is limited due to binding at root surfaces and cell walls (Saifullah et al. 2009); thus, Pb is generally accumulated in roots and its translocation to the shoots is low. Kabata-Pendias (2001) found that Pb accumulation by the aboveground parts of barley, *Hordeum vulgare*, was below 5 % relative to the Pb contents in the roots, thus confirming that Pb was not translocated from the roots to the tops. Lan et al. (1992) used *Typha latifolia* to treat wastewater from a Pb/Zn mine and found that it assimilated significant amounts of Pb and Zn, especially in its roots. However, in this study, limited translocation did not seem to be a problem in *C. murale* and *P. annua*. These were seen to accumulate Pb preferentially in the shoots, and metal content increased with exposure period (Table 13.7). In general, toxicity of Pb in leaves ranges from 30 to 300 mg kg^{-1} (Orcutt and Nilsen 2000), but the plant under investigations developed no visible symptoms of Pb toxicity, showing their tolerance to high Pb contamination in the rhizosphere.

Maximum uptake was observed at 300 mg kg^{-1} Pb which peaked at 442 mg kg^{-1}in *P. annua* and 363 mg kg^{-1} in *C. murale* at 120 DAT. At 500 mg kg^{-1}, *P. annua* performed much better than *C. murale* with 435 mg kg^{-1} Pb in shoots. In the case of *C. procera*, limited transfer from root to shoot was observed, and highest Pb content was observed in roots at 500 mg kg^{-1} at 120 DAT (239 mg kg^{-1}) (Table 13.7). Vickerman et al. (2002) reported that *Atriplex* species are able to accumulate high amounts of Pb. Pb concentrations up to 1,800 mg kg^{-1} have been reported in plants from a dump site by Pitchtel et al. (2000). Yoon et al. (2006) also reported up to 1,183 mg kg^{-1} Pb in 17 species from a metal-contaminated site.

Plants at an abandoned Pb mine in Central Portugal have been reported to tolerate soil Pb concentrations averaging 2,380 mg kg^{-1} and reaching 9,330 mg kg^{-1} (Pratas et al. 2013). Pb concentrations in plants ranged from 1.11 to 548 mg kg^{-1}. This is far above the 100–400 mg kg^{-1} Pb content considered toxic for most plants (Alloway 1990). Significant accumulation of Pb was demonstrated in *Cistus salvifolius* (548 mg kg^{-1}), *Lonicera periclymenum* (318 mg kg^{-1}), *Anarrhinum bellidifolium, Phytolacca americana, Digitalis purpurea, and Mentha suavolens* (217–548 mg kg^{-1}).

13.8.1.2 Cadmium (Cd)

Cadmium is a toxic metal and probable carcinogen. Cd is thought to enter the tissues by both metabolic and non-metabolic processes. Many studies have demonstrated that Cd taken up by plants accumulates at higher concentration in the root than in the stem and leaves (Seregin and Ivanov 2001), including hyperaccumulators (Küpper et al. 2000). Roots seemed to be the organ of choice for Cd accumulation in *C. procera* and *P. annua* with maximum uptake seen in *C. procera* (58 mg kg^{-1}) followed by *P. annua* (32 mg kg^{-1}) at 50 mg kg^{-1} and 120 DAT (Table 13.7). Although *C. murale* translocated Cd more efficiently to the shoot, the uptake trends were similar to *C. procera* and *P. annua*, and root Cd content (49 mg kg^{-1}) peaked at 50 mg kg^{-1} and 120 DAT. Thus, Cd content of all three species was seen to increase with increasing metal concentrations and period of exposure.

Thlaspi caerulescens has been successful in removing Cd from contaminated soils in pilot studies around the world (Brown et al. 1994; Ebbs et al. 1997; Lombi et al. 2001a) though at considerably slow rates. Saraswat and Rai (2009) reported varied capabilities for Cd extraction in *Arabidopsis thaliana*, *Brassica juncea*, *Cynodon dactylon*, *Parthenium integrifolium*, *Phragmitis communis*, and *Crotalaria juncea* with total Cd content ranging from 30.5 to 107 mg kg^{-1} in pot studies using multi-metal-contaminated soil. Accumulation of Cd in plant parts has also been demonstrated in *Calotropis procera*, *Brassica* spp., *Glycine max*, *and Zea mays* (D'Souza et al. 2010; Fellet et al. 2007; Clemente et al. 2005; Marchiol et al. 2004). Phaenark et al. (2009) reported five plant species (*Justicia procumbens*, *Gynura pseudochina*, *Impatiens violaeflora*, *Chromolaena odorata*, *and Brachiaria* sp.) from Thailand with Cd concentrations greater than 100 mg kg^{-1} dry mass in their shoots (the Cd hyperaccumulating level proposed by Baker et al. (1994) with the highest concentration (548 mg kg^{-1} dry mass) in *J. procumbens.*

13.8.1.3 Copper (Cu)

Copper is considered one of the essential micronutrients (Fe, Mn, B, Zn, Cu, Mo, and Cl) to plant growth because it is necessary for several enzymes involved in biological reactions. Cu is essential for plants but may be toxic too. The range of essential Cu is 3–5 mg kg^{-1} while 5–20 mg kg^{-1} is considered sufficient. The toxic range varies from 20 to 100 mg kg^{-1} in plants (Fageria et al. 2002). A gradual increase of Cu accumulation in both roots and shoots with the increase in Cu concentration and passage of time was observed in *P. annua* (Table 13.7). However, in the case of *C. procera* and *C. murale*, though uptake increased with exposure period, the picture was different with increase in Cu dose. In *C. procera*, Cu uptake was actually lowest at the highest dose (150 mg kg^{-1}) and peaked at 100 mg kg^{-1}, while in *C. murale*, Cu uptake peaked at 50 mg kg^{-1}, the lowest dose, and decreased with increasing dosage. Cu was preferentially accumulated in shoots in *C. murale* and *P. annua* and in the roots in *C. procera*. In general, Cu tends to accumulate in the root tissue with little translocated to the shoots (Marschner 1995).

Pratas et al. (2013) reported Cu accumulation in *Digitalis purpurea* (39 mg kg^{-1}), *Phytolacca americana* (30 mg kg^{-1}), and *Mentha suaveolens* (28 mg kg^{-1}) from an abandoned Pb mine in Central Portugal. Shu et al. (2002) and Yoon et al. (2006) have reported much higher Cu concentrations in plants at 7–198 and 6–460 mg kg^{-1}, respectively. Copper concentrations in *Brassica juncea* and *Bidens alba* var. *radiata* up to 3,771 and 879 mg kg^{-1} have been reported in experimental pots with 150 mg Cu kg^{-1} (Ariyakanon and Winaipanich 2006). Beladi et al. (2011) demonstrated Cu concentrations up to 40 mg kg^{-1} in *Onobrychis viciifolia* with soil concentrations ranging from 0 to 450 mg Cu kg^{-1}.

13.8.2 Characterization of Metal Accumulation

Yoon et al. (2006) demonstrated *Phyla nodiflora* having TF values of 12 and 6.3 for Cu and Zn as a suitable phytoextractor and *Gentiana pennelliana* having BCF values of 11, 22, and 2.6 for Pb, Cu, and Zn as a phytostabilizer. On comparing Pb accumulation characteristics of the three species with the criteria for phytoextraction, i.e., BAC>1 and TF>1 (Fitz and Wenzel 2002), only *P. annua* exhibited suitable values (Table 13.8).

Table 13.8 Evaluation of phytoremedial potential: metal accumulation characteristics of test plants at the final day of study

	Pb			Cd			Cu		
	BAC	BCF	TF	BAC	BCF	TF	BAC	BCF	TF
C. procera	0.51	0.89	0.57	1.44	1.62	0.89	0.5	1.67	0.3
	0.33	0.62	0.52	0.99	1.54	0.64	0.26	0.94	0.27
	0.24	0.51	0.48	0.73	1.61	0.45	0.08	0.35	0.22
C. murale	1.13	1.08	1.05	1.89	1.03	1.83	1.21	0.59	2.05
	0.95	0.75	1.26	1.84	1.15	1.6	0.53	0.25	2.16
	0.45	0.27	1.64	1.26	0.71	1.77	0.27	0.11	2.5
P. annua	1.75	0.63	2.79	0.43	1.1	0.4	12.2	1.7	7.21
	1.73	0.45	3.88	0.41	0.92	0.45	8.2	1.01	8.1
	1.02	0.25	4.04	0.47	0.76	0.62	6.61	0.76	8.67

Table 13.9 Evaluation of phytoremedial potential: reduction (%) of heavy metals in soil at the final day of study

Pb		150	300	500
	C. procera	3.17	3.57	4.66
	C. murale	6.7	7.0	7.1
	P. annua	10.45	14.33	13.58
Cd		15	30	50
	C. procera	18.2	20.1	26.5
	C. murale	17.2	20.8	22.3
	P. annua	11.28	14.20	15.38
Cu		50	100	150
	C. procera	8.33	10.23	7.67
	C. murale	5.1	4.5	4.3
	P. annua	13.21	16.72	18.30

Although *C. procera* was categorized as a promising Pb phytoextractor in the survey, it did not perform so well in the greenhouse study albeit *C. murale* exhibited both BAC and TF > 1. However, the potential for the latter could best be described as limited as it was seen only at low Pb concentration (150 mg kg^{-1}). None of the three species tested qualified the phytostabilization criteria for Pb, i.e., BCF > 1 > TF (Mendez and Maier 2008).

In the case of Cd, *C. murale* was categorized as phytoextractor and *C. procera* as phytostabilizer at all three concentrations applied. These results corroborate those of the survey where these two species showed promise in these categories. *P. annua* showed limited potential for phytostabilization of Cd (at 15 mg kg^{-1}) in the greenhouse study though it showed promise in the survey (Table 13.8).

Although *C. murale* showed promise as a Cu phytoextractor (BAC and TF > 1) in the survey, in this study, it crossed the threshold only at low concentration (50 mg kg^{-1}). *P. annua* proved to be a good phytoextractor of Cu as expected from the results of the survey with high BAC values ranging from 6.6 to 12.2 with equally good TFs. The phytostabilization potential of *C. procera* as seen at the survey stage was limited since BCF > 1 > TF was observed only at low dose (50 mg kg^{-1}) of Cu (Mendez and Maier 2008).

Metal tolerance is one of the most important criteria to select an appropriate plant to remediate sites contaminated with heavy metals. Baker (1981) proposed two contrasting strategies of plant tolerance, namely, *exclusion* and *accumulation*. In exclusion, the heavy metal(loid) concentration in the aboveground biomass is maintained at a low concentration with roots acting as barriers. Studies that have examined accumulation and partitioning of heavy metals in mangroves indicate greater heavy metal concentrations in roots (Peters et al. 1997; MacFarlane et al. 2007). Exclusion is effective up to a certain threshold concentration with a leaf-root heavy metal(loid) concentration ratio of <1; after which, the mechanism breaks down resulting in unrestricted uptake and possible death (Baker 1981; Baker et al. 2000). Physiological mechanisms enable these species to survive in the presence of high concentrations of heavy metal(loid)s. Mechanisms may include a change in the metal binding capacity of cell walls, exudation of chelating substances in the rhizosphere, or altered membrane permeability (Ghosh and Singh 2005). In the present study also, *C. procera* may have adopted the exclusion mechanism to tolerate the high contamination of Cd and Cu in soil with decent leaf-root concentrations.

Conversely, in the *accumulation strategy*, plants can actively accumulate high levels of heavy metals in the aboveground biomass without adverse affects on plant growth until soil conditions become toxic and plant growth suppressed. These species are characterized by a leaf-root heavy metal(loid) concentration ratio of >1 (Baker et al. 2000). Metal accumulation in the shoots by *P. annua* (Pb, Cu) and *C. murale* (Cd) indicates the accumulation strategy that helps to tolerate contamination in soil. These findings are supported by similar results obtained by Baker (1981) for *Hordeum vulgare* L. (barley) and *Avena sativa* L. (oats). Both plants are tolerant to Pb up to 600 mg kg^{-1} by accumulating moderate to high amounts of these metals in aboveground tissues. Saraswat and Rai (2009) demonstrated phytoextraction potential for Cd in *Arabidopsis thaliana*, *Brassica juncea*, and *Crotalaria juncea.* All plants exhibited translocation factors greater than one.

13.8.3 Reduction in Heavy Metal Content of Soil

At the final day of study (120 DAT), maximum Pb reduction was observed in *P. annua* (14.33 %) in the order [maximum at 300 mg kg^{-1}; minimum at 150 mg kg^{-1}] followed by *C. murale* and *C. procera* (Table 13.9). In these species, reduction % was found to increase with increasing metal concentration in soil. *P. annua* caused up to 2.5 times higher reduction than *C. murale* and *C. procera*. The reduction of Cd in soil at 120 DAT was found in the order *C. procera* > *C. murale* > *P. annua*. Maximum reduction of 26.5 % was observed at 50 mg

Table 13.10 Evaluation of phytoremedial potential: growth parameters of test plants at the final day of study

		C. procera			*C. murale*			*P. annua*		
		Root length	Shoot length	Biomass	Root length	Shoot length	Biomass	Root length	Shoot length	Biomass
	Control	55.6 ± 1.2	134.1 ± 8.4	310.3 ± 13.8	29.8 ± 2.7	68.4 ± 4.2	163.1 ± 8.3	10.7 ± 0.7	17.2 ± 3.4	5.36 ± 1.2
Pb	150	54.8 ± 0.8	132.3 ± 5.1	306.1 ± 9.20	28.4 ± 1.9	65.1 ± 3.8	155.2 ± 7.2	9.57 ± 1.2	15.7 ± 2.9	4.89 ± 1.5
	300	54.1 ± 2.3	130.6 ± 3.6	302.2 ± 12.3	28.7 ± 3.1	65.8 ± 5.2	156.9 ± 5.3	6.51 ± 1.8	10.6 ± 2.1	3.36 ± 0.7
	500	51.1 ± 0.6	126.2 ± 4.1	285.4 ± 9.71	25.2 ± 2.4	60.1 ± 6.3	143.3 ± 9.2	4.83 ± 0.5	8.80 ± 3.6	2.74 ± 0.4
Cd	15	54.5 ± 4.1	131.5 ± 1.8	304.2 ± 14.2	30.3 ± 1.8	67.2 ± 5.2	161.2 ± 10.2	9.31 ± 1.3	16.1 ± 2.8	5.01 ± 1.6
	30	54.6 ± 2.2	125.7 ± 5.6	304.9 ± 10.3	28.8 ± 3.1	66.1 ± 3.9	156.5 ± 5.2	5.40 ± 1.8	9.71 ± 1.2	3.02 ± 0.9
	50	49.0 ± 1.9	118.3 ± 4.3	273.7 ± 7.32	27.1 ± 0.9	62.2 ± 3.8	147.2 ± 6.6	3.81 ± 0.6	7.36 ± 2.7	2.27 ± 0.6
Cu	50	56.4 ± 4.2	136.2 ± 3.9	315.1 ± 4.8	27.9 ± 1.6	63.7 ± 4.2	150.6 ± 7.2	9.92 ± 1.1	16.6 ± 3.1	5.17 ± 1.3
	100	55.4 ± 2.9	133.7 ± 3.2	309.3 ± 11.4	26.3 ± 3.2	60.3 ± 3.8	143.8 ± 4.4	7.33 ± 0.8	14.2 ± 2.9	4.42 ± 0.7
	150	51.4 ± 3.6	124.1 ± 5.2	287.1 ± 7.51	24.5 ± 4.6	56.2 ± 4.7	135.2 ± 3.8	5.34 ± 1.2	11.3 ± 3.6	3.52 ± 1.2

Cd kg^{-1} in *C. procera*. Reduction in all species increased with increasing Cd concentration. *C. procera* showed ~0.7 times the reduction seen in *P. annua*. The difference in *C. procera* and *C. murale* treatments was not so marked.

Percent reduction trends of Cu content in soil at 120 DAT were different for each species (*P. annua* > *C. procera* > *C. murale)*. In *P. annua*, reduction increased with increasing Cu concentration used. In *C. procera* and *C. murale*, least reduction was observed at 150 mg Cu kg^{-1} in soil. Reduction of Cu in *P. annua* treatments was up to four times higher than in *C. murale* and ~ 1.5 times than in *C. procera*.

The results obtained show different reduction trends for individual metals by the test plants. Among the metals, maximum reduction was observed in Cd (26.5 % by *C. procera*) followed by Cu (18.3 % by *P. annua*) and Pb (14.33 % by *P. annua*). Logically higher phytoextraction of metals should result in a low metal concentration in the soil. As *C. procera* extracted the largest amount of Cd from soil, there was a greater depletion in total Cd concentrations in rhizosphere soils of this species as compared to the *P. annua* (difference not so marked in *C. murale*). Similar high amount of Cd depletion of metal concentration in soils by *Brassica* spp. was also reported by Ebbs and Kochain (1997) and Bennett et al. (2003). The least reduction of Pb in soil can be explained by low bioavailability of Pb in soil, but here again, *P. annua* showed great promise compared to the other two species. Cu being an essential element did not pose difficulty in being absorbed and accumulated in the test species.

13.8.4 Growth Parameters

As plant roots are in direct contact with metals in contaminated soil and must act as the conduit for transfer of metal to the stem and leaves, their response to the high metal concentration is important. Growth patterns of the test species (Table 13.10) indicate that presence of heavy metals in the soil was not conducive to root/shoot elongation and biomass accumulation.

All three metals—Pb, Cd, Cu—reduced these parameters in all test species in comparison to respective controls. In Pb treatments, most reduction in root/shoot length was observed at 500 mg kg^{-1}, followed by 300 mg kg^{-1} and 150 mg kg^{-1} Pb. The difference was not so drastic between the two lower concentrations. Similar patterns were observed in Cd and Cu treatments also. Thus, growth retardation increased with increasing concentrations of metals applied. *P. annua* was the most affected; maximum reduction in growth was noted in this species in response to all three metals tested. *C. murale* was the least affected species.

Chen (2000) conducted a similar experiment on the effect of heavy metals (Cu, Pb, Zn, Cd, and As) on vetiver growth. He found that the high contents of metals (100 mg Cu kg^{-1}, 200 mg Zn kg^{-1}, 300 mg Pb kg^{-1}, 1.5 mg Cd kg^{-1}, 30 mg As kg^{-1}) limited the growth of vetiver grass during the first year, but the effect was reduced in the second year. Cull et al. (2000) found that vetiver was highly tolerant to these heavy metals. Romeiro et al. (2006) showed that adding high levels of Pb to soils resulted in adverse effects on *Ricinus communis* plants such as a reduction in biomass and damage to photosynthetic function. Metals like Cd, Pb, Zn, Cr, etc. when present at high concentrations in soil show potential toxic effects on overall growth and metabolism of plants (Agrawal and Sharma 2006). Phetsombat et al. (2006) reported a significant reduction in the relative growth and biomass of *Salvinia cucullata* supplemented with Cd and Pb, with the increase in concentration and exposure time.

Cadmium is not an essential nutrient and at high concentration inhibits plant growth (Aery and Rana 2003). A significant reduction in fresh biomass and root-shoot length of *Vigna radiata* treated with 35, 50, 75, and 100 mg kg^{-1} of Cd was observed by Shen et al. (1990). Rana and Ahmad (2002) conducted a pot study to investigate the toxic effects of certain heavy metals on the plant growth and grain yield of wheat (*Triticum aestivum* L.). Their result revealed that heavy metals brought about significant reductions in both parameters, Cd being the most toxic metal followed by Cu, Ni, Zn, Pb, and Cr. The presence of cadmium in the soil decreases the growth of soybean and chickpea plants (Hasan et al. 2007).

P. annua (for Pb and Cu) and *C. murale* (for Cd) came out as accumulating tolerant species that hold promise for phytoextraction purposes. *C. procera* showed good potential for phytostabilization of Cd. They were able to tolerate the Pb, Cd, and Cu concentrations applied though their growth was affected to different extents. Hence, these were selected for further testing with soil amendments to improve their growth parameters and phytoremedial potential for the abovementioned metals.

13.9 Enhancement of Phytoremedial Potential: Greenhouse Study

In the greenhouse study mentioned above, test plants were selected based on the survey of a range of metal-contaminated sites. The pot study led to the characterization of metal accumulation by the test species and its quantification. Bioavailability of metals is strongly influenced by soil characteristics—physical like moisture content, texture, etc.; chemical like pH, cation exchange capacity, and organic matter content; and biological like soil microflora and mycorrhizae—any of which may hamper phytoremediation. The effectiveness of phytoremediation efforts can be enhanced by suitable use of soil amendments and agronomic practices. Accordingly, farmyard manure (FYM) a universal organic fertilizer, ethylenediaminetetraacetic acid (EDTA) a common chelating agent, and vesicular arbuscular mycorrhizae (VAM) were used to test their efficacy in enhancing the phytoremedial potential of *C. procera*, *C. murale*, and *P. annua* as characterized in the previous greenhouse study.

Pots for this study were prepared and maintained for 120 days as before, followed by analysis of metal uptake in plants, reduction in soil and growth parameters. The three amendments mentioned above were also added to the respective pots when metals were added after 3 weeks. On the basis of optimum uptake, growth, and tolerance shown by test species in the previous phytoextraction/stabilization screening, the soil-metal concentrations used for this study were 150 mg Pb kg^{-1}, 15 mg Cd kg^{-1}, and 50 mg Cu kg^{-1}. FYM, EDTA, and VAM were applied at the rate of 100 g kg^{-1} soil, 5 mmol kg^{-1}, and 25 g kg^{-1} soil, respectively. Each amended treatment was set up with three replicates. Unamended plants with respective metal treatment were also maintained to compare growth and uptake during the course of the study.

13.9.1 Heavy Metal Uptake in Plants

Accumulation trends of Pb, Cd, and Cu in the test species were plant specific in response to all amendments except EDTA (Fig. 13.3). In *P. annua*, all three amendments—FYM, VAM, and EDTA—increased uptake of Cu (EDTA>VAM>FYM>Control). Uptake of Cd was hindered by the addition of VAM to *C. procera* and *C. murale*. Pb uptake by *P. annua* and Cd uptake by *C. procera* were also hampered by FYM. The translocation patterns remained the same. Metal uptake coefficients—BAC, BCF, and TF—reflected the changes in accumulation in response to amendments.

Application of manure to *P. annua* (for Cu) and *C. murale* (for Cd) increased the uptake and accumulation of metal. del Castilho et al. (1993) reported that manure increases the solubility of Cd, Cu, and Zn in soils. Zhou et al. (2005) reported higher bioavailability of Cd and Zn and their subsequent uptake in (*Raphanus sativus*) and pakchoi (*Brassica chinensis*).

Interestingly, the addition of manure to soil was seen to notably decrease metal uptake and accumulation in *P. annua* (for Pb) and *C. procera* (for Cd). Simeoni et al. (1984) also reported decreased uptake of Pb on addition of compost to alfalfa (*Medicago* sp). Reduction of Cd and Zn availability to plants by the application of manure has also been reported in several studies (Knox et al. 2001, 2003; Chaiyarat et al. 2011). Zn and Cd sorption to manure is explained by surface complexation and coprecipitation, presumably combined with ion exchange and solid diffusion resulting in a decrease of the exchangeable amount of these metals (Mench et al. 1998).

Chelating agents are added to soil to solubilize the metals for enhanced uptake (Liphadzi and Kirkham 2006). EDTA was the only amendment which increased metal uptake in all three test species though it seemed to be metal and plant specific. The increase in Pb uptake in *P. annua* was not very high, though it was significant in the case of Cd in *C. procera* (3 times) and *C. murale* (~2 times) as well as in *P. annua* (~0.5 times) for Cu. Synthetic chelating agents like EDTA are well known in increasing the metal uptake capability in plants like *Pisum sativum*, *Zea mays*, *Medicago sativa*, and *Phaseolus vulgaris* (Chen et al. 2004; Fellet et al. 2007; Pajuelo et al. 2007; Luo et al. 2005, 2008). Increases greater than hundredfold have been observed in Pb concentration in the biomass of crops when EDTA was applied to the contaminated soil (Cunningham and Berti 2000).

However, EDTA is persistent once released into the environment (Tandy et al. 2005) and is generally regarded as nonbiodegradable. Leaching of EDTA complexes is also a risk (Lombi et al. 2001b). Hence, its use should be weighed against any possible negative consequences.

Arbuscular mycorrhizal fungi (AMF) provide a direct physical linkage between the soil and plant roots by their extrametrical mycelia. Apart from other benefits like better nutrient availability, AMF can regulate heavy metal uptake by plants and, therefore, play an important role in phytoextraction (Davies et al. 2001). Uptake of metals by mycorrhizal plants does not have a generalized pattern and depends on factors such as soil properties, the host plant, concentrations of the metals in the soil, and resident AM assemblages (Davies et al. 2001). According to Göhre and Paszkowski (2006), AMF enhance uptake and transfer of heavy metals to the host plant shoots. Other workers have also reported

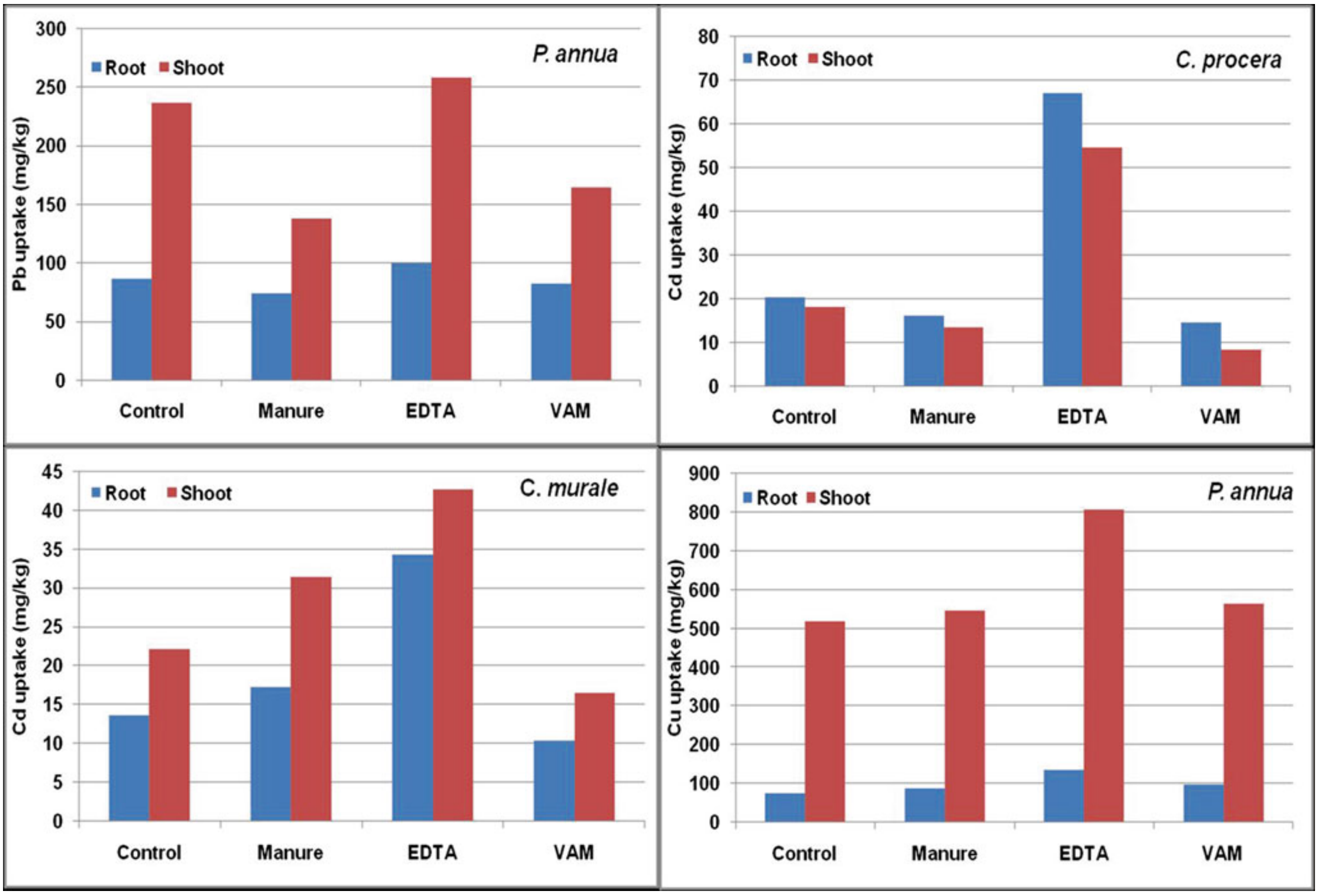

Fig. 13.3 Metal concentrations in test plants (mg kg^{-1}) in response to soil amendments

enhanced metal accumulation in plants inoculated with mycorrhizae (Wang et al. 2005; Akay and Karaarsla 2011). In the present study, VAM addition accounted for slightly greater accumulation of Cu in *P. annua*; however, in all other treatments, it actually decreased the uptake of metals, viz., Cd in *C. procera* and *C. murale* and Pb in *P. annua*.

Lower concentrations of Pb, Zn, and Cd in the shoots of mycorrhizal plants in comparison to non-mycorrhizal plants have also been reported (Andrade et al. 2004; Janoušková et al. 2007). Presumably, the abundant AM might immobilize Pb, Zn, and Cd to reduce the bioavailability and heavy metal toxicity. Joner et al. (2000) demonstrated that AM mycelia had a high metal sorption capacity, thereby minimizing metal translocation to the shoots. The findings of the present investigation are in conformity with the above results.

13.9.2 Heavy Metal Reduction in Soil

Percent reduction of metal content in soil in response to different amendments reflected the plant- and metal-specific trends of uptake and accumulation (Table 13.11). Efficacy of the amendments in reduction of metal was found to vary with the plant. In *P. annua* treatments, reduction of Pb in soil by EDTA application (18.5 %) was twice that of control. Soil amendment with FYM and VAM actually decreased the reduction percent.

Table 13.11 Enhancement of phytoremedial potential: reduction (%) of heavy metals in soil at the final day of study

Metal	Plant	Control	Manure	EDTA	VAM
Pb	*P. annua*	9.3	6.8	18.5	7.7
Cd	*C. procera*	17.0	14.4	21.7	12.6
Cd	*C. murale*	15.7	16.3	18.6	11.9
Cu	*P. annua*	13.6	15.4	23.8	18.7

In the case of Cd, highest reduction in both *C. procera* and *C. murale* treatments was due to EDTA application (21.7 and 18.6 % respectively). FYM increased reduction slightly in the case of *C. murale* but hampered it in *C. procera* treatments. VAM association inhibited metal reduction in both species. Reduction of soil Cu content was enhanced in all amendments in the order DTA>VAM>FYM. Reduction percent in response to EDTA was nearly twice that of control.

Table 13.12 Enhancement of phytoremedial potential: growth parameters of test plants at the final day of study

Metal	Plant	Amendments	Root length	Shoot length	Biomass
Pb	*P. annua*	Control	9.3 ± 1.1	17.7 ± 2.4	5.3 ± 0.8
		Manure	10.5 ± 2.3	20.7 ± 4.1	6.1 ± 1.2
		EDTA	9.5 ± 1.8	17.5 ± 3.2	5.0 ± 2.2
		VAM	9.9 ± 1.4	19.0 ± 3.6	5.6 ± 1.6
Cd	*C. procera*	Control	54.4 ± 6.7	129.8 ± 14.2	294.4 ± 23.6
		Manure	57.7 ± 4.2	136.1 ± 10.8	299.3 ± 18.5
		EDTA	55.8 ± 3.7	132.0 ± 10.2	294.8 ± 20.7
		VAM	58.9 ± 2.9	133.3 ± 8.5	297.3 ± 17.8
Cd	*C. murale*	Control	36.6 ± 3.6	78.8 ± 5.2	186.2 ± 16.4
		Manure	40.2 ± 4.1	81.6 ± 6.9	192.5 ± 22.7
		EDTA	37.2 ± 3.6	80.0 ± 4.7	188.9 ± 11.3
		VAM	33.7 ± 4.4	79.5 ± 5.2	187.7 ± 16.5
Cu	*P. annua*	Control	11.2 ± 1.7	21.4 ± 3.9	6.4 ± 1.1
		Manure	13.7 ± 2.2	27.9 ± 4.1	7.8 ± 1.5
		EDTA	11.2 ± 2.7	21.8 ± 3.6	6.6 ± 0.8
		VAM	12.2 ± 3.1	23.6 ± 3.9	7.1 ± 2.2

13.9.3 Growth Parameters

All soil amendments tested were protective of root and shoot length as well as biomass in all three species (Table 13.12). FYM was the amendment most conducive to plant growth. Zhou et al. (2005) demonstrated that the addition of manure in Cu- and Zn-contaminated soil improved the growth of radish and pakchoi, though difference in biomass among the same manure treatments containing different concentrations of Cu and Zn was insignificant. Application of manure increased the biomass of sunflower (*Helianthus annuus* L.) and canola (*Brassica napus* L.) grown in Pb- and Zn-contaminated soil (Solhi et al. 2005). Simeoni et al. (1984) reported high yields of alfalfa (*Medicago* sp.) grown in Pb-contaminated soil. Chaiyarat et al. (2011) opined that the addition of compost resulted in higher biomass production of *Ocimum gratissimum* (African basil) when Cd and Zn were present in soil, though metal uptake was reduced.

The addition of VAM also improved the plant growth in terms of high biomass and root/shoot length though to a lower extent than FYM. Studies note a positive effect of AM inoculation on the growth and some protection against metal toxicity in plants grown in metal-contaminated soils (Yu et al. 2004; Andrade et al. 2004). This protective benefit may be related to the adsorptive or binding capability for metals of the relatively large fungal biomass associated with the host plant roots, which may physically minimize or exclude the entry of metals into host plant (Meharg and Cairney 2000). AM fungi are also known to improve growth of plants by enhancing nutrient uptake (Khan et al. 2000).

The association between *Elsholtzia splendens* (with Cu, Zn, Pb, and Cd present in soil) and various arbuscular mycorrhizal fungi (*Gigaspora* spp., *Scutellospora* spp., *Acaulospora* spp., *Glomus* spp.) increases both root and shoot growth (Wang et al. 2005). Chen et al. (2005) found that mycorrhizae enhanced biomass and root/shoot ratios of *Kummerowia striata*, *Ixeris denticulata*, and *Echinochloa crus-galli*. They suggested that under an elevated Pb condition, mycorrhizae could promote plant growth by increasing Pb uptake and mitigating Pb toxicity by sequestrating more Pb in roots. Akay and Karaarsla (2011) observed better root-stem growth and uptake when barley grown in multi-metal (Pb, Zn, Cd, As, Ni, and Al)-contaminated soil was inoculated with *Glomus mosseae*.

According to Salido et al. (2003), EDTA may serve to reduce biomass by creating nutrient deficiencies since it forms complexes with many essential minerals for plants including Ca, Mg, and Fe. Chen and Cutright (2002) showed a 50 and 60 % decrease in shoot and root biomass in 4-week-old *H. annuus* with the EDTA versus plants without EDTA. Similar findings were reported by Lesage et al. (2005) for *Helianthus annuus*. However, in the present study, EDTA application was also seen to preserve root/shoot length and biomass of plants, though it was not as effective as the other two amendments. These results are more in line with Liphadzi and Kirkham (2006) who conducted an experiment to determine the uptake or leaching of Cu, Fe, Mn, and Zn due to EDTA added to soil from a sludge farm that had received sludge for 25 years. At harvest, concentrations of metals in the soil and poplar plants with EDTA were very high. Plant biomass increased due to improved supply of nutrient in soil. Saifullah et al. (2010) demonstrated that EDTA application resulted in more solubilization of Pb and increased shoot dry matter in wheat (*Triticum aestivum*).

13.10 Summary

The flora of metal-contaminated soils such as those at mines, dumps, and metal-related industrial areas should be investigated for heavy metal accumulation. It is amply evident from the results of the survey that such species hold promise as candidates for phytoremediation efforts due to their ability to adapt and survive in soils where metal toxicity issues are bound to arise. Metal uptake, translocation, and accumulation are plant as well as metal specific. In most plants surveyed, Zn, Cu, and As were preferentially accumulated in shoots and Co and Cd in roots, while Pb, Cr, and Ni were almost equally partitioned between shoots and roots. Although the plants evaluated in the survey and pot studied did not cross the hyperaccumulation thresholds reported in the literature, they hold promise in view of the fact that most of these yield much higher biomass per plant compared to reported hyperaccumulators. Low BCF values (root to soil ratio of metal) of Co, Pb, Cr, and Ni in the species indicate their limited potential in mobilizing these in the rhizosphere.

Preliminary results from surveys of flora should be corroborated with further studies as metal accumulation in plants is the outcome of diverse plant–soil–metal interactions, and a change in any factor could change the equilibrium. The phytoextraction potential of *C. procera* (for Pb) and *C. murale* (for Pb and Cu) as well as phytostabilization potential of *P. annua* (for Cd) and *C. procera* (for Cu) as seen in the survey were limited and not substantiated at higher metal doses of the subsequent pot study. *P. annua* (for Pb and Cu) and *C. murale* (for Cd) came out as accumulating tolerant species that hold promise for phytoextraction purposes. *C. procera* showed good potential for phytostabilization of Cd. All these point to the synergistic nature of an approach like phytoremediation which makes comprehensive understanding of local conditions even more vital before implementation of any kind.

The same reasoning can be extended to efforts to enhance phytoremediation using soil amendments. This also needs to be planned out in detail as use of soil amendments has a mixed record. Organic matter like manure forms metal complexes, so that it can either reduce metal mobility or increase availability when the complexes are soluble in the soil solution. Application of manure to *P. annua* (for Cu) and *C. murale* (for Cd) increased the uptake of metal but decreased it in *P. annua* (for Pb) and *C. procera* (for Cd). Arbuscular mycorrhizal fungi (AMF) decreased metal uptake except that of Cu in *P. annua*. Manure was most conducive to plant growth followed by VAM, which shows some protection against metal toxicity in plants grown in metal-contaminated soils.

EDTA was the only amendment that increased metal uptake in all three test species though it seemed to be metal and plant specific. But EDTA is generally regarded as non-biodegradable with the added risk of enhanced leaching of EDTA–metal complexes to deeper layers and even groundwater. Hence, its use should be weighed against any possible negative consequences.

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Roles of Brassicaceae in Phytoremediation of Metals and Metalloids

14

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14.1 Introduction

Rapid growth in human population and industrialisation led to the addition of large amounts of toxic wastes into the environment. These effluents affect human health worldwide (Zhuang et al. 2007). Metals, metalloids, radionuclides and other inorganic substances are most prevalent forms of environmental contaminants, and their remediation in soils and sediments is rather a difficult task (Cunningham et al. 1997; Prasad and Freitas 2003). Concerns of their toxicities led to the emphasis on the development of effective techniques to assess the presence and mobility of metals in soil, water and wastewater (Tangahu et al. 2011). Phytoremediation is considered to be cheap and environment friendly technology for removing or reducing toxic pollutants from different components of environment (USEPA 2000; Sarma 2011; Tangahu et al. 2011). In phytoremediation technique, accumulator plants are used to extract, sequester and/or detoxify pollutants. The generic term "phytoremediation" consists of the Greek prefix *phyto* (plant), attached to the Latin word *remedium* (to correct or remove an evil) (Erakhrumen and Agbontalor 2007; Tangahu et al. 2011).

The unique and selective uptake capabilities of plant root systems, effective translocation, bioaccumulation and contaminant degradation abilities of the accumulator plants are utilised in phytoremediation technique (Hinchman et al. 1996). Palmer et al. (2001) suggested the preferences for plants with extensive root system for absorption and transport of large amounts of contaminants to the shoots and finally removal by biomass harvesting. This biological mean of remediation is also referred as phytoextraction and can be used to remove heavy metals from soil. Certain heavy metals with no biological function or rather toxic effects, viz. cadmium (Cd), chromium (Cr), lead (Pb), cobalt (Co), silver (Ag), selenium (Se) and mercury (Hg), can move along with essential elements and get accumulated in plants (Cho-Ruk et al. 2006; Tangahu et al. 2011). Such metal enriched plants can then be removed from the site and disposed off. It is further emphasised that accumulating metal elements be recovered and recycled, if possible (Salt et al. 1998; Palmer et al. 2001). Several such species of plants have so far been identified for phytoremediation, and many of them are hyperaccumulators, efficiently accumulating phytotoxic elements at concentrations 50–500 times higher than average plants (Baker et al. 1994a; Reeves and Baker 2000; Palmer et al. 2001; Sarma 2011; Tangahu et al. 2011; Mellem et al. 2012). Hyperaccumulators with shoot-to-root metal concentration ratio of >1 are highly efficient in transport of metals from belowground to aboveground parts (Baker 1981; Gabbrielli et al. 1990; Homer et al. 1991; Baker et al. 1994a, b; Krämer et al. 1996; Shen et al. 1997; Zhao et al. 2000; McGrath et al. 2002; Alkorta et al. 2004). High bioconcentration factor (the ability of the plant to extract metals from the soil), efficient root-to-shoot transport and high metal tolerance enhance the desired detoxification potential of hyperaccumulators (Krämer 2010). Certain standards of hyperaccumulators have been set taking into account the extent of a specific metal a plant can accumulate without any adverse effect. The hyperaccumulation standards of plants vary with the type of metal. A plant can be called hyperaccumulator of specific metals if it exhibits the ability to accumulate 1,000 μg nickel (Ni) g^{-1} leaf dry mass, 10,000 μg (zinc) Zn or manganese (Mn) g^{-1} shoot dry mass, 100 μg Cd g^{-1} shoot dry matter and 1,000 μg Co/Cu/Pb or Se g^{-1} shoot dry matter (Brooks et al. 1977; Brooks 1998; Baker et al. 2000). About 500 plant species are known for their unique abilities to accumulate heavy metals in such high quantities (Krämer 2010). Most of these are angiosperms (Palmer et al. 2001; Baker and Whiting

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2002; Krämer 2010; Sarma 2011). Among angiosperms, Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae constitute the largest number of hyperaccumulators (Prasad and Freitas 2003). Family Brassicaceae alone represents about 25 % of the total list of hyperaccumulating angiosperms (Palmer et al. 2001; Krämer 2010; Sarma 2011), with 90 species of 11 genera (Prasad and Freitas 2003; Krämer 2010). It is important to mention here that the members of the plant family Brassicaceae have extraordinary potential to accumulate and tolerate high quantities of a number of toxic metals (Kumar et al. 1995; Anjum et al. 2012). In Table 14.1, hyperaccumulating members of Brassicaceae along with the specific accumulating metal(s) are listed.

The majority of the metal hyperaccumulators from the family Brassicaceae so far reported are hyperaccumulators of Ni followed by Zn (Table 14.1). Only six Brassicaceae members have been reported as Cd hyperaccumulators, viz. *Arabis gemmifera* (Kubota and Takenaka 2003), *Arabis paniculata* (Küpper et al. 2000; Tang et al. 2009), *Arabidopsis halleri* (Zhao et al. 2000; Yang et al. 2004), *Noccaea caerulescens* (Basic et al. 2006; Küpper and Kochian 2010), *N. praecox* (Küpper et al. 2000; Yang et al. 2004; Vogel-Mikuŝ et al. 2005; Hassan and Aarts 2010; Maestri et al. 2010) and *N. goesingensis* (Lombi et al. 2000).

Ni hyperaccumulation has been reported in 7 genera and 72 species of Brassicaceae (Reeves et al. 1996, 1999) and Zn in 20 species of 3 genera (Delorme et al. 2001; Prasad 2005). Most of these species are so far known as specialist metal accumulators as they either accumulate Ni or Zn. But some of them accumulate more than one heavy metal (Table 14.1). These hyperaccumulator plants appear to be good indicators of Ni and Zn because they have restricted field distribution and high degree of endemism to serpentine soils and specific rock types (Brooks and Radford 1978; Baker and Brooks 1989; Raskin et al. 1997; Palmer et al. 2001; Alkorta et al. 2004). Serpentine soils are naturally enriched with high levels of heavy metals (essential as well as non-essential), and species that grow on such soils have either developed mechanisms to exclude these metals or are capable of accumulating large quantities (Reeves et al. 1983; Palmer et al. 2001) without any adverse effect on their growth performance.

Among all Brassicaceae members, *Alyssum*, *Noccaea* and *Arabidopsis* are considered as model hyperaccumulator plants for their ability to accumulate excessive quantities of several metals including heavy metals and metalloids (Anjum et al. 2012). The heavy metal accumulation has been recorded in several Brassicaceae members, viz. *Alyssum* (Krämer et al. 1996; Sarma 2011), *Arabidopsis* sp. (Zhao et al. 2000; Chiang et al. 2006), *Brassica* sp. (Blaylock et al. 1997; Huang et al. 1997) and *Noccaea* sp. (Baker et al. 1994b). The findings of a number of workers on the accumulation of heavy metals in some representative plant species of Brassicaceae (*Alyssum* sp., *Arabidopsis* sp., *Brassica* sp. and *Noccaea* sp.) are summarised under the headings of some selected genera and suggested as suitable phytoremediators.

14.2 Genus *Arabidopsis*

Arabidopsis thaliana (L.) is a small, 10–40 cm tall, annual herb and considered as the model organism of flowering plants for experiments and trials of findings almost as guinea pigs (from among the fauna) has been used. The plant has a wide range of distribution; Europe and middle Asian mountains are thought to be its native places (Anjum et al. 2012). This plant is currently used in almost every discipline of experimental biology. Its genome has been completely sequenced (The *Arabidopsis* Genome Initiative 2000), which paved the way to a better understanding of every aspect of plant biology (Buell and Last 2010). In fact, *A. thaliana* is the first plant and the third multicellular organism after *Caenorhabditis elegans* (The *C. elegans* Sequencing Consortium 1998) and *Drosophila melanogaster* (Adams et al. 2000), to be completely sequenced (The *Arabidopsis* Genome Initiative 2000; The *C. elegans* Sequencing Consortium 1998; Adams et al. 2000; Anjum et al. 2012). A more striking finding in *A. thaliana* is the identification of Zn-accumulating genotypes (Reeves 1988; Palmer et al. 2001). *A. halleri* is also reported as hyper metal tolerant (Peer et al. 2003; Krämer 2010) and frequently distributed at Zn-, Cd- and Pb-contaminated sites and specifically hyperaccumulates Cd and Zn (Brooks 1998; Küpper et al. 2000). It is self-incompatible, perennial, stoloniferous diploid species ($2n = 16$ chromosomes) and a genome size of approximately 1.65 times to that of *A. thaliana* (Peer et al. 2003; Krämer 2010). Interestingly, it is closely related to and interfertile with *A. lyrata* subsp. petraea L. (O'Kane and Al-Shehbaz 1997) which is both non-tolerant and a nonaccumulator (Macnair et al. 1999; Anjum et al. 2012).

Populations of *A. halleri* subsp. *halleri* occur in Zn- and Cd-contaminated soils of Harz Mountains of Germany (Krämer 2010), in addition to natural populations of *A. halleri* on nonmetalliferous and noncalcareous soils in mountain regions of Bohemian forests and the central Swiss Alps (Krämer 2010). This wide distribution of *A. halleri* in both contaminated and non-contaminated soil makes Brassicaceae member more interesting for its wide range of tolerance to Zn and Cd in deficient to highly contaminated environment. Root elongation tolerance tests in hydroponics suggested that *A. halleri* plants tolerate at least 76-fold higher Zn concentrations (Willems et al. 2007) and 8 times higher Cd concentrations (Bert et al. 2003) as compared to *A. thaliana* and *A. lyrata*. In Poland, 33 European races of *A. halleri* (of metalliferous and nonmetalliferous sites) differed in their level of tolerance to Zn (Pauwels et al. 2006).

Table 14.1 Brassicaceae species known to hyperaccumulate heavy metals and metalloids

Taxa	Reference
Cadmium (Cd)	
Arabidopsis halleri	Zhao et al. (2000); Bert et al. (2002)
Arabis paniculata	Tang et al. (2009)
Arabis gemmifera	Kubota and Takenaka (2003)
Noccaea caerulescens (T. caerulescens)	Milner and Kochian (2008); Lombi et al. (2000); Küpper and Kochian (2010); Vogel-Mikuŝ et al. (2006)
N. goesingensis (T. goesingense)	Lombi et al. (2000)
N. praecox (T. praecox)	Küpper et al. (2000); Vogel-Mikuŝ et al. 2005
Lead (Pb)	
Alyssum wulfenianum	Reeves and Brooks (1983); Baker and Brooks (1989)
Arabidopsis halleri	Yang et al. (2004)
Arabis paniculata	Tang et al. (2009)
Brassica juncea	Blaylock et al. (1997)
Noccaea caerulescens (T. caerulescens)	Vogel-Mikuŝ et al. (2006)
N. cepaefolia (T. rotundifolium)	Reeves and Brooks (1983); Baker and Brooks (1989); Prasad (2005)
Nickel (Ni)	
Aethionema spicatum	Reeves et al. (2001)
Alyssum akamasicum	Brooks et al. (1979)
A. alpestre	Brooks and Radford (1978)
A. anatolicum	Brooks et al. (1979)
A. argenteum	Brooks and Radford (1978); Brooks et al. (1979)
A. bertolonii	Minguzzi and Vergnano 1948; Barzanti et al. (2011)
A. bracteatum	Ghaderian et al. (2007a)
A. callichroum	Brooks et al. (1979)
A. caricum	Brooks et al. (1979)
A. cassium	Brooks and Radford (1978)
A. chalcidicum	Brooks and Radford (1978)
A. chlorocarpum	Brooks et al. (1979)
A. cilicicum	Brooks et al. (1979)
A. condensatum	Brooks et al. (1979)
A. constellatum	Brooks et al. (1979)
A. corsicum	Brooks et al. (1979); Li et al. (2003)
A. crenulatum	Brooks et al. (1979)
A. cypricum	Brooks et al. (1979)
A. davisianum	Brooks et al. (1979)
A. discolour	Brooks et al. (1979)
A. dubertretii	Brooks et al. (1979)
A. dudleyi	Adigüzel and Reeves (2002)
A. eriophyllum	Brooks et al. (1979)
A. euboeum	Brooks and Radford (1978)
A. floribundum	Brooks et al. (1979)
A. giosnanum	Brooks et al. (1979)
A. heldreichii	Brooks and Radford (1978)
A. huber-morathii	Brooks et al. (1979)
A. inflatum	Ghaderian et al. (2007b)
A. lesbiacum	Brooks et al. (1979)
A. longistylum	Ghaderian et al. (2007b)
A. markgrafi	Brooks and Radford (1978)
A. masmenaeum	Brooks et al. (1979)
A. murale	Reeves et al. (2001); Reeves and Adigüzel (2008)
A. obovatum	Brooks et al. (1979)
A. oxycarpum	Brooks et al. (1979)
A. pateri	Reeves and Adigüzel (2008)
A. peltarioides	Reeves and Adigüzel (2008)
A. penjwinensis	Brooks et al. (1979); Reeves and Adigüzel (2008)

(continued)

Table 14.1 (continued)

Taxa	Reference
A. pinifolium	Brooks et al. (1979)
A. pintodasilvae	Gonclaves et al. (2007)
A. pterocarpum	Brooks et al. (1979)
A. robertianum	Brooks and Radford (1978)
A. samariferum	Brooks et al. (1979)
A. serpyllifolium	Becerra-Castro et al. (2009)
A. sibiricum	Brooks and Radford (1978)
A. singarense	Brooks et al. (1979)
A. smolikanum	Brooks et al. (1979)
A. syriacum	Brooks et al. (1979)
A. tenium	Brooks and Radford (1978)
A. trapeziforme	Brooks et al. (1979)
A. troodii	Brooks et al. (1979)
A. virgatum	Brooks et al. (1979)
Bornmuellera baldacci	Reeves et al. (1983)
B. glabrescens	Reeves et al. (1983)
B. kiyakii	Reeves and Adigüzel (2008)
B. tymphaea	Reeves et al. (1983)
Brassica juncea	Saraswat and Rai (2009)
Cardamine resedifolia	Vergnano Gambi and Gabbrielli (1979)
Leptoplax emarginata	Reeves et al. (1980)
Masmenia rosularis	Reeves (1988)
Microthlaspi perfoliatum (as Thlaspi perfoliatum)	Reeves et al. (2001)
Noccaea caerulescens (as T. caerulescens)	Reeves and Brooks (1983); Milner and Kochian (2008)
N. cariensis (as T. cariense)	Reeves et al. (2001)
N. cepaefolia (T. rotundifolium)	Baker and Brooks (1989); Prasad (2005)
Selenium (Se)	
Stanleya pinnata	Rosenfeld and Beath (1964); Freeman et al. (2010); Hladun et al. (2011)
Zinc (Zn)	
Arabis gemmifera	Kubota and Takenaka (2003)
A. paniculata	Tang et al. (2009)
Arabidopsis thaliana	Cannon (1960); Reeves (1988)
Arabidopsis halleri	Ernst (1968)
Noccaea caerulescens (as T. caerulescens)	Sachs (1865); Ernst (1974); Reeves and Brooks (1983); Milner and Kochian (2008); Küpper and Kochian (2010)
N. cepaefolia (T. rotundifolium)	Reeves and Brooks (1983); Baker and Brooks (1989)
N. goesingensis (T. goesingense)	Lombi et al. (2000)
N. ochroleuca (as T. ochroleucum)	Reeves and Brooks (1983)

Biotechnological and conventional plant breeding attempts have been made to enhance metal (Zn, Cd, Pb, As) accumulating capacity of these plants (Palmer et al. 2001) on the knowledge that trait is genetically controlled (Tomsett and Thurman 1988; Palmer et al. 2001; Alkorta et al. 2004; Song et al. 2003; Dhankher et al. 2002; Sauge-Merle et al. 2003). The metal (As) accumulating capacity of transgenics of *A. thaliana* enhanced many folds (Dhankher et al. 2002) in addition to increase in Cd and Pb remediation abilities of *A. thaliana* (Song et al. 2003; Alkorta et al. 2004) and Hg (Bizily et al. 1999; Palmer et al. 2001; Alkorta et al. 2004; Raskin et al. 1997; Heaton et al. 1998; Prasad and Freitas 2003).

14.3 Genus *Noccaea*

Noccaea caerulescens (formerly *Thlaspi caerulescens*) is a self-compatible, biannual, diploid and Zn/Cd hyperaccumulator with $2n = 14$ chromosomes with approximately 88 % resemblance in nucleotide sequence to that of *Arabidopsis thaliana* (Peer et al. 2003, 2006; Krämer 2010). It is facultative metallophyte and naturally distributed from northern Spain and Italy to the UK, Scandinavia and Poland (Krämer 2010; Anjum et al. 2012). Genus *Noccaea* is described as generalist hyperaccumulator of a varied range of metals and metalloids (Peer et al. 2003). *N. caerulescens* is known as hyperaccumulator for Cd, Ni, Pb and Zn; *N. goesingensis* for Cd and Zn; *N. ochroleuca* (earlier as *T. ochroleucum*) for Zn; and *N. cepaefolia* (*Thlaspi rotundifolium*) for Ni, Pb and Zn (Palmer et al. 2001) (Table 14.1). In particular, *N. caerulescens* is extensively studied species for Cd, Zn and Ni hyperaccumulation (Assunção et al. 2003a, b; Milner and Kochian 2008). *N. caerulescens* is widely distributed in Zn- and Pb-enriched serpentine soils (Garcia-Gonzalez and Clark 1989; Baker and Proctor 1990) and has been recommended as suitable phytoremediator, because it transfers a greater proportion of absorbed heavy metals to its upper parts (Palmer et al. 2001), where from consistence harvesting/removal of toxicant is relatively easier. Zn hyperaccumulation in *N. caerulescens* was first discovered in 1865 (Sachs 1865; Reeves and Baker 2000) but attracted special attention in the early 1990s as alternative metal accumulator (Krämer 2010). The field and pot trials revealed that *N. caerulescens* decreased soil Cd levels by half in a single year, but equal reduction in Zn levels took longer time (Robinson et al. 1998) because soil levels of Zn were much more higher than that of Cd in addition to a lower bioaccumulation coefficient of Zn. This species could remediate about 500 μg Zn g^{-1} soil in 8 years and 20 μg g^{-1} Cd in 2 years (Palmer et al. 2001).

Several ecotypes of *Noccaea caerulescens* have been reported for variable hyperaccumulating capacity, viz. an ecotype of *N. caerulescens* accumulated over 5 % (53.45 mg g^{-1} dry weight) of Zn from mine areas of France (Baker et al. 1994b; Reeves et al. 2001; Besnard et al. 2008). Some ecotypes were shown to accumulate only 3 % (30 mg g^{-1}) Zn (Brown et al. 1995a, b) and 10 mg Cd g^{-1} dry weight (Lombi et al. 2000) without any signs of toxicity (typical shoot levels are 0.1–0.2 mg g^{-1} for Zn and 0.0001–0.01 mg g^{-1} for Cd) (Anjum et al. 2012). The shoot Zn concentrations in Zn-sufficient nonaccumulator plants are around 100 mg g^{-1}, with 30 mg g^{-1} adequate and 300–500 mg g^{-1} toxic (Mengel and Kirkby 1987), but foliar Cd levels above 1–10 mg g^{-1} are usually toxic (Anjum et al. 2012). Therefore, *N. caerulescens* has emerged as a model metal hyperaccumulator species with homeostasis and tolerance (Rigola et al. 2006; Anjum et al. 2012). In this genus another species is also known for Ni and Pb hyperaccumulation. *N. cepaefolia* can accumulate Ni to about 18.5 mg g^{-1} (Prasad and Freitas 2003; Krämer 2010) and Pb to about 8.2 mg g^{-1} dry weight (Reeves and Brooks 1983).

Like *Arabidopsis halleri*, there is considerable variation in leaf Cd, Zn and Ni accumulation within populations of *N. caerulescens* (Reeves et al. 2001; Krämer 2010). Many populations of *N. caerulescens* are hyperaccumulators of Ni which mainly concentrated it in their leaves (Peer et al. 2003, 2006), but Zn was preferably accumulated when both metals (Ni and Zn) were applied in equal concentrations (Taylor and Macnair 2006) in Ni-hyperaccumulating accessions (Krämer 2010). Cd hyperaccumulation has been reported in the majority of populations on contaminated soils (Baker et al. 1994a; Reeves et al. 2001; Krämer 2010). Lombi et al. (2000) reported that *N. caerulescens* may hyperaccumulate Cd (>0.01 % 100 μg g^{-1} shoot dry weight) and Ni (>0.1 % of shoot dry weight) as well. However, the accession of *N. caerulescens*, namely, Ganges (southern France), had superior ability to hyperaccumulate Cd up to 1 % in hydroponics and up to 0.3 % Cd under field conditions (Robinson et al. 1998), while *N. praecox* Wulfen (previously as *T. praecox*) from a trace metal polluted site in Slovenia hyperaccumulated up to 0.6 % Cd in shoots (Vogel-Mikuŝ et al. 2005), up to 0.07 % in flowering and seed stalks (Pongrac et al. 2007) and up to 0.14 % Cd in seeds (Vogel-Mikuŝ et al. 2007) under field conditions. These variable hyperaccumulation capacities of *Noccaea* spp. were genotype and/or growing conditions dependent. Under field conditions, various accessions of *N. caerulescens* could accumulate up to 4–5 % Zn (Baker et al. 1994b; Reeves et al. 2001), but in hydroponically grown plants, only 2–4 % Zn accumulation was recorded (Brown et al. 1995a, b; Shen et al. 1997).

High variability in metal accumulation potential has been reported in two populations of *Noccaea praecox* from Slovenia (Vogel-Mikuŝ et al. 2005). About 1.5 % Zn accumulation was recorded in shoot specimens of *N. praecox* collected from the field (Vogel-Mikuŝ et al. 2005), but 0.3 % Zn was recorded under experimental conditions by them. The "model" hyperaccumulator *N. caerulescens* has been much screened in the search for new and more extreme ecotypes (Lombi et al. 2002; Alkorta et al. 2004). Thus in entire genus, *N. caerulescens* is a potential source of genes for engineering trace metal phytoremediation (Assunção et al. 2003b; Alkorta et al. 2004; Deniau et al. 2006).

14.4 Genus *Alyssum*

Alyssum is a genus of about 172 species (Brooks 1998; Reeves et al. 2001; Ghaderian et al. 2007a, b) and widely distributed in Europe, Asia and northern Africa, with the highest species diversity in the Mediterranean region. The genus *Alyssum* possesses extraordinary Ni hyperaccumulation potential (Brooks 1998; Reeves et al. 2001). All Ni-hyperaccumulating species of genus *Alyssum* are perennials and distributed mostly on serpentine soils in southern Europe and Asia Minor, extending from Portugal to the western parts of Iran (Ghaderian et al. 2007a, b). Only the individuals of this genus growing on serpentine soils had elevated levels of Ni and Co compared to the individuals growing on other soil types (Minguzzi and Vergnano 1948; Brooks and Radford 1978; Brooks et al. 1979; Ghaderian et al. 2007a).

The serpentine soils of Iran had highest Ni concentrations (1,350 μg g^{-1} of soil), *Alyssum inflatum* growing in soil accumulated 3,700 μg and *A. longistylum* 8,100 μg Ni g^{-1} dry weight (Ghaderian et al. 2007b). The Mg/Ca quotient of these *Alyssum* species has always been low due to its exceptional ability to take up Ca, even from soils with low total Ca concentrations (Lombini et al. 1998; Shallari et al. 1998). The Ni hyperaccumulator species of genus *Alyssum* can be considered for their remarkable Ca uptake ability (Ghaderian et al. 2007b). *A. murale* populations from serpentine soils of Serbia emerged as strong Ni hyperaccumulators (Tumi et al. 2012) and be employed for phytoextraction for their high biomass production and Ni hyperaccumulation potential in addition to *A. corsicum* (Chaney et al. 2007).

More detailed studies on sites of Ni hyperaccumulation in different organs of *Alyssum* species have been carried out. Significant concentrations of Ni in epidermal cell vacuoles, trichome bases and the lower parts of the trichome pedicle have been reported in *Alyssum murale* (Smart et al. 2007). Histidine had a key role as a Ni chelator in both Ni hypertolerance and high root-to-shoot Ni flux in the xylem of *Alyssum lesbiacum* (Ingle et al. 2005). Ni hyperaccumulator species of the genus *Alyssum* have constitutively harboured high concentrations of the free amino acid histidine to act as a ligand for Ni ions in the plants (Krämer et al. 1996; Kerkeb and Krämer 2003; Anjum et al. 2012).

14.5 Genus *Brassica*

The genus *Brassica* includes about 35 species (most of them herbs). Several species of *Brassica* exhibit higher tolerance to toxic metals/metalloids and are good accumulators of toxic metals like Cd, Cu, Ni, Pb and Zn (Kumar et al. 1995). *B. juncea* exhibits superior metal accumulation characteristics (Alkorta et al. 2004; Szczygłowska et al. 2011). Field trials of *B. juncea* have been conducted for phytoremediation of Cd, Cu, Cr, Ni, Pb, Se, Zn, radionuclides and other metals (Lasat et al. 1998; Salt et al. 1998). *B. juncea* has been found to accumulate 1,450 μg Cd g^{-1} and *B. napus* 555 μg Cd g^{-1} dry weight (Nouairi et al. 2006). In *B. juncea*, heavy metals, such as Cu^{2+} and Cd^{2+}, increased the mRNA for enzyme γ-glutamylcysteine synthetase (γ-ECS) in roots and shoots which in turn increased phytochelatins and GSH levels even when growth was inhibited (Schäfer et al. 1997, 1998). *B. juncea* also exhibited a high Pb and Se removal efficiency (Salt et al. 1998; Szczygłowska et al. 2011). In another study Kumar et al. (1994) reported that Pb accumulation in *B. juncea* varies from 1,416 to 18,812 μg g^{-1} dry weight. *B. juncea* was superior to other species in the transport of absorbed Pb to shoots and was also found to be highly tolerant to Se even under saline conditions (Banuelos et al. 1996, 1997; Alkorta et al. 2004). This species is more effective in removing Zn from soil due to higher biomass production than *N. caerulescens*, a known hyperaccumulator of zinc (Gisbert et al. 2006; Szczygłowska et al. 2011).

B. juncea may be a good source of suitable genes for phytoremediation in addition to *N. caerulescens*, since tolerance and accumulation are largely independent properties and both taxa should be engineered to get a suitable plant for metal phytoextraction (Macnair et al. 1999; Kärenlampi et al. 2000). Hybrid plants, produced by asymmetric somatic hybridisation between *N. caerulescens* and *B. juncea*, have already shown high metal accumulation potential, tolerance to toxic metals and good biomass production (Dushenkov et al. 2002). In another experiment, it was noted that transgenic Indian mustard (*B. juncea*) plants removed a significant amount of metal from the contaminated soil as compared with wild-type Indian mustard (Bennett et al. 2003). The transgenic *B. juncea* overproduced enzyme gamma-glutamylcysteine synthetase or glutathione synthetase (a metal-binding thiol peptides phytochelatins and glutathione responsible for enhanced Cd tolerance and accumulation) as well as adenosine triphosphate sulfurylase (for higher levels of glutathione and total thiols) as reported by Alkorta et al. (2004).

B. napus grows profusely and accumulates excessive quantities of heavy metals (Ebbs et al. 1997; Marchiol et al. 2004). Grispen et al. (2006) reported the shoot and root accumulation of Zn in *B. napus* grown for 4 months at two locations of metal smelters with an average of 657 μg g^{-1} and 1,151 μg g^{-1} shoot dry weight. A considerable accumulation of Cd in leaves of *B. napus* grown for 47 days in varying levels of Cd-contaminated soil was reported by Carrier et al. (2003).

Brassica campestris L. (syn. *B. rapa* L.), commonly known as turnip rape or turnip mustard, is widely cultivated as a leaf and root vegetable as well as an oilseed crop. The efficiency of *B. campestris* for accumulating heavy metals in its belowground part makes it suitable for phytoremediation (Gleba et al. 1999). Chromium (Cr) accumulation was twice higher in root and four times in shoot of *B. campestris* as compared to *Trigonella foenum-graecum* and *Spinacia oleracea* grown in Cr-contaminated soil (Dheri et al. 2007). On the basis of response of these three crops, the entire family Brassicaceae was considered as most tolerant to Cr followed by family Chenopodiaceae (*S. oleracea*) and Leguminosae (*T. foenum-graecum*), and it was suggested that the members of family Brassicaceae can be considered for the phytoremediation of Cr (Dheri et al. 2007). These conclusions should have better been drawn either after testing large number of members for the family or supported with adequate literature.

Like other *Brassica* species, *B. oleracea* accumulates significant amounts of toxic heavy metals (Kabata-Pendias 2001). In a study, two species of *Brassica* were tested for their ability to accumulate and transport Pb. The Pb accumulation in *B. juncea* and *B. oleracea* varied from 1,416 to 18,812 μg g^{-1} dry weight. *B. oleracea* retained larger amounts of Pb in the roots than in above-ground parts (Kumar et al. 1994). In another study, it was noted that *B. oleracea* accumulated 381 mg Zn kg^{-1} dry weight and 834 mg Cu kg^{-1} dry weight in the shoots (Gisbert et al. 2006). In a recent study, Cd- and Pb-tolerant *B. oleracea* plants extracted significant amounts of Cd and Pb from the heavy metal salt-spiked soil (Addae et al. 2010).

14.6 Mechanism of Heavy Metal Hyperaccumulation

The heavy metal hyperaccumulation not only varies species to species but also varies population to population with the ecotypes (Roosens et al. 2003; Deng et al. 2007). The hyperaccumulation of plants depends on three basic features that distinguish them from related non-hyperaccumulator taxa. These common traits are (a) potential of a species for greater heavy metal uptake from the soil, (b) quick and effective root-to-shoot translocation of metal and (c) a great ability of hyperaccumulating species to detoxify and sequester translocated heavy metals in the leaves (Rascio and Navari-Izzo 2011). The mechanism is explained through Fig. 14.1. To understand the mechanisms which governs metal hyperaccumulation in plants, comparative physiological (Lombi et al. 2002; Zhao et al. 2002a, b), genomic (Kim et al. 2004; Gustin et al. 2009) and proteomic (Assunção et al. 2001, 2010) studies of hyperaccumulators and related non-hyperaccumulator plants have been carried out. A great number of studies (see Table 14.1) are on *N. caerulescens* and *A. halleri*, which have become model plants (Milner and Kochian 2008; Krämer 2010). It is interesting to note that most key steps in hyperaccumulation do not depend only on novel genes; instead the genes common to hyperaccumulators and non-hyperaccumulators were differently expressed and regulated in the two kinds of plants (Baker and Brooks 1989; Rascio and Navari-Izzo 2011).

14.7 Uptake of Metals from the Soil to the Root

Some studies have revealed that the enhanced Zn uptake (in *N. caerulescens* and *A. halleri* roots) takes place by constitutive overexpression of some genes of ZIP (*z*inc-regulated and *i*ron-regulated transporter *p*roteins) members, coding for plasma membrane located cation transporters (Assunção et al. 2001; Krämer et al. 2007) (Fig. 14.1). Moreover, the expression of many ZIP members (ZNT1 and ZNT2 in *N.*

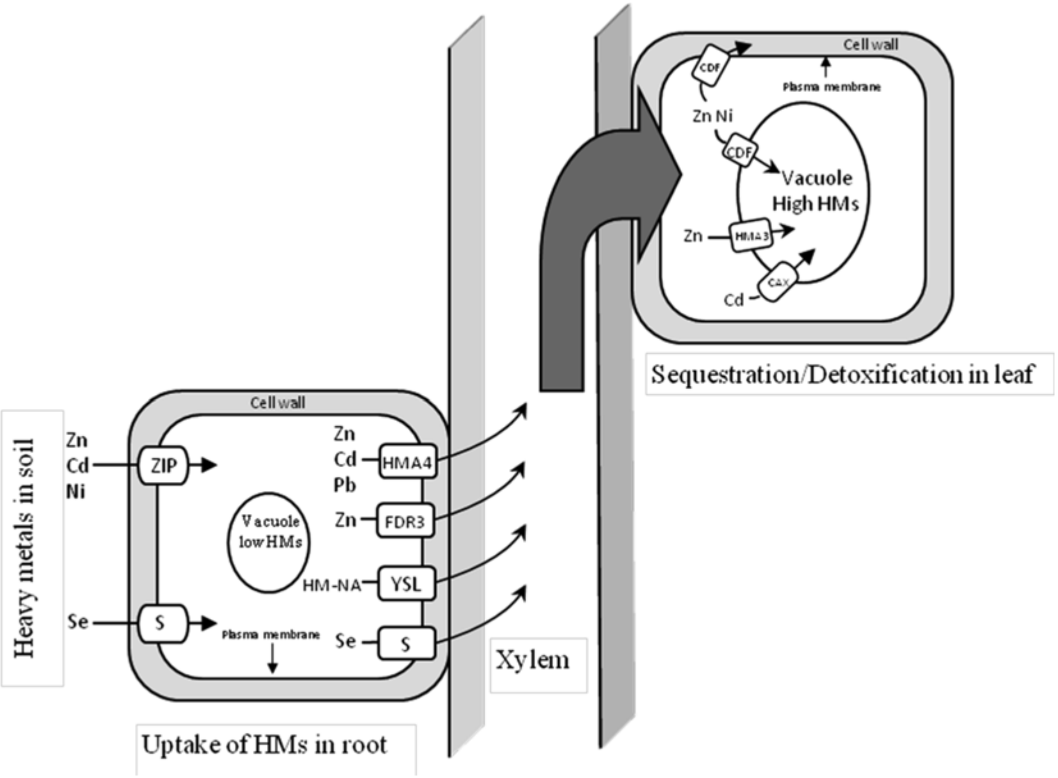

Fig. 14.1 Diagrammatic representation of uptake, transport and sequestration/detoxification of heavy metals in plants (adapted from Rascio and Navari-Izzo 2011 with permission from Elsevier). *CAX* cation exchangers, *CDF* cation diffusion facilitators, *FDR3* a member of the multidrug and toxin efflux family, *HM* heavy metal, *HMA* heavy metal transporting ATPases, *NA* nicotianamine, *S* sulphate transporters, *YSL* yellow strip 1-like proteins, *ZIP* zinc-regulated and iron-regulated transporter proteins

caerulescens and ZIP6 and ZIP9 in *A. halleri*) in non-hyperaccumulating plants is Zn regulated (Assunção et al. 2010) and occurred at detectable levels only under Zn deficiency, whereas in hyperaccumulators, their expression is more or less independent of the Zn supply and they are also expressed even at high Zn availability (Pence et al. 2000; Verbruggen et al. 2009; Rascio and Navari-Izzo 2011).

Some physiological studies on *N. caerulescens* provided strong evidence that multiple uptake systems were involved in Cd and Zn uptake in root (Lombi et al. 2002; Zhao et al. 2002b; Roosens et al. 2004; Verbruggen et al. 2009). The experiments revealed that Cd uptake in root decreased with increasing Zn concentration in Cd/Zn hyperaccumulator *A. halleri* and many ecotypes of *N. caerulescens* (Zhao et al. 2002b) and made it further clear that Cd influx is mainly due to Zn transporters (Fig. 14.1) with a strong preference to Zn uptake over Cd (Zhao et al. 2002b). Surprisingly, Ganges ecotype of *N. caerulescens* has evolved an exceptionally high ability to hyperaccumulate Cd in aboveground tissues independent of Zn inhibition. These findings suggest the presence of a specific and efficient independent Cd transport system in root cells of some ecotypes (Lombi et al. 2001; Verbruggen et al. 2009; Rascio and Navari-Izzo 2011).

The Cd uptake was enhanced by iron (Fe) deficiency in Ganges ecotypes (Lombi et al. 2001), but not in Prayon ecotypes (Roosens et al. 2003). The Cd competed with Fe uptake in former ecotype but not in later where Ca uptake was checked on Cd addition. This finding suggests that in addition to the two Zn uptake systems, at least two other uptake systems are involved in Cd uptake (Roosens et al. 2003; Verbruggen et al. 2009). The Cd hyperaccumulation in the Ganges population was governed by AtIRT1 (Lombi et al. 2001), whereas TcIRT1 was incapable of Cd transport (Plaza et al. 2007). These ecotypes are environmentally (Cd/Zn) induced variants with specific uptake systems. Specific transporters for Ni hyperaccumulation have not yet been identified. But, preference of Zn uptake over Ni by some Zn/Ni hyperaccumulators (Assunção et al. 2001) strongly indicates that a Zn transport system might also be employed in Ni entrance into roots (Fig. 14.1) (Assunção et al. 2008).

A possible mechanism of Se uptake based on chemical similarity has been suggested (Shibagaki et al. 2002). The chemical similarity between sulphate and selenate accounts for the root uptake of Se in the form of selenate through sulphate transporters (Fig. 14.1) and duly regulated by the sulphur status of the plant (Shibagaki et al. 2002). In a Se hyperaccumulator (*Stanleya pinnata* of Brassicaceae), the Se/S ratios in shoots are much higher than in non-hyperaccumulator sister species. This supports the idea of a role of one or more sulphate transporters in Se uptake (Galeas et al. 2007).

14.8 Translocation from the Root to the Shoot

Most of the heavy metals taken up from the soil by non-hyperaccumulator plants are retained in root cells, detoxified through chelation in the cytoplasm or partitioned and stored in vacuoles, whereas hyperaccumulators rapidly and efficiently translocate these elements to the shoot via the xylem (Rascio and Navari-Izzo 2011) as shown in Fig. 14.1. The availability of trace metals for translocation to the shoot implies limited sequestration in vacuoles (Fig. 14.1) of root cells (Verbruggen et al. 2009). It is further evident from that the amount of Zn sequestered into root cell vacuoles was 2–3 times lower and the Zn efflux out of vacuoles was almost twice higher in the hyperaccumulator *N. caerulescens* than in congener non-hyperaccumulator relatives (Lasat et al. 2000; Rascio and Navari-Izzo 2011). The higher metal concentrations in the xylem sap due to enhanced xylem loading have also been recorded in hyperaccumulators (Lasat et al. 1998). And, in this process several types of transporters are involved (Verbruggen et al. 2009).

A large body of evidence indicates that fast and efficient root-to-shoot translocation of heavy metals and metalloids in hyperaccumulator plants depends on enhanced xylem loading due to constitutive overexpression of genes coding for transport systems (Verbruggen et al. 2009). Some heavy metal transporting proteins (P_{1B}-type ATPases or HMAs) play important role in transporting metal ions against their electrochemical gradient using the energy provided by ATP hydrolysis (Axelsen and Palmgren 1998; Verbruggen et al. 2009). Genes encoding bivalent cation transporters (HMAs particularly HMA4) are overexpressed or upregulated in roots and shoots of Zn/Cd hyperaccumulators *N. caerulescens* and *A. halleri* (Papoyan and Kochian 2004; Talke et al. 2006; Hanikenne et al. 2008) and downregulated in non-hyperaccumulator relatives (Papoyan and Kochian 2004). HMAs cluster into two classes: Cu/Ag group (transporting monovalent cations) and Zn/Co/Cd/Pb group transporting divalent cations (Verbruggen et al. 2009). The role of HMA4 proteins (located at plasma membranes of xylem parenchyma) in Zn/Co/Cd/Pb subgroup is enhanced by the overexpression of *HMA4* gene which in turn efflux Cd and Zn from root symplast to xylem vessels for shoot uptake (Mills et al. 2003; Rascio and Navari-Izzo 2011). The HMA4 activity positively affects other candidate genes for hyperaccumulation. Thus the root-to-shoot translocation acts as a driving force of the hyperaccumulation by creating a permanent metal deficiency response in roots (Hanikenne et al. 2008; Rascio and Navari-Izzo 2011).

The MATE (multidrug and toxin efflux), a small organic molecule transporter protein, is active in heavy metal uptake in hyperaccumulator plants (Fig. 14.1). *FDR3* gene belongs to this group and is constitutively overexpressed in roots of *N. caerulescens* and *A. halleri* (Talke et al. 2006). The overexpression of FDR3 protein localised at root pericycle plasma membranes is thought to efflux citrate into the root vascular tissue to transport iron (Durrett et al. 2007) and may also translocate other metals, such as Zn (Krämer et al. 2007).

A large number of constitutive small organic molecules present in hyperaccumulator roots function as metal-binding ligands (Haydon and Cobbett 2007). Free amino acids, such as histidine and nicotianamine, form stable complexes with bivalent cations and play a key role in heavy metal hyperaccumulation (Callahan et al. 2006). Free histidine (His) is regarded as the most important ligand involved in Ni hyperaccumulation (Callahan et al. 2006; Verbruggen et al. 2009). In the Ni hyperaccumulator *Alyssum lesbiacum*, Ni exposure induced a dose-dependent increase in His in the xylem sap (Krämer et al. 1996; Kerkeb and Krämer 2003) due to overexpression of the *TP-PRT1* gene which leads to a larger endogenous pool of His in their roots and thereby favours the Ni loading in xylem as a Ni–His complex (Kerkeb and Krämer 2003; Ingle et al. 2005). Amino acid operates in the same way in Ni-hyperaccumulating *Noccaea* species and other hyperaccumulators (Assunção et al. 2003b). Thus, the Ni–His complexation and sustained Ni release into the xylem prevent the heavy metal entrapment in root cell vacuoles and keep it in the cytosol in detoxified form and, thereby, make it available for translocation to shoot in hyperaccumulator plants (Verbruggen et al. 2009; Rascio and Navari-Izzo 2011). His-overproducing transgenic *A. thaliana* displayed elevated Ni tolerance, but did not exhibit increased Ni concentrations in xylem sap or in leaves (Wycisk et al. 2004; Ingle et al. 2005). This suggests that His-dependent Ni unloading in xylem may not be universal in Brassicaceae (Verbruggen et al. 2009).

Synthesis of nicotianamine (NA) is universal in all plants (Verbruggen et al. 2009) and forms strong complexes with most transition metal ions. The NA seems to be involved in the movement of micronutrients throughout the plant (Stephan and Scholz 1993). Nicotianamine enhances metal hyperaccumulation, both in *A. halleri* and in *N. caerulescens* (Talke et al. 2006; Rascio and Navari-Izzo 2011). Enhanced nicotianamine synthesis and nicotianamine–metal chelation (Colangelo and Guerinot 2006) showed a positive correlation with Ni hyperaccumulation in *N. caerulescens* (Vacchina et al. 2003) and Zn hyperaccumulation in *A. halleri* (Weber et al. 2004).

Three genes (*TcYSL3*, *TcYSL5* and *YSL7*) are constitutively overexpressed in roots and shoots of *N. caerulescens* where the YSL (yellow strip 1-like) proteins participate in vascular loading and translocation of nicotianamine–metal (especially nicotianamine–Ni) complexes (Gendre et al. 2007). The transport system involved in xylem loading of Ni–His complexes occurring in hyperaccumulator roots has not been revealed yet (Rascio and Navari-Izzo 2011). After the uptake of Se by Se hyperaccumulators, Se remains in the form of selenate in root cells. Thus, its root-to-shoot translocation occurs through sulphate transport systems (Sors et al. 2005).

14.9 Detoxification/Sequestration

Effective detoxification and sequestration is a key feature of hyperaccumulator plants and allows them to accumulate huge amounts of heavy metals/metalloids in above-ground organs without any toxicity (Rascio and Navari-Izzo 2011). Among the above-ground parts, leaves are the major sink of heavy metal accumulation where photosynthesis (essential for plant survival) takes place (Rascio and Navari-Izzo 2011). In different hyperaccumulator plants, heavy metals/metalloids are detoxified in different organs or tissues (Table 14.2). The detoxification/sequestration may occur in epidermis (Bidwell et al. 2004; Asemaneh et al. 2006), trichomes (Küpper et al. 2000) or cuticle (Robinson et al. 2003), where photosynthetic machinery is not directly threatened (Rascio and Navari-Izzo 2011) (see Table 14.2). The detoxification mechanisms in aerial organs involves complexation of heavy metals with ligands and/or their removal from metabolically active cytoplasm and by moving them into metabolically inactive sites like vacuoles and cell walls (Fig. 14.1). The detoxification trait of hyperaccumulators depends, in part, on constitutive

Table 14.2 Heavy metal distribution at tissue/cellular level in some hyperaccumulators of Brassicaceae

Plant species	Tissue/organ	Metals	References
Alyssum lesbiacum	Trichome, epidermis	Ni	Krämer et al. (1997)
A. inflatum	Trichome	Ni	Ghasemi et al. (2009)
Arabidopsis halleri	Trichome	Zn, Cd	Küpper et al. (1999)
	Mesophyll	Zn	Küpper et al. (1999)
Brassica juncea	Trichome	Cd	Salt et al. (1995)
Noccaea caerulescens	Epidermis, vacuole	Zn	Vazquez et al. (1994); Küpper et al. (1999)
N. goesingensis	Cell wall	Ni	Krämer et al. (2000)

overexpression of genes to encode proteins involved in heavy metal transfer across the tonoplast and/or plasma membrane and involved in excluding them from cytoplasm (Rascio and Navari-Izzo 2011).

Among these proteins CDF (cation diffusion facilitator) also called MTPs (metal transporter proteins) mainly mediate bivalent cation efflux (Fig. 14.1) from the cytosol (Peiter et al. 2007). *MTP1*, a gene encoding a protein localised at tonoplast, is highly overexpressed in leaves of Zn/Ni hyperaccumulators (Gustin et al. 2009). The Zn transport into the vacuole initiates a systemic Zn deficiency response and thereby enhances the heavy metal uptake and translocation via the increased ZIP transporters (Gustin et al. 2009). MTP members also mediate the Ni vacuolar storage in *N. goesingensis* shoots (Persant et al. 2001). The *MTP1* gene localised both at vacuolar and plasma membrane can facilitate Zn and Ni efflux from cytoplasm to cell wall (Kim et al. 2004; Rascio and Navari-Izzo 2011). The overexpression of another gene *HMA3* coding for a vacuolar P_{1B}-ATPase may also be involved in Zn compartmentation. *CAX* genes encoding cation exchangers mediate Cd sequestration (Fig. 14.1) as found in *N. caerulescens* and *A. halleri* (Craciun et al. 2006; van de Mortel et al. 2008).

Small ligands, such as organic acids, have a major detoxifying role and prevent the persistence of heavy metals as free ions in the cytoplasm by entrapping them in vacuoles. Citrate is the main ligand of Ni in leaves of *N. goesingensis* (Krämer et al. 2000). Zn in *A. halleri* and Cd in *N. caerulescens* are complexed with malate (Salt et al. 1999; Sarret et al. 2002). The heavy metal detoxification in hyperaccumulators, in contrast with tolerant non-hyperaccumulator plants, does not depend on high molecular mass ligands, such as phytochelatins (Raab et al. 2004; Verbruggen et al. 2009), because of the excessive sulphur amounts and the prohibitive metabolic cost that a massive synthesis of this kind of chelators would require (Zhao et al. 2002a, b). Instead, overexpression of antioxidation-related genes (Chiang et al. 2006) and enhanced synthesis of glutathione (GSH) as pivotal antioxidant molecule (van de Mortel et al. 2008) do occur to strengthen the cell antioxidant system and deal with the risk of ROS rise due to heavy metal stress (Rascio and Navari-Izzo 2011). The major detoxification strategy in Se hyperaccumulators is to get rid of selenoamino acids, mainly selenocysteine (Se–Cys), derived from selenate assimilation in leaf chloroplasts. Misincorporation of selenoamino acids in proteins instead of sulphur amino acids leads to Se toxicity (Rascio and Navari-Izzo 2011). This detoxification occurs through methylation of Se–Cys to the harmless non-protein amino acid methylselenocysteine as catalysed by selenocysteine methyltransferase constitutively expressed and activated only in leaves of hyperaccumulator species (Sors et al. 2009).

14.10 Conclusion

From the reviews it is evident that family Brassicaceae is exceptionally important for phytoremediation of heavy metals and metalloids worldwide. This family includes a large number of hyperaccumulating species with better knowledge and state of the art regarding metal tolerance at the molecular, cellular and whole plant level. The use of *Arabidopsis thaliana*, *A. halleri*, *Alyssum* sp. *Brassica* sp and *Noccaea caerulescens* as model species has revealed numerous physiological mechanisms and genetics behind the metal uptake. The phytoremediation technique for cleaning metal contaminated sites needs further refinements. Some generalisations are also required to be made. There should be a set standard for determining useful hyperaccumulators. The relative uptake of heavy metals on above-ground mass basis appears to be more reliable and may be practically harvested and disposed off. The accumulation of heavy metals on per gram dry leaf may be useful in species whose leaves are not used as food, fodder or medicines.

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Part IV

Phytoremediation of Organic Contaminants and Organic–Inorganic Mixtures

PAH Contamination of Urban Soils and Phytoremediation

15

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15.1 Introduction

Accumulation and persistence of toxic materials in the environment is a major concern. Anthropogenic activities have been steadily altering the natural biogeochemical cycles of the environment ever since the industrial revolution. Every compartment of the biosphere, today, is reeling under the ill effects of the rapid pace of industrialisation, urbanisation and intensive agricultural activities. Soil is one of the natural resources being over-exploited globally due to increased industrial, agricultural and other human activities. Soil contamination, both diffuse and localised, can lead to severe damage of soil functions as well as contamination of surface and groundwater. Urban soils are increasingly acting as a sink for a wide range of contaminants due to the rapid pace of development. Various organics are generated as by-products from various industries (petroleum, pulp and paper, chemical industries etc.), which may be released into the environment due to negligence or accidents. Toxic aromatics and their chlorinated derivatives which are difficult to biodegrade are of primary concern. These are generated from petroleum and petrochemicals, e.g. polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), pesticides and herbicides, etc. Fossil fuels are still the major energy source for most industries. Metals, radionuclides and other inorganic contaminants are also very prevalent.

15.1.1 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs), which occur naturally in crude oil and are a part of its aromatic fraction, are mutagenic and carcinogenic contaminants that are widely present in air, water, soils and sediments. PAHs are a large group of organic compounds with two or more fused aromatic rings. There are more than 100 different PAHs which are introduced into the environment mainly via natural and anthropogenic sources. The contribution from natural sources of PAHs is limited, being restricted to spontaneous forest and prairie fires and volcanic emissions (Bourotte et al. 2005). Anthropogenic PAHs are mainly formed as a result of pyrolytic processes such as petroleum refining, chemical manufacturing, air blowing for asphalt, emissions from coal, oil, gas and wood refuse incineration for cooking and heating, biomass burning, power generation and vehicle emissions (Nadal et al. 2004; Durand et al. 2004).

Once they enter the soil, they accumulate in horizons rich in organic matter where they are likely to be retained for many years due to their persistence and hydrophobicity. Sediments and soils are therefore considered the main sinks for PAHs in the environment, and PAHs with four or more aromatic rings are persistent environmental pollutants (Chen et al. 2004). Comparatively the more toxic components of petroleum, PAHs have been placed on the United States Environmental Protection Agency (USEPA) priority pollutant list. The structures of the 16 PAHs currently on the USEPA priority pollutant list are shown in Fig. 15.1. Selected physicochemical properties are given in Table 15.1.

Colourless, white or pale yellow-green solids, PAHs are planar, relatively inert and volatile in nature. They are hydrophobic compounds and their persistence in the environment is mainly due to lower water solubility and electrochemical stability. Their low solubilities in water are expected from their nonpolar character. These decrease dramatically in going from the two- and three-ring compounds to five-ring B(a)P (Mackay and Shiu 1992). Evidence suggests that the lipophilicity, environmental persistence and genotoxicity of

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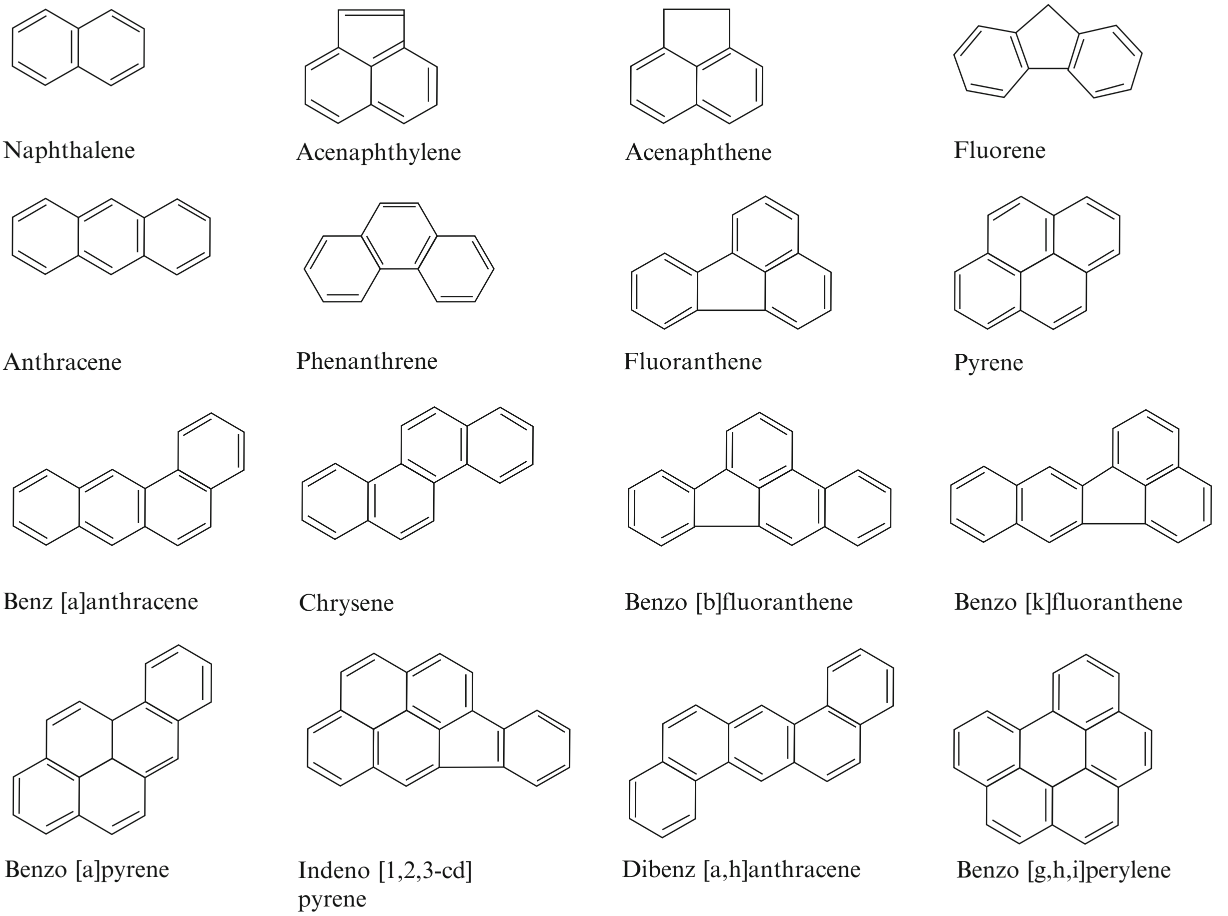

Fig. 15.1 Structure of the 16 USEPA priority PAHs

Table 15.1 Selected properties of 16 USEPA 'priority PAH pollutants'

Common names/abbreviations	Ring number	Empirical formula	MW (g mol^{-1})	Water solubility at 25 °C (mg l^{-1})	Log K_{ow}
Naphthalene (Nap)	2	$C_{10}H_8$	128	31.7	3.35
Acenaphthylene (Acy)	3	$C_{12}H_8$	152	–	–
Acenaphthene (Ace)	3	$C_{12}H_{10}$	154	3.42	3.92
Fluorene (Flu)	3	$C_{13}H_{10}$	166	1.98	4.18
Anthracene (Anth)	3	$C_{14}H_{10}$	178	4.5×10^{-2}	4.54
Phenanthrene (Phen)	3	$C_{14}H_{10}$	178	1.29	4.57
Fluoranthene (Fla)	4	$C_{16}H_{10}$	202	2.6×10^{-1}	5.22
Pyrene (Pyr)	4	$C_{16}H_{10}$	202	1.35×10^{-1}	5.18
Benzo(*a*)anthracene (BaA)	4	$C_{18}H_{12}$	228	5.7×10^{-3}	5.79
Chrysene (Chy)	4	$C_{18}H_{12}$	228	1.9×10^{-3}	5.98
Benzo(*b*)fluoranthene (BbF)	5	$C_{20}H_{12}$	252	1.4×10^{-2}	6.06
Benzo(*k*)fluoranthene (BkF)	5	$C_{20}H_{12}$	252	4.3×10^{-3}	6.06
Benzo(*a*)pyrene (BaP)	5	$C_{20}H_{12}$	252	3.8×10^{-3}	6
Indeno(1,2,3-*cd*)pyrene (IP)	6	$C_{22}H_{12}$	276	5.3×10^{-4}	6.4
Dibenz(*a*,*h*)anthracene (DbA)	6	$C_{22}H_{14}$	278	4.0×10^{-4}	6.86
Benzo(*g*,*h*,*i*)perylene (B(ghi)P)	6	$C_{22}H_{12}$	276	3.0×10^{-4}	7.1

Adapted from MacKay and Shiu (1992); Miller et al. (1985); data on indeno(1,2,3-*cd*)pyrene from Harvey (1997). Structures are based on IUPAC recommendations as described by Loening and Merrit (1990)

PAHs increase as the molecular size of the PAHs increases up to four or five fused benzene rings.

Most of the 16 USEPA priority PAHs included in the present investigation have no known use except as research chemicals. Some, however, find use in myriad ways like naphthalene in 'moth balls'; anthracene in synthetic fibre and dye production, scintillation counter crystals and organic semiconductor research; as the chemotherapeutic agent amsacrine; acenaphthene in the manufacture of dyes, pharmaceuticals and plastics; and as an insecticide and fungicide. Fluorene is used as a chemical intermediate in many chemical processes, in the formation of polyradicals for resins and in the manufacture of dyestuffs. Phenanthrene is used in the manufacture of dyestuffs and explosives and in biological research (Holloway et al. 1987; Wadler et al. 1986; Windholz 1983).

More than 200 compounds have tested positive as possible carcinogens. Among these, 25 % have been found tumorigenic, and about 30 % of these were PAHs. Lamb and Kaplan (1980) reported that BaP is a definite carcinogen with an LD50 of 24 µg. PAHs being highly lipid soluble are absorbed by the lungs and gut of mammals. At sufficient dose levels, laboratory studies show that some PAHs cause adverse health effects including cancer and reproductive difficulties in animals. These are mutagenic and carcinogenic environmental contaminants that are widely present in the air, water and aquatic system, soils and sediments (Zhang et al. 2004). The ubiquitous nature of these 'chemicals of potential environmental concern' is evident from the fact that these 16 priority PAHs pollutants are found in urban airsheds throughout the world (USEPA 1988). It is believed that there is no *threshold* or *safe* level for mutagenic compounds; hence, exposure to these PAHs at any level provides the risk of toxic effects.

The United States Environment Protection Agency has classified B[a]P as a probable human carcinogen in Group B2 (USEPA 2002). A value of 7.3 mg^{-1} kg^{-1} day^{-1} has been defined as the oral cancer slope factor for B[a]P (USEPA 1999). Besides B[a]P, only six other PAHs have been considered to be carcinogenic. All these—benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz[*a*,*h*]anthracene and indeno[1,2,3-*c*,*d*]pyrene—have also been included in Group B2 (USEPA 2002). In turn, apart from B[a]P, the International Agency for Research on Cancer (IARC 1987) considers benzo[*a*]anthracene and dibenz(*a*,*h*) anthracene as probable carcinogenic agents (Group 2A), while the remaining are only classified as possible carcinogens (Group 2B) or even not classifiable (Group 3).

15.2 Case Study

The levels of toxic contaminants in urban soils have been monitored and well documented in developed countries of North America and Europe, but there is an acute paucity of data on soil pollution due to petroleum products and its remediation in developing countries. Phytoremediation is especially suited for countries where such labour, expertise and cost-saving techniques are vital due to constraints of funds for other methods. The mechanisms and synergies of interactions between plants, microbes, soils, contaminants and the environment must be better understood in order to predict the outcome of in situ phytoremediation applications. Variations in climatic and edaphic conditions must also be taken into consideration. It therefore appears logical that studies are taken up to generate feasibility data about such remediation methods in the Indian context especially with regard to sites that appear to be at risk of contamination by petroleum products.

Agra (27°10′N, 78°05′E, 169 m.s.l.) is located in Uttar Pradesh in the north central part of India. It is roughly 200 km southeast of the national capital, New Delhi. Bounded by the Thar desert of Rajasthan on its southeast, west and northwest peripheries, it is a semiarid area (Fig. 15.2).

The world-renowned Mughal monument, the Taj Mahal is situated in Agra, and therefore the area is recognised as a sensitive zone, and a trapezoid area of about 10,400 sq. km. around the Taj, known as Taj Trapezium Zone (TTZ), has been identified by the government where new industries are not permitted and the relevance of existing industries has also been considered for further reducing the total pollution load (CPCB report: CUPS/7/1981-82). The study area is a part of Indo-Gangetic alluvium of Quaternary age and is made up of recent unconsolidated fluviatile formations comprising sand, silt, clay and kankar with occasional beds of gravel.

15.2.1 Experimental Design

Three test sites potentially polluted with PAHs were selected in and around Agra. The Mathura Refinery was designated site I as a petroleum refining site. It lies within the sensitive Taj Trapezium Zone (TTZ) along the National Highway-2, about 50 km away from Agra. The present refining capacity of this refinery is 8.00 million tonnes per annum. In 2005, it became the first refinery in India to produce auto fuels that adhere to 100 % of the Euro-3 rules. Site II is the erstwhile Idgah oil depot [IOCL] dismantled less than a decade ago and happens to be in the middle of a densely populated residential colony. This represented a petroleum storage/transportation site. An interstate bus station is just 600 m away from the site. Site III is a petrol filling station in the heart of the city that faces heavy traffic including heavy diesel vehicles (at night, when these are allowed within city limits). It lies on the road connecting three national highways. Thus, it represented the pollution profile due to petroleum fuel consumption. At each site, soil samples were collected from two depths, i.e. 0–10 and 10–20 cm (depths A and B, respectively)

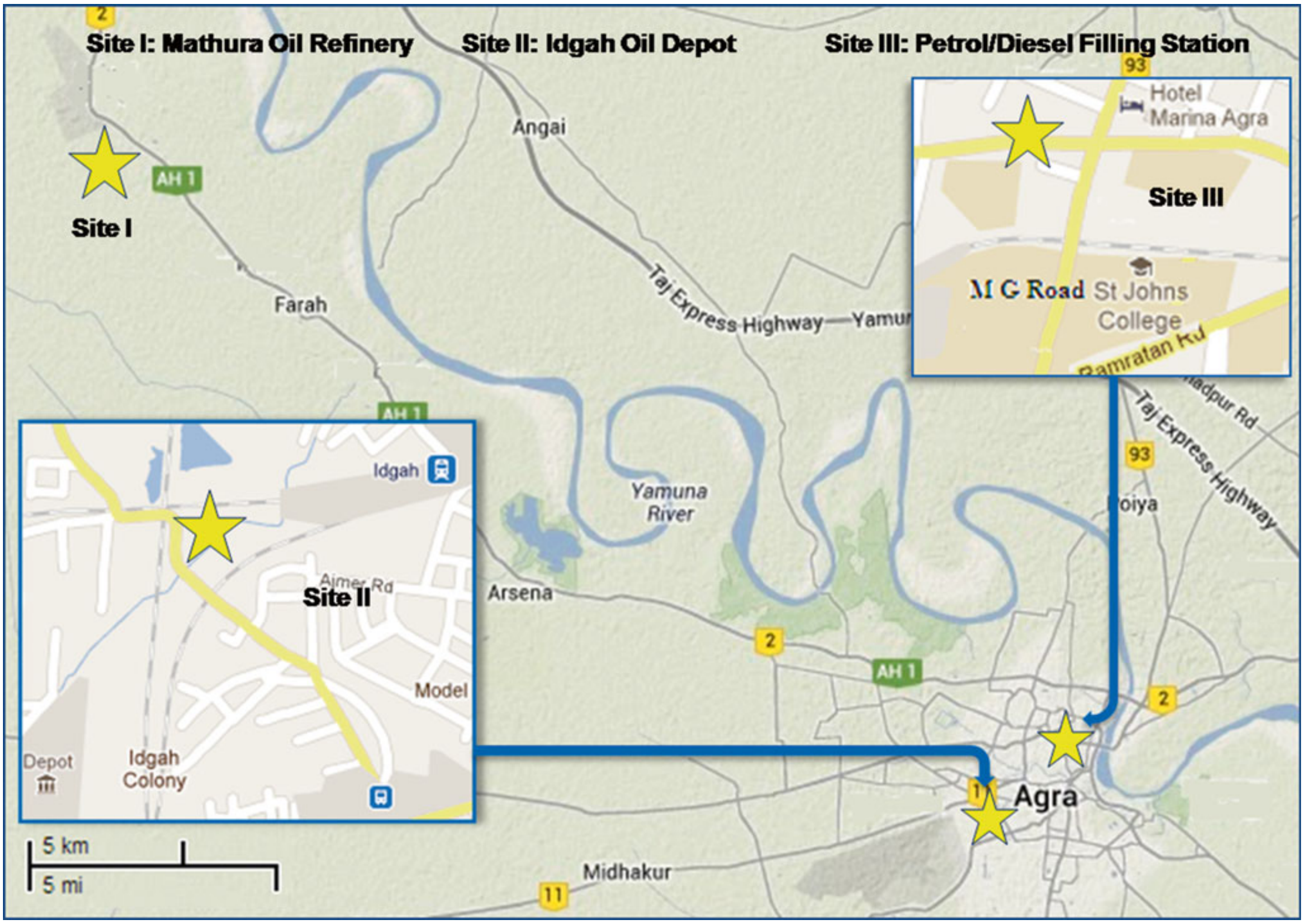

Fig. 15.2 Map of Agra indicating test sites

using a core sampler. Five random cores were collected from each site to obtain five samples for each depth. Two-way analysis of variance (ANOVA) was utilised to assess statistical significance between mean PAH contents at sites.

15.2.2 PAHs in Soils

It has been estimated that soils contain the vast majority (>90 %) of the total environmental burden of polycyclic aromatic hydrocarbons (Wild and Jones 1995; Ribes et al. 2002), and the atmosphere is their main transport vector (Drooge et al. 2002). PAHs with four or more rings persist for a long time in the environment in general and in soils and sediments in particular. The PAH concentrations in the soil samples are presented in Table 15.2. The fact that the topsoil from site I had the highest total PAH concentration followed by sites III and II can be easily noted. This was expected since site I is in the vicinity of the Mathura Refinery, hence, faces emissions from petroleum refining processes. Besides, it is along the busy National Highway-2, an important freight corridor connecting New Delhi to Kolkata, and also faces traffic especially heavy vehicles that run on diesel. It is also important to note that the PAH levels were not very different from those at site III which is a petrol/diesel station in a busy locality with high traffic density and experiences considerable quantities of traffic emissions including those from diesel vehicles. Site II registered lower levels as it has not been in use for nearly a decade since the oil depot here was dismantled and shifted elsewhere. Also, being located within a densely populated residential area, it does not face much traffic of the heavier kind unlike sites I and III. The results obtained indicate that PAH concentrations are linked to land use.

Zhang et al. (2006) analysed 16 EPA priority polycyclic aromatic hydrocarbons (PAHs) in surface soil (0–10 cm) samples collected from rural and urban areas of Hong Kong. Total PAH concentrations were in the range of 7–410 μg kg^{-1} (dry wt.), with higher concentrations in urban soils compared to rural soils. The three predominant PAHs were fluoranthene, naphthalene and pyrene in rural soils, while fluoranthene, naphthalene and benzo(*b*+*k*)fluoranthene dominated the PAHs of urban soils. The workers concluded that though light-duty diesel vehicles represent only 6.5 % of the total vehicular fleet of the metropolitan region of Sao Paulo, they are a significant

Table 15.2 Concentrations of PAHs in soils (in μg g^{-1})

	Soil from depth A			Soil from depth B			
PAHs	Site I	Site II	Site III	Site I	Site II	Site III	Control
Naphthalene	1.13±0.15	0.49±0.13	0.76±0.05	0.75±0.09	0.1±0.05	0.53±0.14	0.33±0.21
Acenaphthylene	0.56±0.11	0.55±0.09	0.35±0.19	0.35±0.09	0.24±0.11	0.33±0.12	0.12±0.11
Acenaphthene	0.93±0.19	0.97±0.09	0.68±0.22	0.45±0.04	0.41±0.12	0.77±0.21	0.35±0.12
Fluorene	0.71±0.18	0.86±0.11	1.08±0.30	0.54±0.03	0.54±0.19	0.57±0.23	0.24±0.06
Phenanthrene	0.44±0.12	0.32±0.09	0.72±0.38	0.22±0.06	0.22±0.05	0.38±0.22	0.21±0.18
Anthracene	0.94±0.16	0.62±0.16	0.57±0.34	0.51±0.06	0.28±0.15	0.85±0.13	0.45±0.10
Fluoranthene	1.39±0.29	0.7±0.15	1.16±0.29	0.96±0.14	0.49±0.17	0.79±0.12	0.17±0.06
Pyrene	0.38±0.15	–	0.85±0.50	0.15±0.05	–	0.10±0.07	–
Benzo(*a*)anthracene	0.61±0.07	0.25±0.13	0.42±0.30	0.42±0.21	0.12±0.13	0.52±0.10	0.14±0.06
Chrysene	1.85±0.23	0.81±0.16	1.73±0.60	0.93±0.10	0.57±0.40	0.86±0.14	0.55±0.23
Benzo(*b*)fluoranthene	0.87±0.09	0.69±0.35	1.83±0.30	0.63±0.10	0.44±0.25	0.67±0.28	0.35±0.23
Benzo(*k*)fluoranthene	0.23±0.04	–	0.17±0.07	0.15±0.07	–	0.11±0.03	0.09±0.12
Benzo(*a*)pyrene	0.58±0.09	–	0.35±0.21	0.23±0.22	–	0.29±0.14	–
Dibenz(*a*,*h*)anthracene	–	–	–	–	–	–	–
Indeno(1,2,3-*cd*) pyrene+Benzo(*g*,*h*,*i*)perylene	1.71±0.19	0.51±0.17	0.84±0.39	0.84±0.09	0.35±0.19	0.94±0.16	0.35±0.11
Total PAHs	12.33±0.52	6.77±1.63	11.51±2.58	7.13±0.46	3.76±0.33	7.71±0.71	3.35±0.53

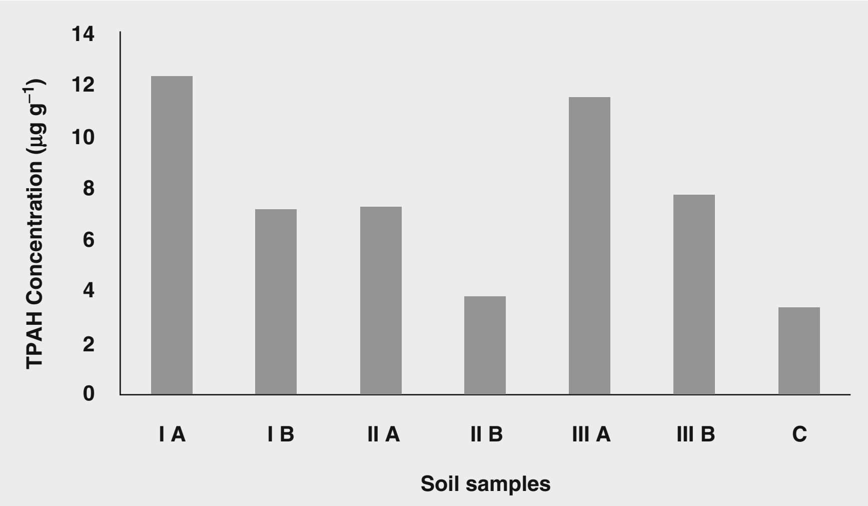

Fig. 15.3 Total PAH concentrations in soils from two depths at sites studied

source of PAHs. Zohair et al. (2006) reported that PAH burden was dominated by the low molecular weight compounds acenaphthene/fluorene ranging from 1.6±0.3 to 9.84±2.9 μg kg^{-1} and phenanthrene in the range 2.07±0.3 to 8.28±1.3 μg kg^{-1}. These compounds are more water soluble and more volatile than the relatively high molecular weight PAHs.

Out of the 16 USEPA priority PAHs, only dibenz(*a*,*h*) anthracene was not detected in the soils from selected sites. On computing two-way analysis of variance (ANOVA), it was observed that for Nap, Acy, Flu, Fla and Chy, the difference in the mean values among the different sites was greater than would be expected by chance after allowing for effects of differences in depth and vice versa. In other words, there was a statistically significant difference in mean concentrations among the sites and depths, independently. The effect of different sites depended on the depth from which the soil sample was collected for Ace, Anth, Pyr, BbF and IP+BghiP. In other words, there was a statistically significant interaction between sites and depth of soil samples. In the case of BaA, BkF and BaP, there was a statistically significant difference in mean concentrations among the sites independent of depth. The difference among depths was insignificant. In the case of Phen, the difference was not significant statistically. The same was the case for depths.

The average TPAH (total PAH) concentrations measured in soils at the two designated depths (A and B) at the studied sites are presented in Fig. 15.3.

Sites I and III showed higher PAH levels at both depths studied. Depth-based variation in the concentrations of individual PAHs in soil at the sites is evident from Fig. 15.4. It is

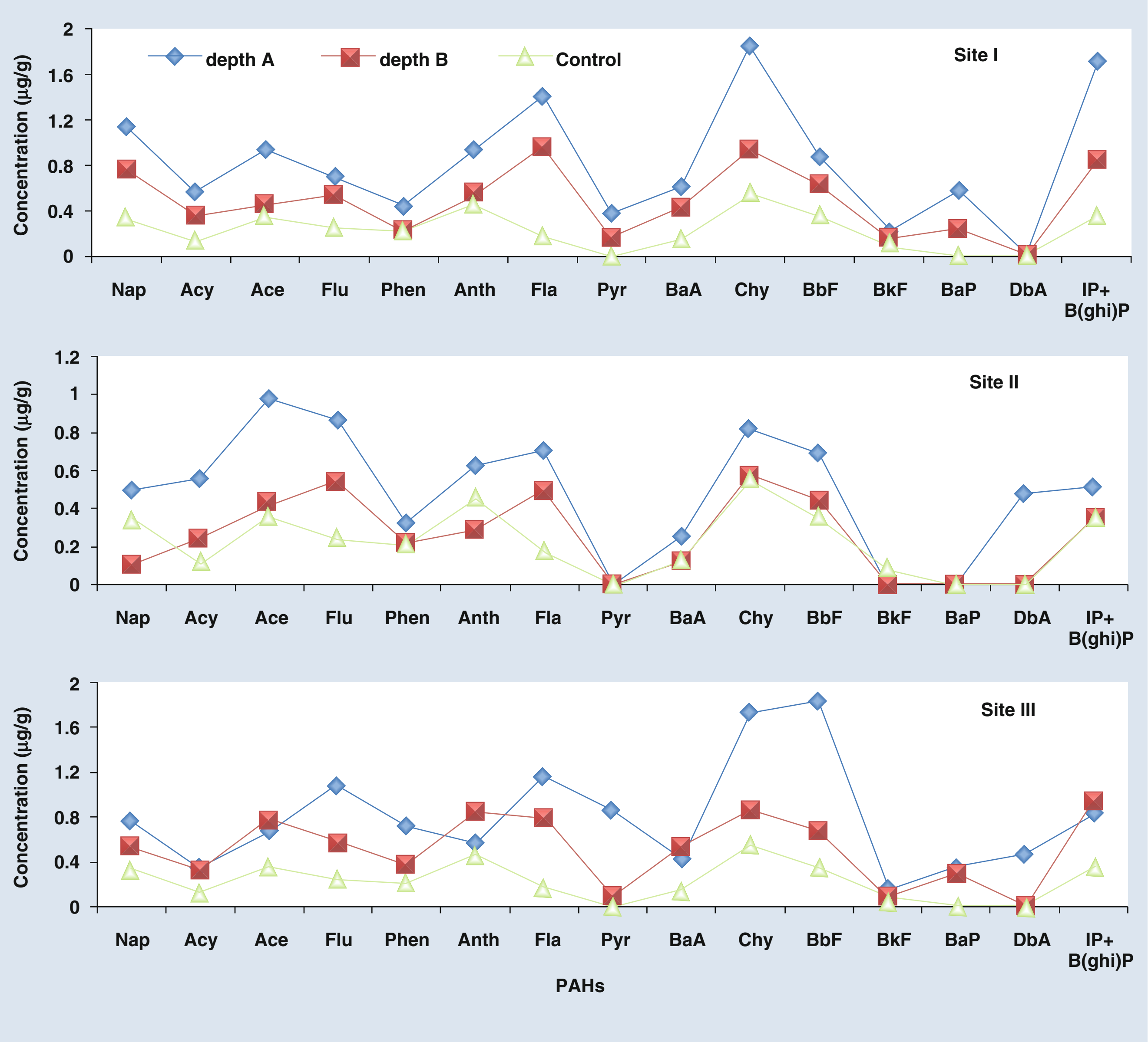

Fig. 15.4 Depth-based variation in the concentrations of individual PAHs in soil

also clear that much variation exists in individual PAH concentrations. Control values showed negligible depth-based variations, and as such, mean values have been used.

The trends of PAH concentrations were different for the two depths; PAH levels were higher in upper layer of soils at all sites. At site I, the top three PAHs were chrysene > indeno(1,2,3-*cd*)pyrene + benzo(*g*,*h*,*i*)perylene > fluoranthene at depth A and fluoranthene > chrysene > indeno(1,2,3-*cd*)pyrene + benzo(*g*,*h*,*i*)perylene at depth B. At site II, the trend was acenaphthene > fluorene > chrysene depth A and chrysene > fluorene > fluoranthene for depth B, whereas at site III, it was benzo(*b*)fluoranthene > chrysene > fluoranthene for depth A and indeno(1,2,3-*cd*)pyrene + benzo(*g*,*h*,*i*)perylene > chrysene > anthracene for depth B. Chrysene and fluoranthene were thus the predominant compounds. Zhang et al. (2006a) also reported that fluoranthene was among the three dominant PAHs in soils from both rural and urban areas in Hong Kong, although higher concentrations can also be attributed to a host of sources other than direct contamination due to petroleum spill/leakage, e.g. industrial-oil burning, wood combustion and emissions from diesel-powered vehicles (Ravindra et al. 2001).

Figure 15.5 shows the relative contribution of 2-, 3-, 4-, 5- and 6-ring PAHs in the upper and lower layers (designated depths A and B) of soils of different locations investigated in this study. The distribution patterns do not show much variation with control except for site II. If the percentage of PAHs

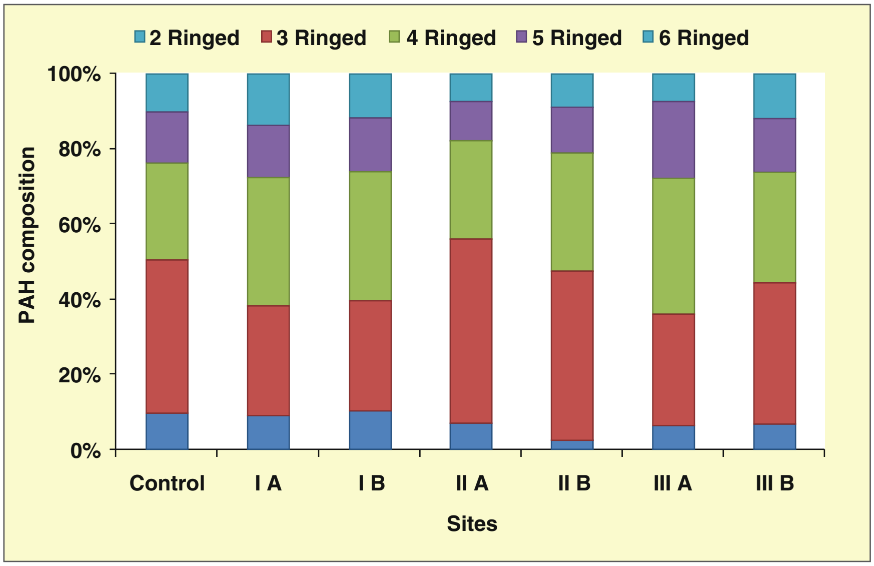

Fig. 15.5 Distribution of PAHs in soils (based on number of benzene rings)

based on the number of benzene rings is considered, 3-ring and 4-ring PAHs were found to be dominant at both depths in soils at all sites.

The concentration of PAHs in the environment varies widely depending on the level of industrial development and contamination due to petroleum products. PAH concentrations range from a low of 5 μg g^{-1} soil in an undeveloped area to 1.79 × 10^{6} μg g^{-1} at an oil refinery (Cerniglia 1992). Nam et al. (2003) determined the content and type of polycyclic aromatic hydrocarbons (PAHs) in soils from paddy fields and upland areas in South Korea. The overall distribution of PAH was found to be closely related to the pollution sources, the size of city and the type of industry. The PAH content ranged from 23.3 to 2,384 μg kg^{-1} with an average of 236 μg kg^{-1}. The highest concentrations were found in soils near iron processing plants. The concentration of PAH decreased in the order fluoranthene > benzo(*b*)fluoranthene > pyrene. Agarwal et al. (2006) studied the distribution, concentration trends and possible sources of PAHs in bank sediment of river Yamuna in Delhi, India. The levels of 16 priority polycyclic aromatic hydrocarbons (PAHs) were analysed during pre-monsoon, monsoon and post-monsoon seasons in the sediment fraction <53 μm. The sum of 16 PAH compounds ranged from 4.50 to 23.53 μg g^{-1} with a mean concentration of 10.15 ± 4.32 μg g^{-1} (dry wt.). From the predominance of 2–4-ring PAHs, the authors have suggested relatively recent local sources of PAHs in the study area. Source apportionment based on molecular indices also illustrates pyrogenic source fingerprint of PAHs.

Table 15.3 shows a comparison of worldwide PAH concentrations with those observed in the present study. The total PAH concentration in upper soil near the refinery along National Highway-2, i.e. site I (12.33 μg g^{-1}), is much less than the concentrations found in Belgium (300 μg g^{-1}), Novi Sad (47.87 μg g^{-1}) and Nigeria (45.9 μg g^{-1}). This is commendable. The Mathura Refinery is the first in Asia and the third in the world to receive the coveted ISO-14001 certification for Environment Management System in 1996. A full-fledged environment protection (EP) cell having qualified engineers is at place to deal with all environmental issues. A well-equipped pollution control lab has also been established to monitor environmental performance on day-to-day basis.

The lab is approved by the Ministry of Environment & Forests (MoEF) and accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL). The picture of PAH contamination was similar at site III, a petrol/diesel station. The values at sites I and III are also similar to those at industrial and roadside sites (13.7 and 12.9 μg g^{-1}, respectively) reported from Agra itself by Masih and Taneja (2006). In the case of site II, a dismantled oil depot, the total PAH concentration (6.77 μg g^{-1} in upper soil) was about half that of sites I and III. This was far below that of refinery locations as seen above. However, this site lies within a densely populated residential zone, and in this context, the levels are higher than several residential localities worldwide as seen in Table 15.3. It is to be noted that the level of contamination was lower than that reported from another residential area (9.37 μg g^{-1}) in the same city (Masih and Taneja 2006).

15.2.3 Toxicity Analysis of PAHs

The environmental occurrence of PAHs has been associated with adverse effects on public health (Fang et al. 2004). A significant health concern resulting from exposure to PAHs is their potential for carcinogenicity, which is chemical-structure dependent. Four- to six-ring PAHs have been shown to be carcinogenic in laboratory animals (DHHS 1995). As mentioned

Table 15.3 Concentrations of PAHs in soils around the world (in μg g^{-1}) (compiled from literature)

Country		PAH conc. (μg g^{-1})	No. of PAHs	Study area	Reference
Brazil		0.096	20	Agricultural	Wilcke et al. (1999)
		0.39	20	Residential	
UK		0.19	12	Agricultural	Wild and Jones (1995)
		4.5	12	Industrial	
		4.2	12	Residential	
Germany		1.9	6	Agricultural	Tebaay et al. (1993)
		16	6	Industrial	
		1.8	6	Residential	
Poland		42,309	1	Petrochemical complex	Siewniak (1975)
		2.64	16	Agricultural	Maliszewska-Kordybach (1996)
Estonia		12.39	16	Industrial	Trapido (1999)
		2.2	12	Urban	
		0.23	12	Rural	
France		1.23	17	Roadside	Crépineau et al. (2003)
		5.65	14	Industrial	
		2.78	14	Urban	Motelay-Massei et al. (2004)
Belgium		300	7	Refinery	Bakker et al. (2000)
		41,699	7	Refinery	
Novi Sad		47.87	–	Refinery	Skrbic and Miljevic (2002)
Italy		0.319	22	Industrial	Capuano et al. (2005)
Spain		1	–	Industrial	Nadal et al. (2004)
Greece		0.3–1.6	13	Coal burning area	Voutsa et al. (2004)
Austria		79	18	Industrial	Weiss et al. (1994)
Australia		3.3	14	Roadside	Yang et al. (1991)
Nigeria		45.9	17	Refinery	Duke and Albert (2007)
		3.83	17	Residential	
USA		58.68	14	Roadside	Rogge et al. (1993)
Canada		1.75	12	Urban	Wong et al. (2004)
Thailand		0.38	20	Residential	Wilcke and Muller (1999)
Japan		6.11	13	Urban	Yang et al. (2002)
Malaysia		0.22	–	Roadside	Omar et al. (2002)
South Korea		2.4	16	Industrial	Nam et al. (2003)
		0.16	16	Agricultural	
China		3.9	16	Urban	Tang et al. (2005)
		0.82	16	Industrial	Wang et al. (2003)
		3.08	16	Agricultural	Chen et al. (2005)
India		6.7	11	Agricultural	Masih and Taneja (2006)
		13.7	11	Industrial	
		9.3	11	Residential	
		12.9	14	Roadside	
Present study	Depth				
	1–10 cm	12.33	15	Refinery/roadside	
	10–20 cm	7.13			
	1–10 cm	6.77	12	Petroleum storage	
	10–20 cm	3.76			
	1–10 cm	11.51	15	Petrol/diesel filling station	
	10–20 cm	7.71			

earlier, International Agency for Research on Cancer has classified PAHs according to carcinogenicity (IARC 1987). These PAHs [benzo(*a*)pyrene, benzo(*a*)anthracene, dibenz(*a*,*h*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and indeno(1,2,3-*cd*)pyrene] are of special concern to human health. BaP is a five-ring ($C_{20}H_{12}$) compound, which is mutagenic for human cells in culture (Osborne et al. 1987) and carcinogenic in whole animal assays (Cerna et al. 2000). It is to be

Table 15.4 Proposed toxic equivalency factors for individual PAHs with their BaPeq at the test sites

		TEF-adjusted concentrations in μg g^{-1} (BaP equivalents)						
		Soil from depth A			Soil from depth B			
PAHs	TEF[a]	Site I	Site II	Site III	Site I	Site II	Site III	Control
Nap	0.001	0.00113	0.00049	0.00076	0.00075	0.0001	0.00053	0.00033
Acy	0.001	0.00056	0.00055	0.00035	0.00035	0.00024	0.00033	0.00012
Ace	0.001	0.00093	0.00097	0.00068	0.00045	0.00041	0.00077	0.00035
Flu	0.001	0.00071	0.00086	0.00108	0.00054	0.00054	0.00057	0.00024
Phen	0.001	0.00044	0.00032	0.00072	0.00022	0.00022	0.00038	0.00021
Anth	0.01	0.0094	0.0062	0.0057	0.0051	0.0028	0.0085	0.0045
Fla	0.001	0.00139	0.0007	0.00116	0.00096	0.00049	0.00079	0.00017
Pyr	0.001	0.00038	–	0.00085	0.00015	–	0.0001	–
BaA	0.1	0.061	0.025	0.042	0.042	0.012	0.052	0.014
Chy	0.01	0.0185	0.0081	0.0173	0.0093	0.0057	0.0086	0.0055
BbF	0.1	0.087	0.069	0.183	0.063	0.044	0.067	0.035
BkF	0.1	0.023	–	0.017	0.015	–	0.011	0.009
BaP	1	0.58	–	0.35	0.23	–	0.29	–
IP + BghiP	0.055[b]	0.09405	0.02805	0.0462	0.0462	0.01925	0.0517	0.01925
Σ BaPeq		0.88	0.14	0.67	0.41	0.09	0.49	0.09

[a] Data from Nisbet and LaGoy (1992)

[b] Mean TEF of IP + BghiP = 0.1 + 0.01; since in the present study, these were co-eluents. Dibenz(*a*,*h*)anthracene was not detected at any site

noted that five-ring compounds including benzo(*a*)pyrene contribute 10–20 % of the TPAH at depth A and 11–14 % at depth B. Percentage carcinogenic content of PAHs (based on IARC 1987) in soil was ~32 % at both depths at site I and 33 % and 33 % at depth A and B, respectively at site III. The proportion was lower at site II (24 % and 28 % at depth A and B, respectively) and control site (~28 %). Percentage carcinogenic content of total PAHs in soil was ~32 % at both depths at site I and 33 % and 33 % at depth A and B, respectively at site III. The proportion was lower at site II (24 % and 28 % at depth A and B, respectively) and control site (~28 %) (Fig. 15.5).

In light of the fact that several PAHs are known human carcinogens, the carcinogenic potencies of PAHs in soils of the three test sites were also determined. In principle, the carcinogenic potency of a given PAH compound is assessed on the basis of its BaPeq (BaP equivalent) concentration. The calculation of BaPeq concentration for a given compound is determined by its toxic equivalency factor (TEF), which represents the relative carcinogenic potency of the given PAH compound, using BaP as a reference compound to adjust its original concentration. The TEF for BaP is one, which is the highest among PAHs. The Nisbet and LaGoy scale was adopted for the present study, and the BaPeq of each PAH was determined by multiplying the concentration of each PAH with its toxic equivalency factor (TEF). In order to calculate the carcinogenic potencies associated with the total PAH exposures from soil, the sum of each BaPeq, i.e. Σ BaPeq, was used as a surrogate indicator. The above method has the main advantage of being relatively easy to apply in the environments affected by human sources; however, it may underestimate risks since limited PAHs are considered (WHO/IPCS 1998). Listed in Table 15.4 are the TEFs for individual PAHs suggested by Nisbet and LaGoy (1992) along with the TEF-adjusted concentrations of PAHs in soils. All these values are below 1 μg g^{-1} indicating that at the present levels, soil PAH exposure in Agra is not carcinogenic. It should be noted that the above inference is based on the exposure of soil to each individual PAH compound not for TPAHs.

15.3 Remediation

15.3.1 Conventional Techniques

Conventional remediation methods employed to clean contaminated soils include natural attenuation, engineering techniques and bioremediation. Each of these technologies has specific benefits and limitations (EPA 1997). Natural attenuation relies entirely on natural processes with no human intervention. These may be physical, chemical and biological in nature such as dilution, dispersion and adsorption of the contaminants (USEPA 1996a) as well as unassisted growth of plants and microbial communities that break down contaminants. However, only a fraction of sites offer such naturally occurring conditions where contaminants are degraded quickly enough to prevent their spreading (Committee on In Situ Bioremediation 1993). Engineering techniques are primarily physical, chemical and mechanical in nature and may involve ex situ and in situ processes. Immobilisation and extraction by physicochemical methods can be expensive; in effect, limiting their suitability for areas where rapid complete decontamination is the prerequisite (Bio-Wise 2000). The most important concern is the prohibitive costs of these techniques. Bioremediation employs a number of bacteria, protozoa and fungi to degrade contaminants into less toxic or nontoxic compounds (USEPA 1996b). The involvement of bacteria, e.g. *Pseudomonas*, *Arthrobacter*, *Alcaligenes* and *Corynebacterium*, and soil fungi, e.g. *Aspergillus ochraceus, Cunninghamella elegans* and *Phanerochaete chrysosporium*, has been documented in the degradation of petroleum hydrocarbons (Bossert and Bartha 1984; Sutherland 1992). It can be applied both ex situ and in situ. Bioremediation does not involve the use of plants and generally employs more invasive engineering techniques than phytoremediation.

15.3.2 Phytoremediation

The global emphasis at present is to use natural materials and methods as far as possible to combat a variety of problems. The same has been extended to soil remediation programmes. It is known that plants can cause profound alteration of surrounding soil by simple life processes like water and mineral uptake, decay, etc. *Phytoremediation* may be defined as 'the engineered use of green plants to remove, contain or render harmless environmental contaminants like heavy metals, trace elements, organic compounds and radioactive compounds in soil or water' (Hinchman et al. 1998). It has been derived from other fields such as agronomy, forestry, chemical and agricultural engineering, microbiology, etc. Since its inception, it has developed into an independent field of study and a widely applicable technology (Tsao 2003).

Phytoremediation has many benefits. It leaves the topsoil in usable condition, and it is aesthetically pleasing. It requires minimal equipment and less energy inputs as plants do most of the work using solar energy. Thus, it is an eco-friendly process. The plants used can later be harvested, processed and disposed off in an environmentally sound manner. It is also potentially cheaper—approximately 90 %—than conventional treatments (Hinchman et al. 1998), which are primarily chemical based and pursue an energy-intensive approach. This approach is hence emerging as an innovative tool with a great potential to decontaminate soil and water and thus achieving a sustainable development status (Desouza et al. 1999). Up to 25 % of the remediation agenda of the United States Environmental Protection Agency (USEPA) included phytoremediation of metals and radionuclides as a thrust area during 2000.

15.3.3 Mechanisms of Phytoremediation

There are three primary mechanisms by which plants and microorganisms remediate contaminated soil and groundwater. These mechanisms include *degradation*, *containment* as well as *transfer* of contaminants from soil to the atmosphere. In the case of oil contamination, the mechanism primarily involved is rhizosphere degradation (Merkl et al. 2004).

In *phytodegradation*, plants and microbes aid, directly or indirectly, in the degradation of organic contaminants into products like alcohols, acids, carbon dioxide and water. These are generally less toxic and less persistent in the environment than the parent compounds (Eweis et al. 1998). Though plants and microorganisms can degrade petroleum hydrocarbons independently of one another, it is the interaction between plants and microorganisms, i.e. the rhizosphere effect, which is the primary mechanism responsible for petrochemical degradation in phytoremediation. Soil redox conditions, organic content, moisture and other soil properties are manipulated by the activity of plant roots. The rhizosphere is the region of soil closest to the roots of the plants and hence under direct influence of the root system. The remediation process wherein contaminants are degraded/transformed by microbes in the rhizosphere is referred to as *rhizodegradation*. Critical components of the rhizosphere, in addition to a variety of free-living microorganisms, include root exudates that provide carbohydrates to 'feed' the microorganisms, organic acids that make the ions of nutrients and contaminants more mobile in the soil and enzymes that have important natural functions including degradation of organic contaminants with nitro groups or halogens (Hinchman et al. 1998). Root exudates of sugars, alcohols and acids can amount to 10–20 % of plant photosynthesis annually (Schnoor et al. 1995), and due to these exudates, microbial population and activities are 5–100 times greater in the rhizosphere than in bulk soil, i.e. soil not in contact with plant roots (Gunther et al. 1996). This plant-induced enhancement of microbial population is termed as the rhizosphere effect (Atlas and Bartha 1998) and is believed to enhance degradation of organic contaminants in the rhizosphere.

Containment involves using plants to reduce or eliminate the bioavailability of both organic and inorganic contaminants to other biota. It includes (1) accumulation of contaminants within the plants, known as *phytoextraction*, (2) adsorption of the contaminants on the root surface and (3) binding of contaminants with soil organic matter or humus by plant enzymes. These two processes are referred to as *phytostabilisation.* When plant roots accumulate or adsorb contaminants from the waste water, this is known as *rhizofiltration*. Contaminants are not necessarily degraded when they are contained. Various studies have documented that plants accumulate petroleum hydrocarbons to a small degree in their roots and shoots as well as adsorb these compounds onto the surface of roots (Edwards 1988; Durmishidze 1977; Ferro et al. 1997; Duxbury et al. 1997; Wild and Jones 1992; Anderson et al. 1993). Plants may also transfer volatile petroleum constituents from the soil to the atmosphere (Wiltse et al. 1998; Watkins et al. 1994; Kroening et al. 2001). This process is known as *phytovolatilisation.*

15.4 Greenhouse Study

In order to evaluate the extent to which plants can be used as phytoremediators either singly or with supplementation using different amendments, studies were also conducted to determine the ability of two plants *Cymbopogon jwarancusa* (lemongrass) and *Helianthus annuus* (sunflower) to degrade/contain PAHs in soil. The former is an aromatic oil-yielding grass with enormous potential for resurrection. The latter is an oil-yielding plant previously documented in phytoremediation studies.

15.4.1 Experimental Design

To assess the potential of selected species to phytoremediate PAHs from soil, pots were prepared with garden soil. Each pot could hold 4 kg of soil and was prepared with a layer of crocks and gravel about 1.5 in. deep at the bottom. The soil had a pH of 8 and electrical conductivity of 0.54 dS m^{-1} with an organic carbon content of 0.64 %. The bulk density was 1.45 g cm^{-3}. Available nitrogen, phosphate and potash were 112.9, 17.5 and 393 kg ha^{-1}, respectively.

In order to compare the raw potential of all species, the treatment designated T1 did not have any enhancing amendments. Uniform seedlings of each species were transplanted from nursery bed to pots, and the enhancement treatments were applied. The sets were allowed to mature for 3 weeks after which diesel were added to each pot near the stem at 10 % w/w. Each treatment was set up with nine replicates, three to be taken apart at each test date. Unplanted, unamended pots were set up as controls to compare degradation of diesel components without plants. All pots were kept in a random block design and watered as and when required in such a way as to prevent loss of contaminants by leaching. Any leachates obtained were added to the pots at next watering. The PAH content was measured in five random pots at day 1, and the average was taken as initial PAH concentration. Subsequently, three replicates of each treatment were taken apart at 60, 120 and 240 days after treatment to obtain a successive picture of PAH degradation. At these days, PAH content of soil, root and shoot as well as physiological parameters were measured. A composite sample was prepared from the three replicates for PAH analysis.

Uncontaminated, unamended plants were maintained as controls to compare chlorophyll and proline content at the testing dates. In addition to the regular testing dates, these parameters were also measured at 10, 20 and 30 DAT to monitor stress symptoms, if any, at the initial stages of the study. Growth parameters, i.e. shoot and root length and biomass (dry), were measured at regular testing dates. Since *H. annuus* is a seasonal, the final testing date for all parameters was 120 DAT for this plant. The various treatment pots were set up as follows:

T0	Unvegetated pot + 10 % diesel w/w (control)
T1	Plant + 10 % diesel w/w
T2	Plant + 10 % diesel w/w + vesicular-arbuscular mycorrhiza (VAM)
T3	Plant + 10 % diesel w/w + *Azospirillum*
T4	Plant + 10 % diesel w/w + phosphorus-solubilising bacteria (PSB)
T5	Plant + 10 % diesel w/w + *Pseudomonas putida*
T6	Plant + 10 % diesel w/w + *Trichoderma viride*
T7	Plant + 10 % diesel w/w + biosurfactant
T8	Plant + 10 % diesel w/w + *Azospirillum* + PSB
T9	Plant + 10 % diesel w/w + maleic acid

15.4.2 Phytoremediation of PAHs

Initial concentrations of individual PAHs ranged from undetectable to 32.42 μg g^{-1}. Initial total PAH (TPAH) content in treatments was 99.77 μg g^{-1}. Low molecular weight PAHs were dominant with concentrations ranging from 1.85 to 32.42 μg g^{-1}; ΣLPAH was 84.58 μg g^{-1}, which is 85 % of the TPAH content. ΣHPAH was 15.19 μg g^{-1}. The co-eluents BghiP and IP were undetectable.

Different treatments had varying effects on PAH-reducing capacity of different plants. In all vegetated treatments, the decline in TPAH levels was not only higher but also more rapid than the unplanted control, more so in T2 (VAM), T5 (*Pseudomonas*), T7 (biosurfactant) and T8 (*Azospirillum* + PSB). Figure 15.6 summarises the PAH concentrations in treatments at initial and final days of study clearly. It is to be noted that *C. jwarancusa* did not survive the dose of diesel in the case of T1, T3, T4, T6 and T8.

The decreasing trends of PAH concentrations in *C. jwarancusa* and *H. annuus* treatments as compared to the unvegetated control demonstrate the fact that contaminated soils can be remediated more rapidly using plants. At successive days of analysis, treatments with plants had lower levels of TPAH compared to unplanted control. Total PAH degradation ranged from 95 to 99 % in *C. jwarancusa* at 240 DAT (days after treatment) and 75 to 84 % in *H. annuus* at 120 DAT. The final reduction of total PAHs in the unplanted control T0 was about 73 %. The PAH degradation in soil of *H. annuus* treatments is lower as being a seasonal it could only be monitored for 4 months. Similar results where vegetated treatments consistently showed a higher and faster decline in PAH concentrations have been reported by many other workers in studies ranging from laboratory and greenhouse experiments to pilot and field-scale demonstrations (Pradhan et al. 1998; Reynolds et al. 1999; Spriggs et al. 2005; Lin 2004; Widdowson et al. 2007).

The behaviour of organic compounds in soils and plants is generally dependent upon whether they are hydrophobic or hydrophilic. To measure this, the log of their octanol-to-water partition coefficient or ‘log K_{ow}’ is often used.

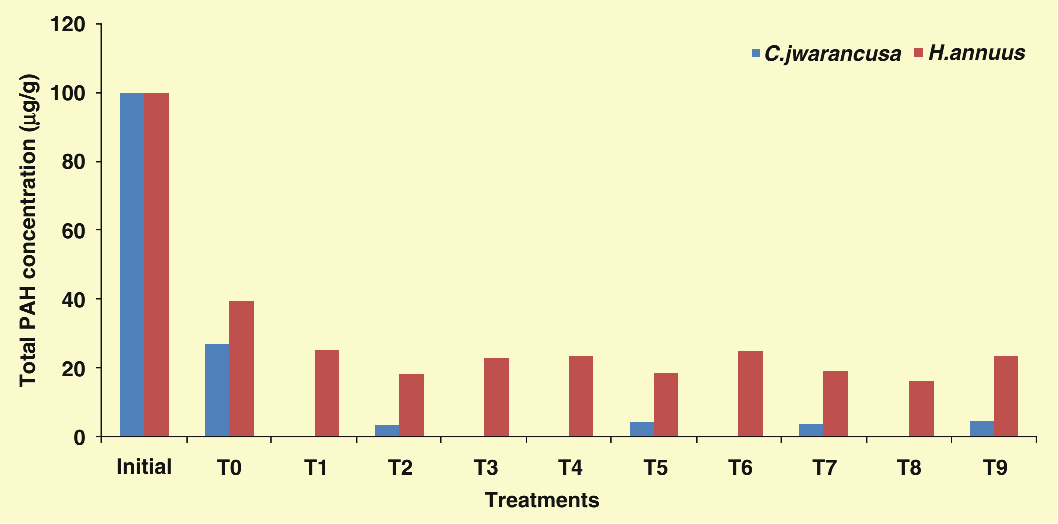

Fig. 15.6 Total PAH concentrations in plant treatments at initial and final days of study

Chemicals that are highly water soluble are *hydrophilic* compounds with a log $K_{ow} < 0.5$ while *hydrophobic* chemicals have a log $K_{ow} > 3.0$ (Kömives and Gullner 2000). Compounds with very low log K_{ow} values are hydrophilic and soluble in the polar soil solution. Over time, hydrophobic organic compounds can become sequestered in soil micropores or tightly bound to soil particles. The log K_{ow} values of PAHs are between 3.5 and 7.66, indicating their moderate to highly hydrophobic behaviour. Higher molecular weight PAHs with more than three benzene rings are less water soluble, have little vaporisation and are more hydrophobic than the smaller PAHs (Maliszewka-Kordybach 1999). The smaller ring PAHs may be volatilised from soil or rapidly biodegraded within several months of application especially if the soil is moist, leaving behind the larger PAHs that are more persistent and resistant to degradation (Hawthorne and Grabanski 2000). In this context, different trends of degradation obtained for individual PAHs were expected. It was not surprising to see that LPAHs were degraded quite efficiently. However BkF, BaP and DbA which happen to be 5–6-ring HPAHs were also degraded to below detection levels by all test plants but not in the unplanted control. In the light of the reported recalcitrance of these high molecular weight PAHs (Li et al. 2010), their degradation below detection level is remarkable. However, (Juhasz et al. 1997) found that for soils contaminated with mixtures of PAHs, the high molecular weight PAHs might be degraded more rapidly in the presence of low molecular weight PAHs, which may serve as carbon sources for soil microbes. This is a phenomenon known as *co-metabolism* (Cunningham and Berti 1993).

The parameter found to have the most influence on PAH retention in soil is the amount of organic matter present, which controls soil sorption affinity towards PAHs (Maliszewka-Kordybach 1999). A high organic carbon content (>5 %) in soil usually leads to strong adsorption and, therefore, low availability, while a moderate organic carbon content (1–5 %) may lead to limited availability (Otten et al. 1997). Uptake of organic contaminants is greatest in soils with low organic matter content, as this provides a strong sorptive surface. The organic carbon content of the soil used in this study was below 1 %, so this certainly enhanced the availability of the PAHs in the treatment pots and their subsequent degradation.

The effect of the agronomic treatments applied to enhance the phytoremedial potential was seen to be plant specific. T7 and T8 were the top two treatments consistently at each successive sampling date (60, 120 and 240 DAT) followed by T2, though the other treatments showed different efficiencies at these dates. Enhancement of phytoremedial potential of plants by surfactants as well as N and P addition has been studied and reported by workers previously. In a study using nutrients with sophorose lipids blended with PAH-contaminated soil, many PAHs were significantly removed (Kosaric 2001). In another study, the addition of a surfactant accelerated initial PAH dissipation but did not attain final PAH concentrations below those obtained with non-mycorrhizal plants (Joner et al. 2001).

Radwan and Dashti (2005) showed that co-inoculation of *Vicia faba* (broad beans) plant roots in oily sand with nodule-forming rhizobia (*Rhizobium leguminosarum*) and plant-growth-promoting rhizobacteria (PGPR) (*Pseudomonas aeruginosa* and *Serratia liquefaciens*) enhances the phytoremediation potential of this plant for oily desert sand through improving plant growth and nitrogen fixation. The addition of *Pseudomonas* seemed to protect *C. jwarancusa* (lemongrass) from succumbing to the diesel content in its growth matrix. This could be attributed to the metabolic activities of the bacterium in the rhizosphere which have degraded or

Table 15.5 Σ BaPeq in vegetated treatments at initial and final days of study

	Σ BaPeq										
Plant	Initial	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
C. jwarancusa	1.83	1.38	–	0.04	–	–	0.06	–	0.04	–	0.12
H. annuus		1.51	0.37	0.31	0.29	0.29	0.38	0.30	0.26	0.25	0.30

Based on Nisbet and LaGoy toxic equivalency factors (1992)

transformed PAHs the fastest. When PAHs become tightly adsorbed to the soil and less available for microbial degradation, a bacterial strategy is to release a biosurfactant. These are small detergent-like molecules with a hydrophilic head and lipophilic tail. They form spherical or lamellar micelles with cores where hydrophobic compounds become solubilised leading to transfer of PAH from solid to liquid phase (Volkering et al. 1997). As a result, it can be reasonably inferred that the root-PAH interaction was decreased in this case. Since the treatment T1 (diesel only) set up to monitor the raw potential of the plant did not survive, the effect of *Pseudomonas* could not be compared with it. In *H. annuus*, it showed no distinguishable difference in total PAH reduction when compared to control T1 though initially (60 DAT) it showed better results.

The other two treatments that showed some enhancement of total PAH reduction were VAM and *Azospirillum* (T2 and T3, respectively). The beneficial effects of these treatments have also been reported. (Joner et al. 2001; Dominguez-Rosado and Pichtel 2004; Rivera-Espinoza and Dendooven 2004).

15.4.3 Toxicity Analysis of PAHs

Initially, the Σ BaPeq in various treatments was 1.83 μg g^{-1}. At 240 DAT (Table 15.5), it was still 1.38 μg g^{-1} in T0. At the same day, it ranged from 0.04 to 0.12 in *C. jwarancusa* treatments. Even in *H. annuus* where the study lasted 120 days, the values ranged from 0.26 to 0.38 μg g^{-1}. All Σ BaPeq values of vegetated treatments are below 1 μg g^{-1} indicating that in all vegetated treatments, soil PAH exposure was not carcinogenic. It should be noted that the above inference is based on the exposure of soil to each individual PAH compound not for TPAHs.

15.4.4 Rhizodegradation v/s Phytoaccumulation

Although plants and microorganisms can degrade petroleum hydrocarbons independently of one another, it is the interaction between plants and microorganisms which is the primary mechanism responsible for petrochemical degradation in phytoremediation. The remediation process wherein contaminants are degraded/transformed by microbes in the rhizosphere is referred to as *rhizodegradation*. Critical components of the rhizosphere, in addition to a variety of free-living microorganisms, include root exudates that provide carbohydrates to 'feed' the microorganisms, organic acids that make the ions of nutrients and contaminants more mobile in the soil and enzymes that have important natural functions including degradation of organic contaminants with nitro groups or halogens (Hinchman et al. 1998). Root exudates of sugars, alcohols and acids can amount to 10–20 % of plant photosynthesis annually (Schnoor et al. 1995), and due to these exudates, microbial population and activities are 5–100 times greater in the rhizosphere than in bulk soil, i.e. soil not in contact with plant roots (Gunther et al. 1996). This plant-induced enhancement of microbial population is termed as the rhizosphere effect (Atlas and Bartha 1998). Plants may also beneficially affect PAH-degrading communities by influencing other important factors, including soil aeration, moisture levels and bioavailability.

Studies have indicated that stimulation of microbial activity in the rhizosphere of plants can stimulate biodegradation of various toxic organic compounds (Liste and Alexander 2000a, b; Daane et al. 2001). In the rhizosphere, soil redox conditions, organic content, moisture and other soil properties are manipulated by the activity of plant roots. Rhizodegradation is responsible for the enhanced removal of petroleum hydrocarbons from soil by deep-rooted trees and other annual species (Kim et al. 2006; Margesin et al. 2007; Teng et al. 2010). In fact, microbial activity or *rhizodegradation* has been deemed the most influential and significant cause of PAH removal from soil (Cerniglia 1997).

Dehydrogenase activity, which indicates the biological oxidation processes in soils and other systems, was used as an indicator of overall microbial activity. Measurement of dehydrogenase activity is related to the presence of viable microorganisms (Ceccanti et al. 2006; Vivas et al. 2008). Compared with control soils, microbial activity was higher in all treated soils (Fig. 15.7). Soil DHA was observed in the order 60 DAT > 120 DAT > 240 DAT in all plants. Correlation coefficients in Table 15.6 show significant positive correlation between decrease in PAH content of soil and microbial activity at the three test dates. Microbial activity was high in treatments with low PAH content.

The differential behaviour in stimulation of microbial activity in the rhizosphere exhibited by the test plants was to be expected as many factors are involved in such synergies. It

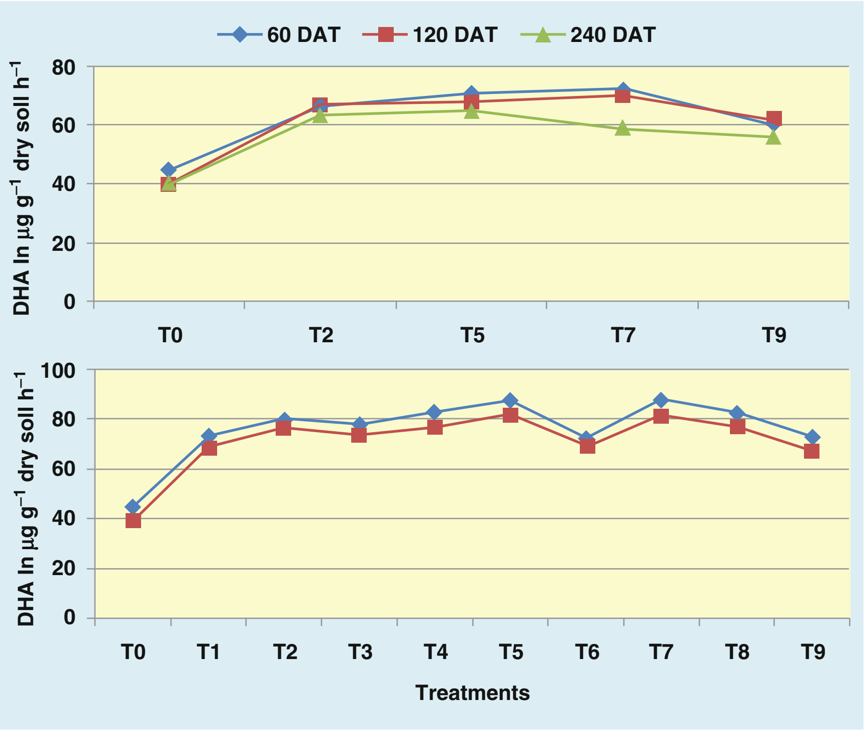

Fig. 15.7 Microbial activity in vegetated treatments at successive days of study

Table 15.6 Correlation coefficients

Plant	60 DAT	120 DAT	240 DAT
Microbial activity and percentage decrease in TPAH in soil			
C. jwarancusa	0.876[a]	0.852[a]	0.937[b]
H. annuus	0.602	0.718[a]	–
TPAH accumulation in roots and percentage decrease in TPAH in soil			
C. jwarancusa	–0.177	0.491	0.302
H. annuus	0.119	0.145	–
TPAH accumulation in shoots and percentage decrease in TPAH in soil			
C. jwarancusa	0.446	0.532	0.242
H. annuus	–0.162	0.074	–

[a] Correlation is significant at the 0.05 level (2-tailed)
[b] Correlation is significant at the 0.01 level (2-tailed)

has been shown that the structure of microbial communities inhabiting the rhizosphere can be affected by root architecture, root age and plant age (Nicol et al. 2003), but the complex interaction between soil type, plant species and root zone location probably is the main factor (Marschner et al. 2001).

In addition to their role in supporting rhizospheric degradative activities, plants may possess a limited capacity to transport some of the more mobile pollutants into roots and shoots via fine roots. In those situations where uptake does occur (i.e. only limited microbial activity in the rhizosphere), there is good evidence that the pollutant may be metabolised. Various vegetables (*Lactuca sativa, Solanum tuberosum, Daucus carota, Brassica oleracea*) and plants like *Plantago major* and *Spartina alterniflora* (a salt-marsh species) and grasses grown in PAH-polluted environments were shown to take up and bioaccumulate these compounds (Bakker et al. 2000; Fismes et al. 2002; Watts et al. 2006). In *Spartina fragilis*, the uptake and bioaccumulation of PAHs from oiled sediments to the plant shoot, wherein only roots were exposed to pollutant, has been demonstrated (Meudec et al. 2006).

Uptake of ΣLPAH was far more (1.5–2 times) than that of ΣHPAH (Tables 15.7 and 15.8). In *C. jwarancusa*, it was nearly equal in shoots. More efficient uptake of HPAHs was observed in roots v/s shoots. The actual accumulation of PAHs did not vary much between the three dates, i.e. the uptake was not related to PAH concentration of soil.

Thus, better accumulation characteristics were obtained at 240 DAT, when PAH content was lowest. In *C. jwarancusa*, the presence of VAM (T2) seemed to favour the accumulation of HPAHs over LPAHs in roots at all days. Accumulation patterns of PAHs also indicate that the treatments T2 (VAM), T3 (*Azospirillum*) and T7 (biosurfactant) in general enhanced the mobilisation of PAHs in the rhizosphere or otherwise improved their uptake by roots of the test plants. The beneficial effects of these treatments have been reported (Gamal 2005; Joner et al. 2001; Bossert and Bartha 1984).

To assess the phytoaccumulation potential of plants, some factors were employed based on simple ratios of contaminant concentration in plant parts and growth matrix. Bioabsorption coefficient (BAC) is the ratio of PAH content in shoot to soil, translocation factor (TF) is the ratio of PAH content in shoot to root, and root concentration factor (RCF)

Table 15.7 PAH concentrations (μg g^{-1}) in *C. jwarancusa* treatments

		60 DAT			120 DAT			240 DAT		
Treatments	Matrix	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH
T2	Soil	37.06	20.34	57.40	10.58	6.22	16.80	2.43	1.3	3.73
	Shoot	3.58	3.65	7.23	3.02	3.28	6.30	1.79	1.86	3.65
	Root	8.73	5.50	14.23	7.38	4.97	12.35	6.85	3.92	10.77
T5	Soil	27.10	22.75	49.85	9.66	8.64	18.30	2.88	2.74	5.62
	Shoot	3.70	1.64	5.34	3.00	1.43	4.43	1.71	0.7	2.41
	Root	7.15	3.63	10.78	4.16	2.26	6.42	2.91	1.61	4.52
T7	Soil	26.33	7.27	33.60	7.83	2.37	10.20	2.89	0.71	3.6
	Shoot	4.79	0.88	5.67	2.94	0.60	3.54	1.30	0.21	1.51
	Root	7.49	1.96	9.46	5.93	1.70	7.63	2.16	0.48	2.64
T9	Soil	23.53	40.67	64.20	11.99	22.21	34.20	1.59	2.98	4.57
	Shoot	2.92	1.83	4.75	1.40	0.94	2.34	0.89	0.91	1.8
	Root	6.77	1.90	8.67	4.01	1.24	5.25	1.67	2.11	3.78

ΣLPAH: Sum of low molecular weight PAHs, here naphthalene to anthracene (Table 15.1)
ΣHPAH: Sum of heavy molecular weight PAHs, here fluoranthene to indeno(1,2,3-*cd*)pyrene (Table 15.1)
TPAH: Total PAH; sum of LPAHs and HPAHs
Initial TPAH = 99.77 μg g^{-1}; ΣLPAH = 84.58 μg g^{-1}; ΣHPAH = 15.19 μg g^{-1}

Table 15.8 PAH concentrations (μg g^{-1}) in *H. annuus* treatments

		60 DAT			120 DAT		
Treatments	Matrix	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH
T1	Soil	31.17	19.74	50.91	15.09	10.21	25.30
	Shoot	3.55	0.98	4.53	1.80	0.54	2.34
	Root	7.46	0.99	8.45	3.96	0.61	4.57
T2	Soil	28.08	20.52	48.60	10.32	8.05	18.37
	Shoot	2.90	0.66	3.56	2.29	0.58	2.87
	Root	6.48	2.87	9.35	2.41	1.15	3.56
T3	Soil	46.18	15.94	62.12	15.72	7.38	23.10
	Shoot	2.43	1.03	3.46	1.35	0.62	1.97
	Root	5.94	2.51	8.45	3.13	1.43	4.56
T4	Soil	37.17	23.43	60.60	15.48	7.84	23.32
	Shoot	3.43	1.24	4.67	1.39	0.55	1.94
	Root	6.18	3.27	9.45	2.47	1.40	3.87
T5	Soil	22.28	23.92	46.20	9.75	8.95	18.70
	Shoot	2.88	1.10	3.98	2.36	0.98	3.34
	Root	5.61	2.84	8.45	2.96	1.61	4.57
T6	Soil	31.72	10.38	42.10	17.47	7.74	25.21
	Shoot	3.75	0.92	4.67	2.02	0.55	2.57
	Root	6.76	2.70	9.45	3.19	1.37	4.56
T7	Soil	38.43	14.77	53.20	12.93	6.26	19.19
	Shoot	4.31	1.04	5.35	1.85	0.49	2.34
	Root	9.95	2.28	12.23	3.70	0.94	4.64
T8	Soil	23.98	11.62	35.60	9.34	6.86	16.20
	Shoot	2.56	0.89	3.45	1.06	0.40	1.46
	Root	6.10	3.25	9.35	2.90	1.66	4.56
T9	Soil	37.33	17.87	55.20	14.94	8.81	23.75
	Shoot	2.93	0.96	3.89	1.57	0.56	2.13
	Root	5.21	1.36	6.57	4.33	1.24	5.57

ΣLPAH: Sum of low molecular weight PAHs, here naphthalene to anthracene (Table 15.1)
ΣHPAH: Sum of heavy molecular weight PAHs, here fluoranthene to indeno(1,2,3-*cd*)pyrene (Table 15.1)
TPAH: Total PAH; sum of LPAHs and HPAHs
Initial TPAH = 99.77 μg g^{-1}; ΣLPAH = 84.58 μg g^{-1}; ΣHPAH = 15.19 μg g^{-1}

Table 15.9 PAH accumulation characteristics of *C. jwarancusa*

	60 DAT			120 DAT			240 DAT		
Treatments	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH
BAC = shoot/soil									
T2	0.1	0.18	0.13	0.29	0.53	0.38	0.74	1.43	0.98
T5	0.14	0.07	0.11	0.31	0.17	0.24	0.59	0.26	0.43
T7	0.18	0.12	0.17	0.38	0.25	0.35	0.45	0.3	0.42
T9	0.12	0.04	0.07	0.12	0.04	0.07	0.56	0.31	0.39
RCF = root/soil									
T2	0.24	0.27	0.25	0.7	0.8	0.74	2.82	3.02	2.89
T5	0.26	0.16	0.22	0.43	0.26	0.35	1.01	0.59	0.8
T7	0.28	0.27	0.28	0.76	0.72	0.75	0.75	0.68	0.73
T9	0.29	0.05	0.14	0.33	0.06	0.15	1.05	0.71	0.83
TF = shoot/root									
T2	0.41	0.66	0.51	0.41	0.66	0.51	0.26	0.47	0.34
T5	0.52	0.45	0.5	0.72	0.63	0.69	0.59	0.43	0.53
T7	0.64	0.45	0.6	0.5	0.35	0.46	0.6	0.44	0.57
T9	0.43	0.96	0.55	0.35	0.76	0.45	0.53	0.43	0.48

is the ratio of PAH content in root to soil. Plants exhibiting TF and BAC values greater than 1 are generally considered promising phytoextractors of metals (Fitz and Wenzel 2002). Here, we extend the same logic in the context of PAHs to see if the uptake is as low as reported. The RCF describes the capability of roots to accumulate contaminants from culture/growth medium (Briggs et al. 1983; Polder et al. 1995).

Due to their low solubility in water and tendency to bind to soil particles, PAHs are generally not absorbed by plant roots in large quantities. A lack of PAH accumulation in shoot was exhibited by BAC values of all treatments except for T2 (VAM) in *C. jwarancusa* (ΣHPAH) at 240 DAT. RCF values at 240 DAT indicate some potential for accumulation in roots in few treatments of *C. jwarancusa* (Tables 15.8, 15.9 and 15.10). However, it is to be noted that this could be simply due to the low concentrations of PAHs in soils at that date as the PAH concentrations in plants were not seen to vary much at the various testing dates. TF values of all species clearly indicate limited potential in translocation of PAHs from root to soil as no value is above 1.

TPAH accumulation in plants and percentage decrease in PAH content at the testing dates did not show much correlation ($P < 0.05$) (Table 15.6). In light of other accumulation characteristics, especially the consideration that the actual uptake values did not change much during the study irrespective of the TPAH concentrations in soil, we can safely conclude that accumulation does not a play a major role in the phytoremediative potential of these plants. On the other hand, significant positive correlation between decrease in PAH content of soil and microbial activity was obtained at the three test dates (Table 15.6). In other words, microbial activity was high in treatments in which more PAH degradation had been achieved. This indicates that rhizodegradation is in fact the major pathway for phytoremediation of PAHs in the soil, at least for the four species assessed here. In fact, rhizodegradation has been deemed the most influential and significant mechanism in phytoremediation of PAH-contaminated soil.

15.4.5 Physiology and Growth

PAHs are known to affect germination and growth as well as physiological processes such as photosynthesis or mineral uptake, inducing a gradual deterioration of plant metabolism and the disturbance of their development. For instance, benzo(*a*)pyrene affects plant photosynthesis and respiration by a chlorophyll breakdown and an enzymatic inhibition of the electron transport, respectively (Huang et al. 1996; Marwood et al. 2001). Meudec et al. (2007) investigated the relationship between heavy fuel oil phytotoxicity and PAH contamination in *Salicornia fragilis* in an artificial experiment. They hypothesised that the observed symptoms of chlorosis, yellowing and/or degeneration of tissues may have been the result of chemical stress at the tissue and cellular level. This view is supported by Alkio et al. (2005) who reported chlorosis and necrosis because of a localised H_2O_2 production, oxidative stress and cell death in *Arabidopsis thaliana* exposed to phenanthrene.

Li et al. (2008) used phenanthrene and pyrene in soil to investigate physiological and biochemical responses of rice (*Oryza sativa* L.) to PAH stress, in the presence or absence of a PAH-degrading bacteria (*Acinetobacter* sp.). A number of parameters including biomass and water, chlorophyll and chlorophyll a/b ratio, electrolyte leakage, activities of superoxide dismutase (SOD) and peroxidase and soluble carbohydrate and soluble protein contents were monitored. Results show that rice plants have good resistance and tolerance to

Table 15.10 PAH accumulation characteristics of *H. annuus*

	60 DAT			120 DAT		
Treatments	ΣLPAH	ΣHPAH	TPAH	ΣLPAH	ΣHPAH	TPAH
BAC = shoot/soil						
T1	0.11	0.05	0.09	0.12	0.05	0.09
T2	0.1	0.03	0.07	0.22	0.07	0.16
T3	0.05	0.06	0.06	0.09	0.08	0.09
T4	0.09	0.05	0.08	0.09	0.07	0.08
T5	0.13	0.05	0.09	0.24	0.11	0.18
T6	0.12	0.09	0.11	0.12	0.07	0.1
T7	0.11	0.07	0.1	0.14	0.08	0.12
T8	0.11	0.08	0.1	0.11	0.06	0.09
T9	0.08	0.05	0.07	0.11	0.06	0.09
RCF = root/soil						
T1	0.24	0.05	0.17	0.26	0.06	0.18
T2	0.23	0.14	0.19	0.23	0.14	0.19
T3	0.13	0.16	0.14	0.2	0.19	0.2
T4	0.17	0.14	0.16	0.16	0.18	0.17
T5	0.25	0.12	0.18	0.3	0.18	0.24
T6	0.21	0.26	0.22	0.18	0.18	0.18
T7	0.26	0.15	0.23	0.29	0.15	0.24
T8	0.25	0.28	0.26	0.31	0.24	0.28
T9	0.14	0.08	0.12	0.29	0.14	0.23
TF = shoot/root						
T1	0.48	0.99	0.54	0.45	0.89	0.51
T2	0.45	0.23	0.38	0.95	0.5	0.81
T3	0.41	0.41	0.41	0.43	0.43	0.43
T4	0.56	0.38	0.49	0.56	0.39	0.5
T5	0.51	0.39	0.47	0.8	0.61	0.73
T6	0.55	0.34	0.49	0.63	0.4	0.56
T7	0.43	0.46	0.44	0.5	0.52	0.5
T8	0.42	0.27	0.37	0.37	0.24	0.32
T9	0.56	0.71	0.59	0.36	0.45	0.38

lower levels of PAHs stress, while adding high levels of PAHs to soils resulted in adverse effects on rice plants such as a reduction in biomass and damage to photosynthetic function. Inoculation with PAH-degrading bacteria promoted growth and photosynthesis of rice.

Chlorophyll ‘a’ content in all test plants was found to be higher than chlorophyll ‘b’ across all treatments. Total chlorophyll content trends were more varied. *C. jwarancusa* was most susceptible to diesel toxicity; most plants succumbed to diesel contamination. In these, broad bands of chlorosis followed by necrosis were observed prior to death. The treatments T2 (VAM), T5 (*Pseudomonas*), T7 (biosurfactant) and T9 (Maleic acid) survived. The efficacy of T5 and T7 was more marked. In *H. annuus*, the trends of chl ‘a’ and chl ‘b’ as well as total chlorophyll content in leaves were not very different as far as effect of diesel contamination in the growth matrix is concerned. T5 (*Pseudomonas*) and T3 (*Azospirillum*) seemed to be the most effective in overcoming PAH toxicity.

It has been reported that plants exposed to sublethal doses of used engine oil had low chlorophyll content (Odjegba and Sadiq 2002; Odjegba and Atebe 2007), and this could have a direct effect on the carbon-fixing efficiency of the plant. Rosso et al. (2005) exposed *S. virginica* to sediments polluted by two types of crude oils and reported reductions in growth and photosynthesis.

All surviving treatments of *C. jwarancusa* (Fig. 15.8) exhibited greater proline accumulation in the shoots till 60 DAT. The addition of diesel to the growth matrix was seen to promote proline accumulation in *H. annuus* too (Fig. 15.9). The magnitude of accumulation was many times more than the control. There was a gradual decline in proline accumulation as the study progressed, and by 120 DAT, all plants exhibited values similar to control.

Maximum accumulation of proline was noticeable in T7 (biosurfactant) in both species with T9 (Maleic acid) a close second. This could be due to the increased bioavailability of pollutants in the rhizosphere which magnified the stress on the plants. The treatment T5 (*Pseudomonas*) which showed most rapid degradation of PAHs in the soil also registered the lowest levels of proline in the plants. This combined with the higher

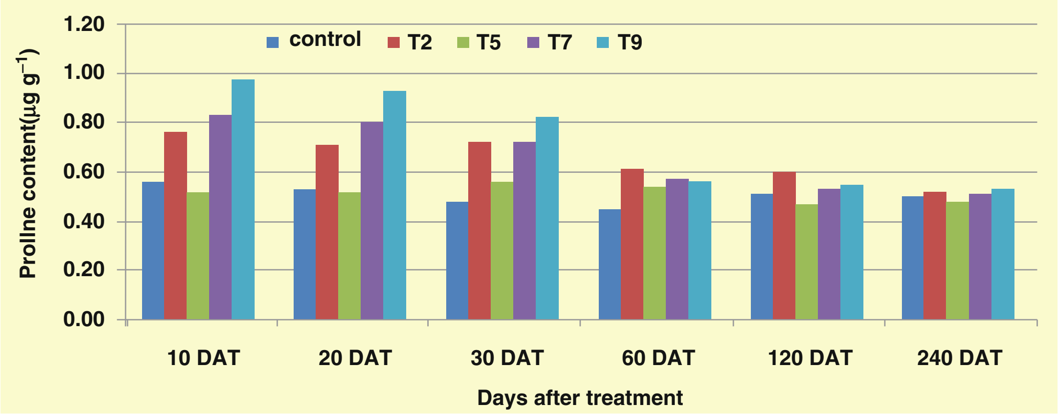

Fig. 15.8 Proline content in *C. jwarancusa* at successive days of study

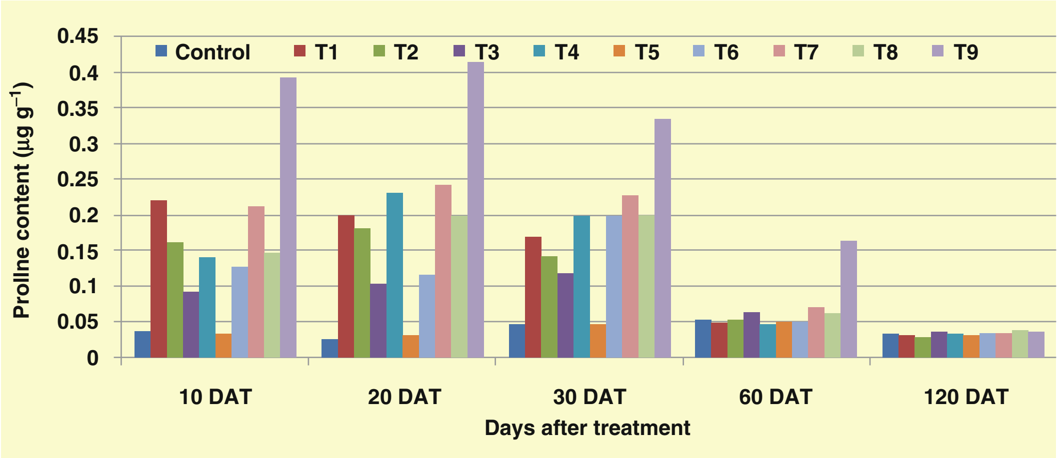

Fig. 15.9 Proline content in *H. annuus* at successive days of study

accumulation seen in increased bioavailability treatments substantiates the hypothesis that proline accumulation in plants was actually in response to contaminants in the rhizosphere.

Detailed studies have shown the accumulation of free proline in plant, viz. *Crinum asiaticum, Phaseolus vulgaris*, *Eruca sativa*, *Helianthus annuus* and *Cajanus cajan* due to metals like Pb, Cu, Co, Cd, Zn and Hg (Varun et al. 2011; Zengin and Munzuroglu 2005; Faheed 2005). Methionine, proline and phenylalanine were found to be the amino acids. Proline and methionine have been found to indicate stress in PAH-treated spruce seedlings (Berteigne et al. 1989). Needle and root growth were also inhibited by the presence of PAHs in the growth matrix.

The addition of diesel to the growth matrix (T1) was seen to inhibit growth in terms of shoot and root length (Table 15.11). More variations in these parameters among the various treatments were visible as the study progressed. Some amendments were able to provide resistance/protect plants from diesel contamination. A gradual stabilising trend was established as the study progressed.

Biomass was negatively affected to varying degrees when diesel was added to the growth matrix without any soil amendment. Both stimulatory (Wieczorek et al. 2001; Maliszewska-Kordybach and Smreczak 2000) and inhibitory effects of PAHs in the growth matrix (Wieczorek et al. 2001; Henner et al. 1999) on stem and root length, their biomass and dry matter have been observed. Smith et al. (2006) observed growth reduction but no significant effect on germination rate of seven oleaginous and grass species after 12 weeks in the presence of PAHs in soils. A similar behaviour—no effect on germination but biomass reduction—was described by Sverdrup et al. (2007) working with *L. perenna*, *T. pretense* and *Brassica alba* and by Besalatpour et al. (2008) with another group of species.

Some amendments were more potent in providing resistance and/or protecting plants from diesel contamination as compared to treatment T1 where only diesel was applied. In general, the treatments T2 (VAM), T3 (*Azospirillum*), T4 (PSB), T5 (*Pseudomonas*), T7 (biosurfactant) and T8 (*Azospirillum* + PSB)

Table 15.11 Growth parameters of test plants at successive days of study

		Shoot length (cm)			Root length (cm)			Total biomass (g)
Plant	Treatments	60 DAT	120 DAT	240 DAT	60 DAT	120 DAT	240 DAT	240 DAT
C. jwarancusa	Control	16.5 ± 0.06	38.26 ± 4.04	73.0 ± 5.7	9.5 ± 0.6	13.4 ± 1.1	17.2 ± 0.7	88.5 ± 8.3
	T2	16.2 ± 0.54	35.96 ± 4.34	58.5 ± 1.5	8.6 ± 1.0	14.1 ± 0.2	18.5 ± 1.0	55 ± 10.7
	T5	16.4 ± 0.04	34.16 ± 4.24	61.2 ± 3.3	9.1 ± 1.1	13.6 ± 1.0	18.7 ± 0.7	39.2 ± 6.6
	T7	16.1 ± 0.34	33.86 ± 0.64	63.3 ± 3.9	7.9 ± 0.5	13.5 ± 1.3	17.6 ± 0.9	24.1 ± 9.7
	T9	16.6 ± 0.26	34.36 ± 2.04	65.4 ± 3.3	8.7 ± 1.1	12.9 ± 0.9	17.2 ± 1.1	35.6 ± 10.1
								120 DAT
H. annuus	Control	60.1 ± 5.3	93.3 ± 5.2	–	18.4 ± 0.5	24.2 ± 1.1	–	101.5 ± 6.8
	T1	49.7 ± 6.8	73.2 ± 5.7	–	12.5 ± 1.8	22.3 ± 1.4	–	90.2 ± 7.3
	T2	53.5 ± 5.2	88.2 ± 6.6	–	15.4 ± 2.0	24.5 ± 1.7	–	104.1 ± 10
	T3	50.3 ± 2.9	75.1 ± 5.2	–	13.9 ± 0.7	21.7 ± 3.0	–	87.1 ± 6.6
	T4	58.8 ± 4.8	112.3 ± 5.5	–	14.8 ± 1.1	29.6 ± 3.7	–	129.4 ± 9.7
	T5	57.4 ± 3.2	79.2 ± 5.0	–	17.3 ± 0.6	24.3 ± 0.8	–	92.5 ± 8.6
	T6	51.8 ± 0.5	86.1 ± 4.1	–	15.3 ± 1.1	20.4 ± 3.5	–	97.6 ± 6.7
	T7	54.1 ± 6.3	87.2 ± 6.7	–	14.2 ± 1.7	23.8 ± 1.0	–	91.6 ± 5.1
	T8	59.2 ± 7.8	78.2 ± 7.1	–	17.7 ± 1.4	24.5 ± 1.4	–	73.6 ± 7.0
	T9	57.5 ± 1.8	88.2 ± 7.0	–	12.9 ± 4.3	22.5 ± 1.4	–	82.6 ± 6.0

seemed to be more protective of plant growth in terms of root length, shoot length as well as total biomass.

Vesicular-arbuscular mycorrhiza (VAM) are known to improve plant growth and health by improving mineral nutrition and increasing resistance or tolerance to biotic and abiotic stresses. PAH-contaminated soils are more or less hydrophobic, and thus plant growth may be limited by water uptake and access to mineral nutrients dissolved in inaccessible soil water. Mycorrhiza-inoculated plants show better tolerance of water stress. In addition, indirect effects of mycorrhizae such as modified root architecture (Hooker and Atkinson 1996), improved membrane integrity (Graham et al. 1981) or enhanced production of oxidative enzymes (Salzer et al. 1999) may improve the performance of inoculated plants in the presence of organic compounds.

Gamal (2005) demonstrated that AM inoculation increased the total biomass of wheat, mung bean and eggplant grown in soil spiked with PAHs. The chlorophyll content of mycorrhiza-inoculated plants also increased in spiked as well as unspiked treatments. Similarly, inoculants used as phytostimulators (*Azospirillum*) or as biological control agents of fungi (*Pseudomonas* and *Trichoderma*) have shown beneficial effects on plant growth and health. Modifications of the microbial community structure in the rhizosphere have also been reported subsequent to such amendments (Mar Vázquez et al. 2000).

15.5 Conclusions

The PAH profile at test sites indicates that levels were much below many contaminated sites worldwide. Yet, these concentrations need to be monitored in the future so that timely interventions may be made. Based on the greenhouse study, it may be concluded that the plant-contaminant-microbe synergy in growth matrices is highly individualistic. Not only the metabolic activities but also the growth of plants can be modified by the presence of pollutants in the rhizosphere as well as by soil amendments and microbiota that are protective/growth promoting in nature. This needs to be extensively studied to gain insight about individual species and their tolerance of soil contamination for phytoremedial strategies to be effective.

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Phytoremediation of Petroleum-Polluted Soils

16

Felix A. Aisien, Eki T. Aisien, and Innocent O. Oboh

16.1 Introduction

Oil exploration, refinery and transportation activities in the Niger Delta areas of Nigeria have resulted in surface and near-surface soil, sediment, groundwater and underground-water contamination with petroleum hydrocarbons (PHCs) including crude oil, gasoline, diesel and creosote causing serious environmental problems (Aisien and Aisien 2005; Aisien et al. 2009). Petroleum contamination is among the most extensive environmentally damaging pollution problem constituting potential threats to humans and ecosystem (Aisien et al. 2009; Bossert and Bartha 1984). Petroleum hydrocarbons (PHCs) are complex mixtures of aliphatic, alicyclic and aromatic compounds plus constituents that contain nitrogen, sulphur or oxygen in addition to H and C (Sparks 1995; Potter and Simmons 1998). Petroleum hydrocarbon contamination of soil occurs through extraction, accidents, transportation, leakage from tanks and operational equipment, pipeline ruptures, pipeline vandalisation and saboteur, consumption and refining (Aisien et al. 2006; Scott 2003). The presence of petroleum and its refined products in the environment (soils, waters and air) is of great concern due to extensive contamination of thousands of sites around the world (Alexander 2000; Jain et al. 2011). The pollution of the soil environment usually results in extreme harsh surroundings, including large qualities of crude oils, refined petroleum products and other associate pollutants such as heavy metals and severe infertile soil. These adverse conditions usually inhibit plant growth and development, threatening human health and the ecosystem (Jain et al. 2011; Besalatpour et al. 2008; Cunningham et al. 1996; Frick et al. 1999; Hutchinson et al. 2001). Previously, chemical and physical methods have been applied for remediation of petroleum-polluted soils such as thermal treatment, soil washing, solidification and stabilisation. However, these methods are expensive and disruptive to the environment and also involve high energy consumption (Kaimi et al. 2007). Therefore, natural remediation techniques have been developed to provide more environmentally friendly and cost-effective cleanup of sites impacted by petroleum spills (Alkorta and Garbisu 2001; Aisien et al. 2013). Recently, phytoremediation of petroleum-polluted soils is becoming a very competitive soil remediation technology because of its advantages over other technologies (Prasad 2006). Phytotechnologies are a set of technologies using plants to remediate or contain contaminants in soil, groundwater, surface water or sediments. Some of these technologies have become attractive alternatives to conventional cleanup technologies due to relatively low costs and the inherently aesthetic nature of planted sites (ITRC 2001; USEPA 2000; Schnoor et al. 1995; Cunningham et al. 1996). Phytoremediation has been proven to be a very effective cleanup method for various classes of contaminants and different media in situ. The typical organic contaminants ('organics') that can be addressed using this technology include petroleum hydrocarbons, gas condensates, crude oil, chlorinated compounds, pesticides and explosive compounds. Also, the inorganic contaminants ('inorganics') include salts (salinity), heavy metals, metalloids and radioactive materials. The affected media in which phytotechnologies can be used to address include soils, sediments, groundwater and surface water. In addition, several emerging applications of phytotechnologies are being developed, including the capabilities of vegetation to utilise atmospheric carbon emissions for greenhouse gas mitigation (USEPA 2000; Cunningham et al. 1996; Cunningham et al. 1997; Mattina et al. 2003; Ndimele 2010).

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The mechanisms of phytoremediation, which is the use of plants to contain, sequester, remove or degrade organics in petroleum-polluted soils, depend on two regions of activity in plants. These are the root zone (phytostabilisation, rhizodegradation and rhizofiltration) and plant tissue (phytodegradation, phytoextraction and phytovolatisation). In plants, these cleanup mechanisms depend on well-developed healthy extensive root system that can allow the plant to explore the soil, affect the soil conditions by increasing soil aeration and moderating soil moisture content, release exudates that affect microbial activity and numbers and be a home to larger and more diverse microbial population than are present in the bulk soil (Aisien et al. 2013; Cunningham et al. 1997; Marmiroli et al. 2006).

16.2 Hydrocarbons Linked with Phytoremediation of Petroleum-Polluted Soils

Petroleum hydrocarbons are widespread in our environment as fuel and chemical compounds. The uncontrolled release of petroleum hydrocarbons has several negative impacts on the soil and water resources (Bossert and Bartha 1984). Petroleum hydrocarbons are a mixture of hydrocarbons obtained from reservoir of crude petroleum. Petroleum hydrocarbons are generally divided into two groups: aliphatics and aromatics. Aliphatics include alkanes that contain single bonds between carbon atoms and have the formula C_nH_{2n+2}, alkenes that contain one or more double bonds between atoms and have the formula C_nH_{2n} and cycloalkanes that contain carbon atoms in cyclic structures. Aromatics have one or more benzene rings as part of their structure. Monoaromatics are aromatics with one benzene ring as part of their structure; polycyclic aromatic hydrocarbons (PAHs) are aromatics with two or more fused benzene rings. Monoaromatics, such as benzene, toluene, ethylbenzene and xylene (BTEX), are some of the most common aromatic compounds in petroleum (Bossert and Bartha 1984; Drake 1997; Chaineau et al. 1997; Mueller and Shann 2006). Crude oil contains less BTEX than gasoline, but combining the average percentage weights for individual BTEX compounds indicates that on average, BTEX compounds represent about 2 % crude oil (by weight). Typically, crude oil contains high concentrations of aliphatic hydrocarbons and lower concentrations of aromatic hydrocarbons (Potter and Simmons 1998). Saturated and aromatic hydrocarbons (mainly *n*-alkanes; branched alkanes; mono-, bi- and polycyclic alkanes; and mono-, bi- and polyaromatics) usually account for 75 % of the mass of crude oils (McGill et al. 1981; Potter a Simmons 1998; Pilon-Smits 2005). Monocyclic aromatic compounds (e.g. toluene, benzene and xylene) and bicyclic types (e.g. naphthalene, biphenol) represent 1–2 %; polycyclic aromatics (usually methylated derivatives of fluorene, phenanthrene, anthracene, chrysene, benzofluorene and pyrene) are present in lower amounts. Aliphatic compounds are generally less toxic than aromatics, and toxicity has been found to vary with compound size (McGill et al. 1981; Edwards et al. 1998; Singh and Jain 2003).

Natural gas condensates also are composed primarily of aliphatic hydrocarbons; however, the condensates may contain substantial amounts of BTEX. When petroleum compounds such as crude oil are released into the environment, the compounds undergo physical, chemical and biological changes. The degree to which the various types of petroleum hydrocarbons degrade depends on the physical and chemical properties of the hydrocarbons (Mueller and Shann 2006; Collins et al. 2002). Hydrocarbons with a low molecular weight are relatively easy to biodegrade. Branched hydrocarbons degrade more slowly than the corresponding straight-chain hydrocarbons. With a molecule size increase the rate of biodegradation decreases. Also, monoaromatic compounds are more rapidly degraded than the two-, three-, four- and five-ring compounds, and lighter mixtures such as gasoline can be readily biodegraded to low levels (Phillips et al. 2009; Radwan et al. 1995).

Water insolubility, hydrophobicity and soil sorptive properties increase with increasing size (number of aromatic rings) and complexity (molecule topology or pattern of ring linkages) of chemicals; PAHs with three or more rings tend to be strongly absorbed to the soil (Reilley et al. 1996; Alexander 2000).

16.3 Categories of Phytoremediation of Petroleum-Polluted Soils

The specific phytotechnology mechanism used to address specific contaminant is dependent not only on the type of constituents and the media that is affected but also on the remediation goals. Typical goals include containment, stabilisation, sequestration, assimilation, reduction, detoxification, degradation, metabolisation and/or mineralisation. To achieve these goals, a proper phytotechnology system must be designed, developed and implemented using detailed knowledge of the site layout, soil characteristics, hydrology, climate conditions, analytical needs, operations and maintenance requirements, economics, public perception and regulatory environment (Peng et al. 2009; Shimp et al. 1993).

Some phytotechnology applications could be primary methods of cleaning up or stabilising contamination, while others will supplement primary remedies. Phytotechnologies may potentially serve the following:

(a) Clean up moderate to low levels of selected elemental and organic contaminants over large areas.
(b) Maintain sites by treating residual contamination after completion of a cleanup.
(c) Act as a buffer against potential waste releases.
(d) Aid voluntary cleanup efforts.

(e) Facilitate nonpoint source pollution control.

(f) Offer a more active form of monitored natural attenuation (McCutcheon and Schnoor 2003). Plants and trees remove organic contaminants provided they are moderately hydrophobic utilising two major mechanisms:

  (i) Direct uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites into the plant tissue.

  (ii) Release of exudates and enzymes that stimulate microbial activity and the resulting enhancement of microbial transformations in the rhizosphere (Merkl et al. 2004b; Shirdam et al. 2009). There are five categories of plant-based technologies applied in phytoremediation of petroleum-polluted soils. These include:

    1. **Rhizodegradation**: Rhizodegradation, where plants break down organic contaminants in the soil by internal and external plant processes through microbial activity. It is enhanced by the presence of the rhizosphere and is a much slower process than phytodegradation. Microorganisms (yeast, fungi or bacteria) consume and digest organic substances for nutrition and energy. Certain microorganisms can digest organic substances such as fuels or solvents that are hazardous to humans and break them down into harmless products through biodegradation. Natural substances released by the plant roots are sugars, alcohols and acids which contain organic carbon that provides food for soil microorganisms, and the additional nutrients enhance their activity. When petroleum compounds such as crude oil are released into the environment, the compounds undergo physical, chemical and biological changes. The degree to which the various types of petroleum hydrocarbons degrade depends on the physical and chemical properties of the hydrocarbons (Aisien et al. 2013; ITRC 2001; Banks et al. 2003; Gerhardt et al. 2009; Shirdam et al. 2009).
    2. **Phytoextraction**: Phytoextraction involves plants' translocation/concentration of organic contaminants in the soil by plant roots into the aboveground portion of the plants (shoots and leaves). There are two types of phytoextraction; these are continuous phytoextraction, where hyperaccumulating plants accumulate high concentrations of contaminants throughout their lifetime, and the induced phytoextraction, which involves enhancing contaminant accumulation at a single time point by addition of an 'accelerants' or 'chelators'. The hyperaccumulating plants or hyperaccumulators absorb unusually high concentration of contaminants into their shoots during normal growth and reproduction. These plants absorbed 100-fold more than non-accumulating plants. Also, hyperaccumulators have additional detoxification mechanisms (Aisien et al. 2013; Ndimele 2010).
    3. **Phytodegradation**: Phytodegradation utilises plants to uptake organic contaminants from soils and subsequent transformation through various internal, metabolic processes that help catalyse degradation. In phytodegradation the breakdown of contaminants is by internal and external plant processes. The internal process by the plants is through metabolic processes within the plant, and in the external process, compounds (enzymes) produced by the plant break down contaminants (Aisien et al. 2013; McGill et al. 1981).
    4. **Phytostabilisation**: Phytostabilisation involves applying plant species to immobilise organic contaminants in the soil through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone and physical stabilisation of soils. This process reduces the mobility of contaminants and prevents migration to groundwater or air. This can reestablish vegetative cover at sites where natural vegetation is lacking due to high contaminant concentrations. Unlike other phytoremediative techniques, phytostabilisation is not intended to remove contaminants from a site but rather to stabilise them by accumulation in roots or precipitation within root zones, reducing the risk to human health and the environment. It is applied in situations where there are potential human health impacts, and exposure to substances of concern can be reduced to acceptable levels by containment. Phytostabilisation is most effective for fine-textured soils with high organic-matter content, but it is suitable for treating a wide range of sites where large areas are subject to surface contamination (Cunningham et al. 1997; Shimp et al. 2009). However, some highly contaminated sites are not suitable for phytostabilisation because plant growth and survival are impossible.
    5. **Phytovolatilisation**: Phytovolatilisation refers to plants being capable of absorbing organic contaminants from the soil, biologically converting them to gaseous species. Unlike other remediation techniques, once contaminants have been removed via volatilisation, there is a loss of control over their migration to other areas within the plant, releasing them into the atmosphere (Khan and Husain 2004; Tsao 2003).

16.4 Enhancement of Phytoremediation of Petroleum-Polluted Soils

Despite the phytoremediation is the in situ use of vegetation in the remediation of petroleum-polluted soils (PPSs). The applications of vegetation alone have a limiting influence on the technology. Presently especially in laboratory scale, attention has been focused on the enhanced phytoremediation in order to improve the efficacy as well as to reduce treatment time for petroleum-polluted soils. These processes include soil amendment, inoculation of plants with microbes, plant growth-promoting rhizobacteria, genetic engineering technology and combined approaches.

1. **Soil amendment for enhancing phytoremediation**
   One of the promising options for the phytoremediation of petroleum-polluted soil (PPS) is the use of soil amendment to enhance the process. This will not only enable great vegetative coverage but also increase the rate of PPS removal in soil. Vouillamoz and Milke (2001) reported that the addition of compost to soil helps reduce the negative effects of PHCs on ryegrass growth and increases PHC removal from the soil. Also, Palmroth et al. (2006) confirmed that in soil amended with NPK fertiliser, 65 % of hydrocarbons were removed and the addition of municipal biowaste compost removed 60 % of hydrocarbons over 39 months. However, hydrocarbons did not significantly decline in non-amended soil. The addition of *Jatropha curcas* amended with organic wastes to soil greatly increases the removal of waste-lubricating oil to 89.6 % and 96.6 % in soil contaminated with 2.5 % and 1.0 % oil, respectively. Besides, loss of 56.6 % and 67.3 % was observed in the corresponding planted soils without organic amendment over 180 days (Agamuthu et al. 2010). Kang et al. (2010) reported that though conventional amendments such as NPK fertiliser have contributed to plant productivity and effective degradation of PHC pollutants, when overused the soil-remaining fertilisers not taken up by the plants usually 'burn' the plants and can even cause environmental problems. Natural biosurfactants such as rhamnolipids, which have no phytotoxicity to plants and can increase petroleum hydrocarbon bioavailability, are proven to enhance petroleum hydrocarbon degradation (Zhang et al. 2010). The advantages of using biosurfactants indicate that biosurfactant-enhanced phytoremediation has the potential to become a promising technology for remediation of contaminated soil (Zhu and Zhang 2008).
2. **Inoculation of plants with microbes for enhancing phytoremediation**
   It has been reported that inoculation of plants with microbes isolated from vegetated soils greatly enhanced the phytoremediation processes. Euliss et al. (2008) reported that different plants may enhance rhizosphere degradation by selecting for a variety of microbial communities. Also, Aprill and Sims (1990) stated that grasses are preferred for phytoremediation due to their large root surface area, which can help establish active microbial activity and populations. Besides, Fang et al. (2001) observed that microbes isolated from grass-planted soils were more effective at degrading petroleum hydrocarbons than those from non-vegetated soils. As a result, inoculation of plants with microbes in rhizosphere may not only protect plant roots from pollutant toxicity (Robert et al. 2008) but also will enhance phytoremediation efficacy. Presently, studies have been concentrated on enhancing rhizodegradation efficiency by inoculating microbes, particularly indigenous microbes isolated from contaminated habitats. Autochthonous microbes are more compatible with local contaminated sites than allochthonous microbes, which do not occupy a functional niche (Atlas and Bartha 1998). Efficient hydrocarbon-degrading bacterial strains that can compete with the native habitat and are closely linked to plants will also serve as promising options for phytoremediation. Also, previous studies have focused on inoculation using hydrocarbon-degrading bacteria to enhance phytoremediation. However, plants with added fungal strains such as *Fusarium acuminatum*, *F. equiseti*, *F. oxysporum*, *F. solani* and *F. reticulatum* are also more effective at increasing petroleum hydrocarbon decontamination than phytoremediation alone (Hashem 2007). There are mutual benefits between plants and inoculated hydrocarbon-degrading microbes that greatly promote phytoremediation of petroleum hydrocarbon. Plant characteristics and phytoremediation efficiency can be greatly improved with the addition of some special microbes.
3. **Plant growth-promoting rhizobacteria for enhancing phytoremediation**
   Plant growth-promoting rhizobacteria are bacteria capable of promoting plant growth by colonising the plant root surface and the closely adhering soil interface (Kloepper and Schroth 1981). Plant growth-promoting rhizobacteria strains can produce indoleacetic acid (an auxin), siderophores and enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Soil contamination generally stimulates ethylene production in plants, leading to plant growth retardation. Enzyme ACC deaminase can convert ACC, the precursor of ethylene into 2-oxobutanoate and ammonia (Glick 2005). Decreased ethylene levels allow plants used in phytoremediation to grow and survive better in heavily contaminated soils. The introduction of plant growth-promoting rhizobacteria strains in phytoremediation can provide better plant growth and thereby increases plant resistance to contaminants in the soil than using plants alone (Kang et al. 2010; Koo et al. 2010). As a

result, plant growth-promoting rhizobacteria can help accelerate degradation of contaminants. Moreover, the introduction of plant growth-promoting rhizobacteria greatly enhanced the PHC (polycyclic aromatic hydrocarbons) and creosote removal when compared with phytoremediation alone. Plant growth-promoting rhizobacteria strains can enhance the grass germination frequency and stimulate grasses to grow better in heavily contaminated soils, thus promoting decontamination of petroleum hydrocarbons (Zhou et al. 2011).

4. **Genetic engineering technology for enhancing phytoremediation**
The utilisation of plants for the cleanup of toxic compound-contaminated soils is limited by the slow growth rate of the plants' action, as it usually takes several years for restoration of the contaminated sites. Kawahigashi (2009) reported that the efficiency of using plants can be substantially improved through genetic engineering technologies. There have been several efforts towards the development of transgenic plants for phytoremediation. These transgenic plants when properly developed greatly assist in remedying heavy metal (Hg)- and organic (trichloroethylene, chloroform, benzene, carbon tetrachloride and halogenated hydrocarbons)-contaminated sites (Doty et al. 2007). However, using transgenic weeds for phytoremediation of petroleum hydrocarbon-contaminated soils has not been given adequate attention.
5. **Combined approaches for enhancing phytoremediation**
Remediation technology using plants and only one enhancement approach may still be inefficient in most cases. For a phytoremediation system to be more effective, plant tolerance and total petroleum hydrocarbon degradation need to be improved by the use of a combination of all the approaches earlier discussed. A multiprocess phytoremediation system has been suggested to combine agronomic treatment, inoculation with contaminant-degrading bacteria and growth of the contaminant-tolerant plants such as tall fescue (*Festuca arundinacea)* with plant growth-promoting rhizobacteria. Huang et al. (2005) reported that during the first 4 months in culture, the removal of total petroleum hydrocarbons and 16 priority polycyclic aromatic hydrocarbons by the multiprocess phytoremediation system was twice that of agronomic treatment, 50 % more than inoculation with microbes and 45 % more than phytoremediation alone. A combined approach consisting of phytoremediation, surfactant, flushing and microbial degradation effectively dissipates oil pollutants from soil and is recommended for quick restoration of petroleum hydrocarbon-contaminated sites (Zhu et al. 2010). Also, Zhang et al. (2010) reported an introduction of a multi-technique phytoremediation system consisting of mycorrhizal fungi, aromatic hydrocarbon-degrading bacteria and rhamnolipids for the bioremediation of polycyclic aromatic hydrocarbons. According to them, after 90 days the total polycyclic aromatic hydrocarbon removal by the multi-technique phytoremediation system was 251.83 % greater than that of phytoremediation alone. Their studies show that applying one approach alone is not very efficient, but combining multiple processes can remedy defects. Therefore, phytoremediation in conjunction with multiple approaches may be an optimal solution for enhancing petroleum hydrocarbon removal.

16.5 Environmental Conditions Affecting Phytoremediation of Petroleum-Polluted Soils

The control and optimisation of phytoremediation processes is influenced by a complex system of many environmental factors. These factors include: type of soil, temperature, pH, organic-matter content, water and oxygen availability, sunlight and nutrients. Some of the factors affect degradation processes directly, while others impact phytoremediation by altering the bioavailability of the pollutants (Infante et al. 2013; Susarla et al. 2002).

1. **Soil type**: Soil type which includes structure, texture and organic-matter content can limit the bioavailability of petroleum contaminants and may influence the quality and quantity of root exudates, which may influence phytoremediation efforts. Microorganisms require low clay or silt content for good activity. A proportion of petroleum hydrocarbon contaminants can be strongly adsorbed on organic matter in the soil system. Plant root exudates increase the organic-matter content in contaminated soils, and this may affect contaminant bioavailability through sorption (Hutchinson et al. 2001; Issoufi et al. 2006). In some cases, petroleum hydrocarbons are not readily desorbed and are therefore not available for phytoremediation. Also, increased soil petroleum hydrocarbon concentration will not only have a negative impact on plant growth but will deteriorate human and animal health through ingestion of water, soil and food produced on the contaminated soils. Also, high concentration of contaminants will even lead to plant death (Adam and Duncan 2003; Susarla et al. 2002).
2. **Soil quality**: This is another important factor for determining successful germination, growth and health of plants. Heavily contaminated soils have a tendency towards poor physical conditioning which is unsuitable for vigorous growth of vegetation and rhizosphere bacteria. It is therefore critical to use amendments to improve the quality of soil before planting. Common limitations are poor moisture-holding capacity, insufficient aeration, low permeability and nutrient deficiencies. Organic

Table 16.1 Summary of the optimum environmental conditions for the degradation of hydrocarbon contaminants in the soil (Hutchinson et al. 2001; McGill et al. 1981)

Parameter	Optimum value for hydrocarbon degradation	Condition required for microbial activity
Soil moisture	30–90 %	25–28 % water-holding capacity
Soil pH	6.5–8.0	5.5–8.8
Oxygen content	10–40 %	Aerobic
Temperature (°C)	20–30	15–45
Nutrient content	C/N/P = 100:10:1	N and P for microbial growth
Type of soil		Low clay or silt content

Table 16.2 Summary of remediation technologies available for cleanup of the petroleum hydrocarbons (Kujat 1999; Gerhardt et al. 2009; McCutcheon and Schnoor 2003; USEPA 2006)

Strategy	Application	Advantages	Limitations	Costs
Air sparging	Gasoline	Can remove some compounds, capital cost, resistant to biodegradation	VOCs only	Low: low capital cost, short treatment time
Bioremediation	Gasoline, fuel oils	Effective on some nonvolatile compounds	Possible lengthy cleanup time	Moderate: because of capital outlay and management
Land farming	Gasoline, fuel oils, coal tar residues	Uses natural degradation processes	Some residuals remain	Moderate: because of initial capital outlay
Phytoremediation	Gasoline, fuel oils	Uses natural degradation process, ecologically benign and soil stabilisation	Efficacy not really known	Low: low capital cost

amendments such as aged manure, sewage sludge, compost, straw or mulch can be used to increase the water-holding capacity of a contaminated soil. Soil pH can be increased and decreased by the addition of lime and sulphur, respectively (Smith et al. 2006; Alkorta and Garbisu 2001; Gerhardt et al. 2009). Contaminated soils are usually deficient in macronutrients (nitrogen, phosphorus, potassium, magnesium, calcium and sulphur) and micronutrients (iron, boron, zinc, copper, manganese and molybdenum) necessary for establishing healthy vigorously growing plants and stimulating microbial contaminant degradation (Prasad 2006). Also, contamination with hydrocarbon-based compounds affects the carbon to nitrogen (C/N) ratio in soil and can lead to nitrogen immobilisation (Newman and Reynolds 2004). The inorganic mineral nutrients that are most often reported to limit the breakdown on petroleum hydrocarbons in soil are nitrogen and phosphorus (Hutchinson et al. 2001).

3. **Soil moisture content**: This is the amount of water the soil contains or can hold. The percentage of soil moisture content for optimum value of hydrocarbon degradation and that for microbial activity is 30–90 % and 25–28 %, respectively, as indicated in Table 16.1 (Infante et al. 2013).
4. **Temperature**: The prevailing temperature and sunlight will greatly influence the weathering process of the soil such as volatilisation, evapotranspiration, photomodification, hydrolysis and biotransformation. These processes selectively reduce the concentration of easily degradable contaminants with the more recalcitrant compounds remaining in the soil. Temperature affects the rate at which the various processes occur. Thus, phytoremediation might be best suited for tropical countries where plant growth occurs all year round than in temperate climates, where active contribution of phytoremediation is restricted to the plants' growing period only. Winter operations may pose problems for phytoremediation when deciduous vegetation loses its leaves, transformation and uptake cease and soil water is no longer transpired. The temperature for the optimum values of hydrocarbon degradation and that for microbial activity is 20–30 °C and 15–45 °C, respectively (see Table 16.1). Generally, a high temperature induces a high rate of biological degradation processes in soil. A very low rate of hydrocarbon degradation/utilisation is observed at low temperature, since low temperature leads to a slow rate of microbial growth. Table 16.1 gives a summary of the optimum conditions required for the degradation of hydrocarbon contaminants' presence in petroleum-polluted soils (Issoufi et al. 2006; Euliss et al. 2008; Khan et al. 2004).

   Table 16.2 represents the summary of remediation technologies for the cleanup of petroleum hydrocarbons.

16.6 Some Plants and Legumes Associated with the Phytoremediation of Petroleum-Polluted Soils

The following plants and legumes represented in Table 16.3 have been implicated in the phytoremediation of petroleum-polluted soils.

Table 16.3 Plants/legumes, contaminants and mechanisms of phytoremediation of petroleum-polluted soils (USEPA 2006; Al-Ghazawi et al. 2005; Issoufi et al. 2006; Merkl et al. 2004a; Huang et al. 2004; Adam and Duncan 2003)

Plants (common/botanical name)	Contaminants	Mechanisms
Fescue grass (*Cynodon dactylon*)	Total petroleum hydrocarbon (TPH), PAHs, diesel	Rhizodegradation, phytostabilisation, phytodegradation
Tall fescue (*Festuca arundinacea*), Kentucky bluegrass (*Poa pratensis*), wild rye (*Elymus canadensis*)	Creosote, TPH, PAHs, diesel fuel	Rhizodegradation, phytostabilisation
Red mulberry trees (*Morus rubra* L.)	Weathered crude oil	Rhizodegradation, phytodegradation
Brachiaria brizantha (*Panicum maximum*)	Crude oil	Rhizodegradation, phytostabilisation, phytodegradation
Hard fescue (*Festuca ovina*), red fescue (*Festuca rubra*), perennial ryegrass (*Lolium perenne*)	Diesel, TPH, PAHs, BTEX	Rhizodegradation, phytostabilisation, phytodegradation
Westerwolds ryegrass (*Lolium multiflorum* L.)	Diesel, TPH, PAHs, BTEX	Rhizodegradation, phytostabilisation, phytodegradation
Hybrid poplar (*Populus charkowiieensis*), understory grasses	Benzene, toluene, TCE, PCBs, PAHs, BTEX, MTBE, TPH (gasoline and diesel range), aged lubricating oil	Phytodegradation, rhizodegradation, phytostabilisation, phytovolatilisation, phytoextraction
White willows (*Trifolium* (genus))	BTEX, MTBE, PAHs, TPH (diesel range), gasoline, aged lubricating oil	Phytodegradation, rhizodegradation, phytostabilisation, phytoextraction, phytovolatilisation
Alfalfa (*Medicago sativa*), volunteer grasses and forb species	TPH, PAHs, BTEX	Rhizodegradation, phytostabilisation, phytodegradation, phytovolatilisation
White clover (*Trifolium* (genus))	BTEX, TPH, PAHs, total residual petroleum (TRP), diesel fuel	Rhizodegradation, phytostabilisation, phytodegradation
Bermuda grass (*Cynodon dactylon*)	TPH, PAHs, diesel fuel, BTEX	Rhizodegradation, phytodegradation
Annual rye (*Lolium multiflorum* Lam.)	PCBs, PAHs	Phytoextraction, phytodegradation, phytoextraction
Black willow (*Salix nigra*)	PCBs, PAHs, gasoline, BTEX	Phytoextraction, rhizodegradation, phytodegradation, phytovolatilisation
Ironwood, buffelgrass	TPH (diesel and gasoline range), BTEX, PAHs	Rhizodegradation

16.7 Advantages and Limitations of Phytoremediation Applications over Conventional Remediation Methods in Petroleum-Polluted Soils

16.7.1 Advantages

- It is less costly and if properly managed is both environmentally friendly and aesthetically pleasing to the public.
- Organic contaminants adsorbed or trapped within the micropores in the soil matrix can be effectively removed.
- It can be used either in in situ or ex situ application. However, to reduce the spread of contaminants through air- and waterborne waste, the in situ application is frequently used.
- It is less disruptive to the environment and economically competitive.
- It can be applied to more multiple and mixed contaminants and media.
- It has relatively low maintenance, has easier implementation and is self-regulating.
- It has the potential to be rapid and suited for large areas of surface contaminations.
- It preserves natural structure and texture of the soil thereby improving soil quality and prevents soil erosion.
- It minimised the leaching of contaminants and soil stabilisation.
- It does not require expensive equipment or highly specialised personnel (Merkl et al. 2005; Newman et al. 1998; Flathman and Lanza 1998; Aisien et al. 2013; Phillips et al. 2009).

16.7.2 Limitations

- Phytoremediation is most effective only at sites with shallow contamination in the soils and/or sites with shallow water table.
- It might require use of a greater land area than other remedial methods. This might interfere with other remediation or site activities.
- It can be applied only under warmer climates for 12 months per year remediation; hence the system can lose its effectiveness during winter (when plant growth slows

or stops) or when damage occurs to the vegetation from weather, disease or pests. A backup remedial technology might be necessary.

- There may be a possibility that the plant shows adverse effects such as potential transfer of contaminants to another medium in the environment and/or the food chain and on introduction or spread of an inappropriate or invasive plant species.
- This technique may not be applicable for highly hydrophobic contaminants due to the tendency of the contaminants to remain adsorbed to the soil particles.
- A longer time period is likely to be required for phytoremediation, as this technology is dependent on plant growth rates for establishment of an extensive root system or significant aboveground biomass.
- High initial contaminant concentrations can be phytotoxic, will prevent plant growth and cause the death of the plant.
- It requires proper handling and disposal methods or an analysis of risk pathways for the harvested hyperaccumulator plants.
- Enhancement of detoxification in the plant by cloning the plant with bacterial genes.
- Plant species or varieties of one species can vary significantly in their efficacy for phytoremediation.
- Phytoremediation might require use of a greater land area than other remedial methods. This might interfere with other remediation or site activities.
- The amendments and cultivation practices in phytoremediation might have unintended consequences on contaminant mobility (Merkl et al. 2005; Newman et al. 1998; Flathman and Lanza 1998; Aisien et al. 2013; Phillips et al. 2009).

16.8 Conclusions

Petroleum-polluted soils are common sites all over the world especially in areas where there are oil exploration activities. Several methods have been used in the past to treat petroleum-polluted soils. However, the application of phytoremediation has proven to be one of the most efficient, cost-effective and environmentally friendly technologies, which should be encouraged. The mechanisms of phytoremediation of petroleum-polluted soils include phytodegradation, rhizodegradation, phytostabilisation, phytoextraction and phytovolatilisation. Many plants, legumes and microorganisms have been implicated in the phytoremediation processes. Currently, researches on other potential plants and microorganisms which can remediate better and faster are in progress. The prospect of this technology is very high; hence in the near future the environmental problems linked with petroleum-polluted soils worldwide will be a thing of the past especially with enhancement of phytoremediation.

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Phytoremediation and Biochar Application as an Amendment

17

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17.1 Introduction

Since the onset of the Industrial Revolution, trace elements (TE) such as Cd, Cu, Hg, Zn, and Pb and organic contaminants such as PAHs, PCB, chlorophenols, and dioxins, originating from waste emissions from industrial production, mining activities, waste (i.e., biosolid and manure) application, wastewater irrigation, and inadequate management of pesticides and chemicals in agricultural production, have contaminated soils worldwide and adversely affected environmental resources and human health (Zhang et al. 2013a). Worldwide more than 80,000,000 ha of land have been degraded by contamination (Evangelou et al. 2012). In the European Union potentially soil-contaminating activities have occurred on nearly three million sites (EEA 2007a). Trace elements are the most important contaminants at 37 % of these sites, followed by mineral oil with 33.7 % and organic pollutants with 29 % (EEA 2007b).

The remediation of this hazardous soil contamination remains to be an important but often elusive task. The use of the most traditional remediation techniques, including excavation and landfilling, is unfeasible on a large scale due to high costs, adverse environmental side effects, or irreversible damages to soil properties that are essential for soil's ecological functions. Phytoremediation was proposed as a cost-effective method which, moreover, is accepted by the public as it is aesthetically pleasing, causes minimal environmental disturbance, and does not impair the ecological quality of soil. Raskin et al. (1997) defined phytoremediation as the use of green plants to remove pollutants from the environment or to render them harmless. Often however, the cleaning soils by phytoremediation is not an option, as the concentrations of the contaminants are too high and thus too toxic for the applied phytoremediation plants or rendering the duration of the cleanup process too long. Kayser et al. (2000), for example, estimated that it would take more than 180 years to reduce the Cd concentration of their study soil from 6.6 to 0.8 mg kg^{-1}. With such long time spans to achieve the target, low-cost phytoremediation (5–10 \$ ton^{-1}) compared to hard remediation methods, which are generally in the range of 100–500 \$ ton^{-1} (Glass 1999), would be lost due to the loss of revenue resulting from the longer-term restrictions in land use. These opportunity costs are often neglected in comparative evaluation of phytoremediation as a treatment option (Robinson et al. 2009).

In many cases, the application of soil amendments immobilizing contaminants in situ may be more advantageous. Immobilization aims at eliminating the risk of groundwater contamination, plant uptake, and exposure of other living organisms, while providing soil conditions that promote plant growth and stimulate ecological restoration (Adriano et al. 2004; Ruttens et al. 2006; Vangronsveld et al. 2009). For in situ TE immobilization, a variety of organic and inorganic soil amendments, such as organic matter, natural and synthetic zeolites, clays and related substances, Fe-rich materials, Mn oxides, and P-rich compounds, have been tested (Ruttens et al. 2006). For organic contaminants also a variety of amendments, such as sludge, compost, organic matter, and activated coal, have been tested, not only to immobilize but also to accelerate their degradation (White et al. 2003).

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17.2 Soil Amendments for Phytoremediation and Bioremediation

17.2.1 Amendments for TE-Contaminated Soils

For the phytoremediation of TE-contaminated soils, two main techniques are applied: phytoextraction and phytostabilization. Phytoextraction describes the use of plants to remove/extract pollutants from soil. In phytostabilization, however, the pollutants are immobilized and remain in the soil. In both techniques various amendments are applied to the soil to either accelerate the uptake of TE by plants (chelant-assisted phytoextraction) or to immobilize TE in soil. In chelant-assisted phytoextraction various synthetic and natural chelating agents are applied. These form extremely stable chelates with metals and are commercially available in sufficiently large quantities at sufficiently low prices such as ethylenediaminetetraacetic acid (EDTA), diethylene triamino pentaacetic acid (DTPA), trans-1,2-cyclohexylene dinitrilo tetraacetic acid (CDTA), ethylenediamine disuccinate (EDDS), or nitrilotriacetic acid (NTA) that were applied. Although the application of chelating agents increases TE plant uptake, the risk of TE displacement in the depth and thus their leaching into groundwater or surface water is greater than its uptake (Evangelou et al. 2007a; Lai and Chen 2005; Luo et al. 2005; Meers et al. 2005). Nowack (2002) showed that EDTA occurred in higher concentrations in river water than any other identified organic compound.

To immobilize TE and in turn to reduce the risk of TE leaching, naturally occurring or artificial soil amendments, such as liming material, phosphate, zeolite, bentonite, clay, Fe metal, Fe and Mn oxides, and organic matter, have been applied (Cheng and Hseu 2002). These amendments reduce contaminant solubility by promoting the formation of insoluble precipitates or by enhancing the soil's capacity to bind them. The latter can be achieved directly through the addition of adsorbent material or indirectly by adjusting the soil's pH–Eh conditions to promote contaminant absorption onto the soil's matrix. Phosphate amendments such as hydroxyapatite are effective in reducing the solubility of Pb, Cd, Zn, Al, Ba, Co, Mn, Ni, and U. However, phosphate has been shown to promote the solubility of As and Cr (Seaman et al. 2001), possibly through reduced sorption of the oxyanions due to an increase in pH and competition from PO_4^{3-}. A variety of inorganic and organic amendments have been used to reduce TE to less soluble, less toxic TE species. Bolan and Duraisamy (2003) showed that organic amendments, such as animal and poultry manures rich in dissolved organic carbon, are effective in reducing Cr(VI) to Cr(III). Also the addition of bauxite residue, "red mud," as demonstrated by Lombi et al. (2002), reduced the solubility of Cd, Pb, Ni, and Zn by raising the soil pH and adsorbing the TE to Fe and Mn oxides. Organic soil amendments, such as compost, poultry litter, natural/commercial humic substances, or industrial sewage sludge, have also been applied to TE-contaminated soils in order to reduce TE mobility and ameliorate TE toxicity to plants (Shuman 1999). However, their application can either increase or decrease TE mobility, depending on the nature of the organic amendment. Insoluble or solid organic matter can immobilize TE through physical and chemical adsorption (Cheng and Hseu 2002), while soluble organic matter can solubilize TE and promote TE leaching but render them unavailable for plant uptake (Robinson et al. 2009).

17.2.2 Amendments for Soils Contaminated with Organic Pollutants

The mechanisms involved in the phytoremediation of soils contaminated with organic pollutants are degradation, adsorption, accumulation, and volatilization. The prevailing mechanism not only depends on the medium to be remediated (air, soil, groundwater, surface water) as well as the type of plant used but also on the physical properties of the contaminant (Newman and Reynolds 2004). In contrast to TE phytoextraction, the phytoremediation of organic solvents such as trichloroethylene (TCE), 2,4-dichlorophenol, 1,1,2,2-tetrachloroethane, and carbon tetrachloride is being successfully applied and is almost at the point of being considered an accepted technology (Newman and Reynolds 2004). For other organic pollutants such as 1,1,1-trichloro-2,2,bis(*p*-chlorophenyl)ethane (DDT), atrazine, polycyclic aromatic hydrocarbons (PAHs), petroleum compounds, and polychlorinated biphenyls (PCBs), advances in their phytoremediation have progressed more slowly. In the case of the very persistent DDT, its degradation by plants and associated microorganisms leads to metabolites such as dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) which are of identical persistence. Furthermore the uptake of DDR as well as its metabolites is too slow to resolve the problem of DDT pollution. For atrazine and some of its metabolites, which are mainly pollutants of hydraulic networks, plants can help to reduce atrazine pollution by (1) accelerating its microbial degradation and (2) mitigating water pollution by reducing runoff of atrazine derivates (Pascal-Lorber and Laurent 2011). With compounds such as the polycyclic aromatic hydrocarbons (PAHs), petroleum compounds, and polychlorinated biphenyls (PCBs), the rhizosphere plays an important role. Flavonoids and other compounds released by roots can stimulate growth and activity of bacteria, which degrade PCBs and PAHs. Furthermore, root growth and death promotes soil aeration, which can enhance oxidative degradation of such recalcitrant organic compounds (Gerhardt et al. 2009).

Various soil amendments such as paper mill sludge, broiler litter, sawdust (White et al. 2003), nutrients, or biosolids have been used to stabilize or to accelerate the degradation of organic contaminants. McLeod et al. (2004) used activated carbon, coke, peat, anthracite, char, and wood to immobilize benzo[*a*]pyrene (BaP) and 2,2′,5,5′-tetrachlorobiphenyl (PCB-52), with wood having the highest absorption efficiency and activated carbon the lowest. Besides organic compounds also a series of inorganic materials, such as sesquioxides, clay, oxides, and oxyhydroxides of iron, silica, and allophane, have been applied to catalyze the process of organic contaminant incorporation (Liu et al. 2007). Thus, the sequestrated organic chemicals may lose their original chemical and biological activity and constitute no threat to human health and the environment.

17.3 Biochar

Biochar is produced by pyrolysis (heat-induced carbonization in oxygen-poor atmosphere) of organic material (e.g., wood, poultry litter, crop residues, etc.). Characteristic for char in general, and hence for biochar, is its mainly stable aromatic form of organic carbon. Compared to the carbon in a pyrolysis feedstock, it cannot readily be returned to the atmosphere as CO_2 even under favorable environmental and biological conditions, such as those that may prevail in soils (Sohi et al. 2010). It is distinguished from charcoal by its main purposes, which are (1) to amend agricultural soils for crop production and at the same time (2) to sequester carbon from organic matter and avoid its release as CO_2 into the atmosphere through mineralization (Laird 2008; Lehmann 2007; Lehmann and Joseph 2009; Sohi et al. 2010). Given that the pyrolysis process can be used in addition (3) to produce energy, the biochar technology in various respects has the potential to make valuable use of organic residues and thus also (4) offer an attractive option to dispose of organic wastes (Kwapinski et al. 2010).

The positive effect of biochar on soil fertility dates back to the practice of pre-Columbian Amazonian Indians, who added charred organics to the surrounding soils (Downie et al. 2011; Graber et al. 2010). This practice formed the fertile *terra preta* of the central Amazon. These soils subsequently recolonized by natural forest were uncovered relatively recently and are locally popular for the production of cash crops. As anecdotal evidence suggests papaya and mango grow three times faster on this land compared to the surrounding soil (Sohi et al. 2010). The enhanced fertility of *terra preta* in the Amazon has been explained by higher levels of soil organic matter (SOM); improved retention of soil nutrients such as N, P, Ca, and K, higher pH; and higher water-holding capacity compared to the more infertile surrounding soils (Downie et al. 2011; Glaser et al. 2001).

The application of biochar to soils outside of the Amazon area in general has resulted in an improvement of plant performance (Chan et al. 2007, 2008; Major et al. 2010; Zhang et al. 2010a), although there are notable exceptions (Gaskin et al. 2010; Van Zwieten et al. 2010).

In general biochar is characterized by a high cation exchange capacity (CEC), high pH, and high surface area. Thus, in recent years, biochar has also been proposed for the immobilization of TE as well as organic contaminants. Its physical and chemical properties, which subsequently influence the types and rates of interactions (e.g., adsorption–desorption, precipitation–dissolution, redox reactions), depend on the following factors: (1) feedstock composition, in particular the total percentage and specific composition of the mineral fraction, (2) pyrolysis process conditions, (3) biochar particle size and delivery system, and (4) biochar age (Joseph et al. 2010). Fresh biochars can have net positive or net negative surface charge, but typically have initially low CEC compared to soil organic matter on a mass basis. Notably, there is an initially measurable anion exchange capacity which disappears over time. In time, biochars (those produced at or below 600–700 °C) seem to oxidize rapidly and attain greater amounts of CEC (Cheng et al. 2008; Lehmann 2007; Lehmann et al. 2011; Nguyen et al. 2010). At higher temperatures of up to 1,000 °C, biochars become mainly hydrophobic and do not sorb appreciable amounts of nutrients or polar organic substances (Lehmann et al. 2011). For all feedstocks, pH increases with greater pyrolysis temperature. Over time, the pH of biochars may change and either decrease or increase depending on the type of feedstock. Typically, biochars with high mineral ash content such as manures or crop residues have greater pH values than those with lower ash contents such as mineral-poor woody materials. The driving force behind a pH decrease is the oxidation of C to form acidic carboxyl groups, whereas the increase in pH is likely related to the dissolution of alkaline minerals. Surface area is governed by two main factors: pyrolysis temperature and feedstock. Independent of the feedstock the specific surface area increases with increasing biochar production temperature (Table 17.1). The value of the specific surface area for the same temperature can vary significantly depending on the feedstock, e.g., at 600 °C the specific area for biochar from pine needles was 207 $m^2 g^{-1}$, for oak wood 642 $m^2 g^{-1}$, for corn stover 527 $m^2 g^{-1}$, and for soybean stalk 179 $m^2 g^{-1}$ (Tang et al. 2013).

Additionally to the physical and chemical properties of biochar, important for the interactions between biochar and contaminant are the soil properties and local environmental conditions as well as the chemical and physical properties of the contaminants themselves. Surface area and pore volume

Table 17.1 Effect of biochar production temperature on sorption of organic pollutants

Feedstock	Temp. (°C)	Surface area ($m^2\ g^{-1}$)[a]	Contaminant	Effect	Reference
Pine needle	100 400 600 700	0.65 112.4 206.7 490.8	Naphthalene, nitrobenzene *m*-dinitrobenzene	Increase of sorption from 100 to 700 °C	Chen et al. (2008)
P. radiata wood	350 700	164 187	Phenanthrene	K_d[b] increased by a factor of up to 51 K_d[b] increased by a factor of up to 700	Zhang et al. (2010a, b)
Red gum wood (*Eucalyptus* spp.)	450 850	27 566	Diuron	Sorption of soils increased by up to 80-fold Sorption of soils increased by up to 125-fold	Yu et al. (2006)
Cotton straw (*Gossypium* spp.)	450 850	3.9 158.8	Chlorpyrifos (C) Fipronil (F)	Plant uptake reduced by 56 % (C) and 20 % (F) Plant uptake reduced by 81 % (C) and 52 % (F)	Yang et al. (2010)
Red gum wood (*Eucalyptus* spp.)	450 850	27 566	Chlorpyrifos (C) Carbofuran (B)	Plant uptake reduced by 30 % (C) and 33 % (B) Plant uptake reduced by 90 % (C) and 75 % (B)	Yu et al. (2009)
P. radiata wood	350 700		Terbuthylazine	K_d[b] increased by a factor of 3.7 K_d[b] increased by a factor of 64	Wang et al. (2010)
Pine needles	100 300 400 700	0.65 19.95 122.4 490.8	Naphthalene Phenanthrene Pyrene	Increase of sorption capacity with increasing pyrolysis temperature	Chen and Yuan (2011)
Pig manure	350 700	23.8 32.6	Carbaryl Atrazine	Sorption capacity increased with increasing pyrolysis temperature	Zhang et al. (2013b)

[a] BET specific surface
[b] Sorption coefficient estimated for Freundlich sorption isotherm

may change upon contact with soil by pore clogging from sorbed organic and mineral material or, conversely, possibly by mineralization of volatile matter that may be blocking pores (Lehmann et al. 2011), thus changing the sorption behavior of TE and organic contaminants.

17.4 Potential of Biochar as a Soil Amendment for Contaminated Soils

17.4.1 Biochar as an Amendment for TE-Contaminated Soils

17.4.1.1 Biochar, TE, and Soil Characteristics Affecting TE Sorption

Surface functional groups of biochar (volatile matter, pH_{pzc}, O:C and N:C ratios) play a governing role in TE sequestration, while a minor role is played by BET surface area, fixed carbon, and ash content (Uchimiya et al. 2011b). In order to achieve a long-term TE stabilization, it is furthermore important that the biochar should itself have high stability (high fixed carbon content) (Uchimiya et al. 2011a). The main parameter determining biochar stability and metal ion-coordinating functional group content is the pyrolysis temperature. With increasing temperature, the feedstock (plant biomass) undergoes dehydration and depolymerization, favoring the formation of graphitic structures (Keiluweit et al. 2010). These in turn increase recalcitrance of biochar towards microbial and chemical degradation (Zimmerman 2010). However, the O/C ratio, which is positively correlated (Uchimiya et al. 2011a), with the ability to retain TE, decreases with increasing temperature (Uchimiya et al. 2011b). Thus, the pyrolysis temperature has to be chosen appropriately to obtain a good balance between TE stabilization and biochar stability.

The application of biochar promotes an increase, in absolute terms, of the negative charge of the soil (Jiang et al. 2012), thus increasing the cation exchange capacity (CEC) of the soil (Fellet et al. 2011; Sohi et al. 2010). The increased CEC is responsible for the adsorption capacity of soil for cations. Uchimiya et al. (2011a) proved that biochar in polluted soils can adsorb TE via complexation by functional groups, thus reducing their bioavailability. This in turn leads to a reduced uptake of TE by the plants and consequently a reduction of their transfer to the food chain.

Lu et al. (2012) and Zhang et al. (2013b) have summarized possible mechanisms which could be involved in the stabilization of TE in soils (Fig. 17.1). Taking Pb^{2+} as an

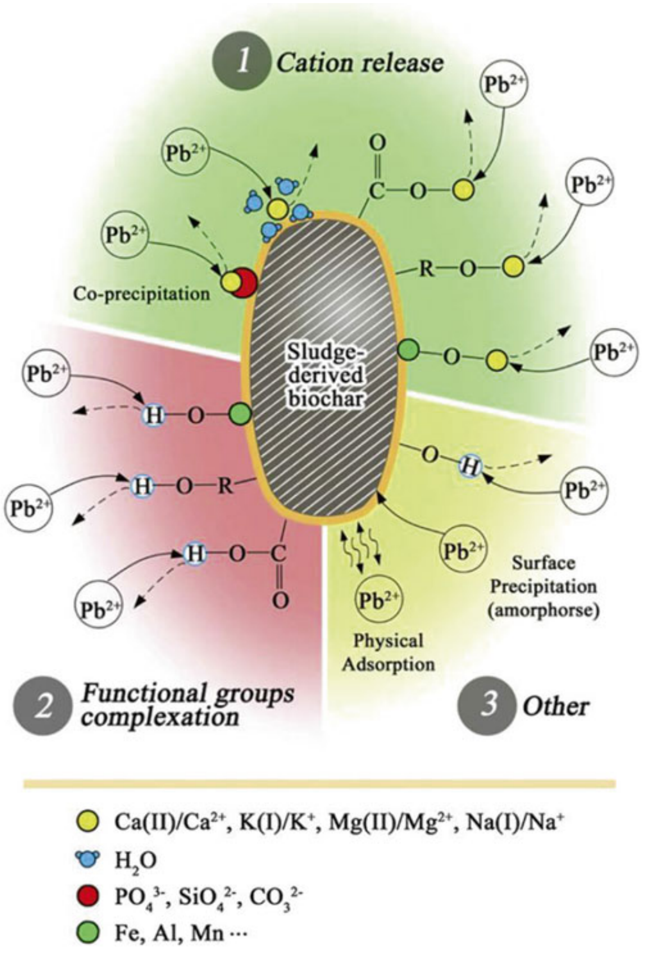

Fig. 17.1 Conceptual illustration of Pb adsorption mechanism on SDBC, including (1) metal exchange with Ca^{2+} and Mg^{2+}, attributing to coprecipitation and inner sphere complexation with complexed humic matter and mineral oxides of SDBC; (2) surface complexation with free carboxyl and hydroxyl functional groups and inner sphere complexation with the free hydroxyl of mineral oxides and other surface precipitation; (3) other inner sphere complexation with the free hydroxyl of mineral oxides and other surface precipitation (from Lu et al. 2012)

example, the authors proposed various mechanisms for Pb^{2+} sorption by sludge-derived biochar that could include (1) the TE exchange with Ca^{2+}, Mg^{2+}, and other cations associated with biochar, attributing to coprecipitation and inner sphere complexation with complexed humic matter and mineral oxides of biochar, (2) the surface complexation of TE with different functional groups and inner sphere complexation with the free hydroxyl of mineral oxides and other surface precipitation, and (3) the physical adsorption and surface precipitation that contribute to the stabilization of Pb^{2+} (Lu et al. 2012; Zhang et al. 2013b).

In order to improve the metal ion-coordinating ability of biochar, its treatment has been considered. Treatments such as oxidation with specific reagents, oxygen, or ozone level during activation were tested and proved to be effective in increasing the adsorption effect of biochar (Tang et al. 2013). Furthermore, the composting of biochar induces a higher sorption of Cu(II) due to the uptake of compost-derived organic matter (Borchard et al. 2012).

The efficiency of biochar to stabilize TE in soil is also influenced by soil properties. In acidic, low CEC, low TOC sandy soils, for example, biochars are more effective in stabilizing Cu (Uchimiya et al. 2011b). In contrast, in soils with high organic matter content, the Cu concentration increased. This could be the result of Cu complexation by organic matter or due to sorption of organic matter on biochar.

17.4.1.2 Applications

Charcoal has a long history of serving as an efficient filter medium for removing impurities from aqueous systems (Ippolito et al. 2012). Consequently, various studies have explored the potential use of biochar as a medium for TE sorption and quality improvement of contaminated soils. Jiang et al. (2012) showed that the incorporation of rice straw biochar (3–5 %) reduced the acid-extractable Cu and Pb by 19.7–100 % and 18.8–77.0 %, respectively. Similarly, Beesley et al. (2010) showed that the amendment of hardwood-derived biochar on a Cd- and Zn-contaminated sediment-derived canal bank soil significantly reduced the concentrations of both metals in pore water during 60 days field exposure. Fellet et al. (2011) amended mine tailings with 0–10 % orchard prune-derived biochar, finding that, as well as increasing pH and CEC, biochar reduced bioavailable (DTPA-extractable) concentrations of Cd, Pb, and Zn (Table 17.1).

The decrease in mobility and bioavailability of soil TE by biochar application has in turn an effect on TE plant uptake. Méndez et al. (2012) assessed the effect of biochar from sewage sludge on solubility and bioavailability of several TE in a Mediterranean agricultural soil. Biochar application decreased the plant-available Cu, Ni, Zn, and Pb; the mobile forms of Cu, Ni, Zn, Cd, and Pb; and also the risk of leaching of Cu, Ni, Zn, and Cd (Table 17.2). Park et al. (2011) reported that both chicken manure- and green waste-derived biochars significantly reduced Cd, Cu, and Pb uptake by Indian mustard. The study also found that the reduction of the plant TE concentrations increased with increasing biochar application rates except for Cu. Karami et al. (2011) used green waste compost and biochar mixture amendments to evaluate their assistance in regulating the mobility of Cu and Pb and the resultant uptake of them into ryegrass (*Lolium perenne* L. var. Cadix). As a result, with biochar and compost addition, both Cu and Pb levels in ryegrass (*Lolium perenne* L. var. Cadix) decreased.

When biochar is applied to soil for TE immobilization not only the biochar characteristics have to be taken into account but also the type of TE present in the contaminated soil. Namgay et al. (2010) reported that biochar application resulted in increased extractable As and Zn soil concentrations. The concentration of extractable Pb however was

Table 17.2 Effect of biochar on sorption and availability of TE

Feedstock	Temp (°C)	Contaminant	Effect	Reference
Various hardwoods	400	As	Increase of As concentration in soil pore water	Hartley et al. (2009)
Eucalyptus saligna wood	550	As, Cd, Cu, Pb, Zn	Sorption in order: Pb > Cu > Cd > Zn > As Decrease of As, Cd, Cu, Pb plant uptake	Namgay et al. (2010)
Various hardwoods	450	As, Cd, Cu, Zn	Tenfold decrease of Cd in pore water	Beesley et al. (2010)
Orchard prune residue	500	Cd, Cr, Cu, Ni, Pb, Zn	Reduction of DTPA-extractable Cd, Pb, and Zn	Fellet et al. (2011)
Chicken manure, green waste	550	Cd, Cu, Pb	Reduction of NH_4NO_3-extractable Cd, Cu, and Pb concentrations of soils	Park et al. (2011)
Sewage sludge	550	Cd, Cu, Ni, Pb, Zn	Reduction of DTPA-extractable Cu, Ni, Zn, and Pb	Méndez et al. (2012)
Rice straw	ND	Cd, Pb	Decrease of acetic acid (0.11 M)-extractable Cd (19.7–100 %) and Pb (18.8–77.0 %)	Jiang et al. (2012)

decreased, for Cu it did not change, and for Cd it showed an inconsistent trend. They also found that sorption of TE on biochar with initial loadings up to 200 µmol at pH 7 occurred in the order: Pb > Cu > Cd > Zn > As.

Although many studies showed that biochar can reduce TE mobility and their plant uptake, majority of these studies were conducted either under controlled laboratory conditions or were pot trials and greenhouse experiments. Pot experiments do not reflect field conditions; thus, before a full-scale application of biochar is attempted, field trials are needed to confirm or redefine what has been discovered and observed in experiments under control conditions. However up to date, only very few field trials have been designed and performed.

17.4.1.3 Risks

The application of biochar for agricultural or environmental purposes (C-sequestration, remediation, etc.) has great potentials but may not be free of adverse effects. During the process of pyrolysis, the formation of complex molecules such as PAH can occur which can pose a risk (see Sect. 17.4.2.3). Furthermore, due to dehydration and volatilization of easily volatilized compounds such as C, the concentration of less readily volatile compounds such as TE increases. Koppolu et al. (2003) observed an increase of Ni, Zn, Cu, Co, and Cr concentration by 4–6 times in a synthetic hyperaccumulator biomass.

Biochars may also have an indirect effect on the behavior of TE in soil or wastes. In a study by Hartley et al. (2009), the application of biochar to soil increased As concentrations in soil pore water. The As mobilization was the result of soil pH increase due to the application of biochar. Similar results were obtained by Beesley et al. (2013); however, As plant uptake was reduced and toxicity-transfer risk was negligible. Leaching therefore appears to be the most probable immediate consequence of biochar addition to As-contaminated soils.

In conclusion biochar may (1) be a point source of TE and (2) change soil properties resulting in the mobilization of soil TE.

17.4.2 Biochar as an Amendment for Soils Contaminated with Organic Pollutants

17.4.2.1 Biochar, Organic Pollutants, and Soil Characteristics Affecting Sorption of Pollutants

For the sorption of organic contaminants, very specific physical and chemical biochar properties, such as hydrophobicity, pore volume, and surface area, influence the sorption capacity and the sorptive properties. A higher biochar surface area facilitates higher sorption of chemicals such as pesticides. For example, chars produced from wheat residue at 500–700 °C, which were well carbonized and with a relatively high surface area (>300 m^2 g^{-1}), exhibited higher sorption capability for organic compounds than chars formed at 300–400 °C which had a lower surface area (<200 m^2 g^{-1}) (Chun et al. 2004). One has to keep in mind though that surface area measured by gas adsorption, however, is influenced by micropores (nm scale) that are not relevant to plant roots, to microbes, or to the mobile soil solution.

With increasing temperature the surface properties change and thus also the sorption mechanisms change. Biochars produced at high temperatures are nearly completely carbonized and exhibit a relatively high surface area, little organic carbon, low oxygen content, and low content of polar surface groups, while biochars obtained at low temperatures are only partially carbonized and show the opposite surface properties in each of the above respects (Chun et al. 2004). The carbonized organic matter is expected to behave as an adsorbent and the non-carbonized organic matter as a partition (absorption) phase; adsorption is typically nonlinear, whereas partition is essentially linear. In this sense, the sorptive uptakes of biochars are determined by the relative carbonized and non-carbonized fractions and their surface and bulk properties. Thus, sorption mechanisms of biochars are evolved from partitioning dominant at low pyrolytic temperatures (200–400 °C) to adsorption dominant at higher pyrolytic temperatures (500–700 °C) (Chen et al. 2008).

In general, sorption of comparatively polar substances, such as catechol or humic acids, to biochars increases in the range of 400–650 °C due to greater surface area. At even higher temperatures of up to 1,000 °C, carbons are mainly hydrophobic and do not sorb appreciable amounts of nutrients or polar organic substances, such as sugars. Such high-temperature carbons sorb mainly nonpolar or weakly polar organic solutes, notably those bearing aromatic structures (Lehmann et al. 2011). Thus, the sorption to biochars does not only depend on biochar properties but also on the properties of the adsorbate itself, e.g., polarity, hydrophobicity, aromaticity, etc.

The sorption of organic contaminants on biochar can be influenced by soil organic matter, by available ions, and/or by the competition between organic contaminants. The interactions between biochars and other soil constituents such as natural organic molecules and clay minerals contribute to the aging of biochars. It has been suggested that natural organic matter can block the micropores of biochars and suppress sorption of organic contaminants (Zhang et al. 2013b). This is supported by Wang et al. (2010) who observed that biochar-enhanced soil adsorption of herbicide terbuthylazine is much greater in a soil with low organic matter than in a soil with higher organic matter content. The presence of ions such as Cu^{2+} can extensively affect the sorption of various organic contaminants as shown by Chen et al. (2007). The authors found that co-adsorption of Cu^{2+} (50 mg L^{-1}) decreases sorption of both polar compound (2,4-dichlorophenol) and nonpolar compounds (1,2-dichlorobenzene and naphthalene), by 30–60 %. Furthermore, the presence of other organic contaminants can hinder the sorption of the target contaminant. Yang and Sheng (2003b) showed that the adsorption of diuron was reduced by 10–30 % due to surface competition with atrazine.

17.4.2.2 Applications

The ability of chars binding organic contaminants such as the precursor of atrazine, s-triazine, was stated by Hilton and Yuen (1963). They discovered that the retained sorptivity of many Hawaiian soils for s-triazines was a result of the soil chars arising from burning of sugarcane trash. Since then biochars have been applied for the sorption of various organic compounds such as pesticides, dioxins, PAHs, PCBs, and chlorophenols.

Several studies have investigated the effect of biochar on pesticides such as diuron or atrazine as they are applied to soils on a great scale. Biochars produced from wheat and rice residues were reported to be up to 2,500 times more effective than soil in sorbing diuron herbicide (Yang and Sheng 2003a). Thus, biochar addition to soil has been shown to generally decrease the bioavailability of pesticides and increase the biomass of targeted plants (Table 17.1). Yu et al. (2006) reported that the addition of biochar (0.1–5 %) increased the sorption capacity of biochar-amended soils for diuron by 7–80 times for biochar produced at 450 °C and by 5–125 times for biochar produced at 850 °C, in comparison to that of biochar-free soil. In consequence, plant uptake was decreased, while plant survival rate and plant biomass were increased. The addition of cotton straw-derived biochars (1 %, 850 °C) reduced the total plant uptake of chlorpyrifos by 81 % and that of fipronil by 52 %, compared to the control treatment (Yang et al. 2010). Yang et al. (2006) showed that at a diuron application rate of 1.5 kg ha^{-1}, a biochar soil content of only 0.05 % increased the fresh weight of barnyard grass fourfold (Fig. 17.2). The decreased bioavailability and plant uptake may reduce pesticide residues in crops; however, since pesticides are applied on agricultural soils in order to control target pests or weeds, they need to be bioavailable to be effective. Thus, a decreased efficacy of pesticides due to application of biochar is undesirable.

The addition of biochar to soil also has an effect on the availability of PAHs. The application of biochar (0.5 %, 700 °C) to soil increased the sorption of phenanthrene significantly (K_d 4.2 $\times 10^3$ L kg^{-1}) compared to the non-amended soil (K_d 8.6 $\times 10^2$ L kg^{-1}) (Zhang et al. 2010b). These results are concurrent with the results by Chen and Yuan (2011) who showed that biochar addition (2 %, 700 °C) to soil enhanced the sorption of phenanthrene by up to 85.5 times. This decrease in phenanthrene availability can also result in a decreased phenanthrene bioaccumulation. Biochar reduced bioavailable (cyclodextrin-extractable) soil concentrations of 2-, 3-, 4-, and 5-ring PAHs from 276 to 182 mg kg^{-1}. Subsequently, PAH concentrations were also reduced in the earthworm *Eisenia fetida* by up to 45 % (Gomez-Eyles et al. 2011).

17.4.2.3 Risks

Although soil biochar application has been shown to bind organic contaminants in soil, there are still many unknowns, with regard to sorption stability and degradation of organic contaminants. Furthermore, biochar could also be the cause of soil contamination, as biochar may contain considerable amounts of PAH. For instance, Brown et al. (2006) quantified concentrations from 3 to 28 mg kg^{-1} in synthetic wood char (sum of 40 individual PAHs), Jonker and Koelmans (2002) reported a charcoal to contain 45 mg kg^{-1} PAHs (sum of 13 individual compounds), Schimmelpfennig and Glaser (2012) found mean concentrations that ranged from 3.9 (Pyreg) to 2,945 mg kg^{-1} (wood gasifier), and in the biochars analyzed by Hilber et al. (2012), PAH concentrations ranging from 9.1 to 355 mg kg^{-1} were found. There have also been reports such as by Singh et al. (2010) who determined "negligible" concentrations of <0.5 mg kg^{-1} PAHs and concluded that these low levels "make these biochars safe for soil application." However, Singh et al. (2010) used a 12 h Soxhlet extraction with dichloromethane, which may not be

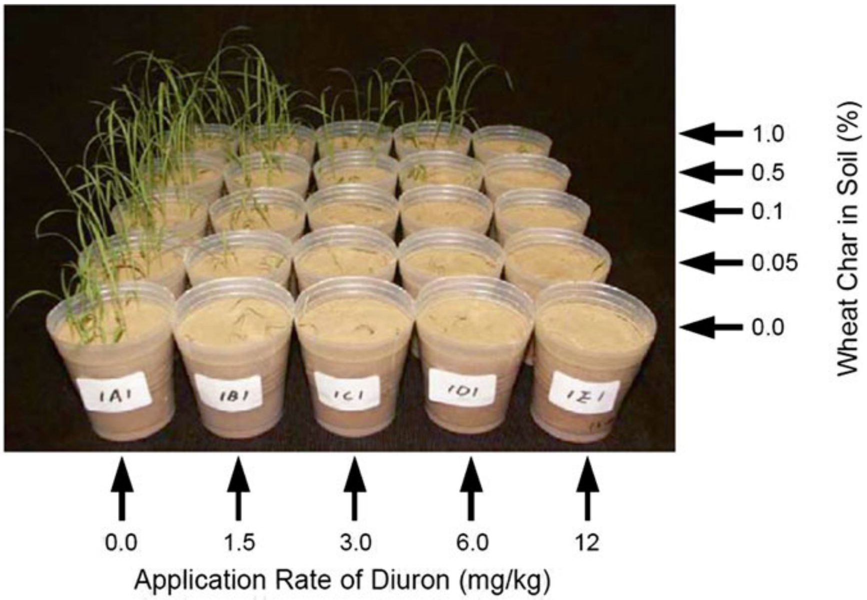

Fig. 17.2 Photograph showing barnyard grass growth in soils as a function of diuron application rate and wheat char content 4 weeks after planting (from Yang et al. 2006)

the ideal extraction solvent (Hilber et al. 2012), thus showing that previously used generic methods might not be suitable for PAH extraction from biochars and new methods specifically adapted and validated for PAH analysis in biochar have to be developed.

Biochars have displayed a high sorption capacity for organic pesticides, thus reducing their availability in soils (Graber et al. 2012; Graber et al. 2011; Nag et al. 2011; Yang et al. 2010; Yu et al. 2009). This can be beneficial especially in an agricultural context as it will reduce the uptake of pesticides in crops. On the other hand this high sorption capacity could also have detrimental effects on agricultural productivity, as it could reduce the efficiency of herbicides, or even render soil-applied pest control agents ineffective, thus making higher application rates of these chemicals necessary. In soils containing 1 % biochar, the application rate of the herbicide atrazine had to be increased fourfold to achieve the same effects as in soils without biochar addition (Nag et al. 2011). Yang et al. (2006) showed that herbicidal efficacy of diuron to barnyard grass was completely lost when the biochar content in soil was 0.5 % or higher (Fig. 17.2). In soils amended with biochars, which have high specific surface area, decreased herbicide phytoavailability cannot be offset by increased doses of soil-applied herbicides. Thus, biochars with low specific surface area are more suitable to meet the needs of pest control (Graber et al. 2012). Not only decrease in efficacy but also increase in the half-life of organic contaminants, due to their sorption on biochars, has been found. In a study by Yang et al. (2010), the addition of biochar (1 %, 850 °C) increased the half-life of chlorpyrifos and fipronil by up to 161 % and 129 %, respectively. Similar results were reported by Zhang et al. (2004) and Yu et al. (2009) who stated that incorporation of a 1 % biochar in soil inhibited the microbial degradation of benzonitrile (Zhang et al. 2004) by >40 % and of chlorpyrifos and carbofuran (Yu et al. 2009) by approximately 50 %. Most of the experiments were performed with freshly produced biochar, and it is not clear whether the effectiveness of biochars in sorbing pesticides/herbicides would increase or decrease with time and what implications it would have on the accumulation of pesticide residues or herbicide efficacy in char-amended soils. Martin et al. (2012) showed that freshly produced biochars are likely to lose their sorption capability over time. Thus, the herbicide application rate may have to be adjusted every cropping season until biochars lose their extraordinary herbicide deactivation capacity. Also Zhang et al. (2010b) stated that aging decreased the overall sorption of phenanthrene in biochar-amended soils. On the other hand many studies have shown that organic compounds become less bioavailable (lower bioaccumulation, biodegradation, or toxicity) with increased residence time in soils or sediments (Kookana 2010). It is difficult to draw a consensus about the sorption effect of a biochar on an organic compound, as the sorption of herbicides to biochar depends on the herbicide, the soil, and the biochar properties and can thus vary greatly (Nag et al. 2011).

17.5 Sustainability Aspects: Biochar Production

Biochar is promoted under the label of sustainability. Sustainability is based on the principle that the requirements of the present must be satisfied without compromising the ability of future generations to meet their own needs (WCED 1987).

The possible contamination of soils through the input of PAHs, PCBs, dioxins, furans, and/or TE originating from biochar, the origin of the feedstock, and in several cases the stated increase in emissions of greenhouse gases from biochar-amended soils make the sustainability of biochar questionable.

To date, the production of biochar is not yet standardized or certified, and its registration not yet institutionalized, but there are initiatives which aim at the reduction of organic and inorganic contaminant concentrations in biochar. Since January 2012 biochar producers can receive a European Biochar Certificate issued by the control board q.inspecta (Frick, Switzerland). Premium biochar should contain <4 mg kg^{-1} of the Σ16 EPA PAH and basic biochar <12 mg kg^{-1} dry matter (DM). The TE thresholds are 150 mg kg^{-1} DM for Pb, 1.5 mg kg^{-1} DM for Cd, 90 mg kg^{-1} DM for Cr, 100 mg kg^{-1} DM for Cu, 1 mg kg^{-1} DM for Hg, and 400 mg kg^{-1} DM for Zn. Also PCB content must be below 0.2 mg kg^{-1} DM, and levels of dioxins and furans must be below 20 ng kg^{-1}. Additionally, on May 29, 2013, the International Biochar Initiative (IBI) launched the IBI Biochar Certification Program. The range of maximum allowed thresholds for PAH are 6–20 mg kg^{-1} DM, for PCB 0.2–0.5 mg kg^{-1} DM, and for dioxins and furans 9 ng kg^{-1}. For TE the maximum allowed thresholds are for Cd 1.4–39 mg kg^{-1} DM, for Cr 64–1,200 mg kg^{-1} DM, for Cu 63–1,500 mg kg^{-1} DM, for Pb 70–500 mg kg^{-1} DM, for Hg 1–17 mg kg^{-1} DM, and for Zn 200–700 mg kg^{-1} DM. Values such as the proposed thresholds for PAHs will be probably difficult to meet if improved extraction methods such as the one proposed by Hilber et al. (2012) are followed. In order however to reach a realistic evaluation of the PAHs in biochar and their potential threat, the assessment of the bioavailable fraction of the PAHs and other organic contaminants is necessary.

The origin of the biomass intended for biochar plays a pivotal role in the sustainability of biochar. The use of crop residues such as corn stover or wheat straw has been suggested and used for biochar production. Crop residues however, which are left on the soil surface, are vital for soil protection from wind erosion, as well as SOC management. If residues are completely removed, the risk of soil erosion for plowed soils and even no-tillage soils can be significantly increased (Blanco-Canqui 2010). As a compromise, Blanco-Canqui and Lal (2009) propose that a partial removal of 25 % of stover may be the threshold removal rate from erosion-prone soils. However, the removal of 25 % of stover needs a careful and objective consideration because although it might be enough to avoid soil erosion, it might be too low to maintain SOC content. Wilhelm et al. (2007) found that the amounts of corn stover needed to maintain SOC, and thus productivity, exceed by far the amounts needed to control water and wind erosion. Additionally, possible future large-scale appropriations of land, for global climate change mitigation benefits and profits from carbon markets, have to be viewed critically, or else biochar production will be involved in the similar "biomass production vs. food" discussions as biofuels are involved in.

Biochar has mainly received so much attention due to its potential to alleviate climate change by sequestrating C and reducing greenhouse gas emissions (Lehmann et al. 2008). Although most of the studies verify its potential, there are also noticeable studies which show otherwise. Zhang et al. (2010a) stated an increase of CH_4 release from rice paddy soils after biochar application. Wardle et al. (2008) showed in a 10-year experiment that fire-derived charcoal promotes loss of boreal forest humus and that this is associated with enhancement of microbial activity by charcoal. This result shows that charcoal-induced losses of belowground carbon in forests can partially offset the benefits of charcoal as a long-term carbon sink.

17.6 Conclusions

Biochar reduces the availability and plant uptake of inorganic and organic contaminants, thus reducing their toxic potential and the risk that they are posing. Biochar itself however can pose a risk, as depending on the feedstock and the production conditions, it can contain high concentrations of TE as well as PAH, PCB, and dioxins. Furthermore, the degradation of the sorbed organic contaminants is also reduced, thus prolonging the remediation of the soils. The stability of the TE sorption on biochar with time has not been assessed, thus it is unclear how the bioavailability of TE will change with time.

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Phytoremediation of RDX

18

Neerja Srivastava

18.1 Introduction

The explosive compounds hexahydro-1,3,5-trinitro-1,3,5triazine (RDX) and 2,4,6-trinitrotoluene (TNT) are significant environmental pollutants, contaminating an estimated 16 million hectares of military land in the USA alone (US Defense Science Board Task Force 1998; US General Accounting Office 2004). Contamination on training ranges mainly arises from incomplete detonation of munitions. The concentration of explosive pollutants is heterogeneous with hot spots of between 100 and 1,000 mg/kg (Talmage et al. 1999; Jenkins et al. 2006), although the majority is below this level. Pollution has also historically arisen from the manufacture and storage of explosives. Although RDX is less toxic than TNT (Woody et al. 1986; Burdette et al. 1988; Kucukardali et al. 2003), it is still classified as a possible human carcinogen by the Environmental Protection Agency (EPA). Within the soil, RDX is highly mobile and readily leaches into groundwater with the potential to pollute subsequent waterways. This route of pollution has led to the contamination of a sole source aquifer below Massachusetts Military Reservation on Cape Cod (USA) (Clausen et al. 2004). Studies have shown that RDX is readily taken up and translocated to the aerial tissues of plants (Vila et al. 2007) and is reduced to hexahydro-1-nitroso-3,5-dinitro-1,3,5triazine and hexahydro-1,3-nitroso-5-nitro-1,3,5-triazine in the leaf, with subsequent mineralization of the heterocyclic ring which requires light (Van Aken et al. 2004a). However, despite high uptake rates, plants have inherently low abilities to degrade RDX (Best et al. 1999a; Winfield et al. 2004). Microorganisms with the ability to degrade RDX have been isolated, including *Rhodococcus rhodochrous* strain 11Y (Seth-Smith et al. 2002). The RDX-degrading ability of this bacterium, encoded by XplA, has been shown to be the result of a cytochrome P450, which catalyzes the aerobic degradation of RDX to 4-nitro-2,4-diazabutanal (NDAB), nitrite, and formaldehyde, whereas, anaerobically, methylene dinitramine is produced instead of NDAB (Jackson et al. 2007) (Fig. 18.1). *Arabidopsis thaliana* plants expressing XplA have been shown to remove RDX from a saturating (180 μM) solution (Rylott et al. 2006). This concentration is more than three times that measured in wastewater from manufacturing sites (Jackson et al. 1978), suggesting that this approach could be successfully incorporated into a phytoremediation program. Soil studies have demonstrated that plant biomass is enhanced in XplA-expressing plants growing on RDX-contaminated soil compared with uncontaminated soil, indicating that XplA-expressing plants can utilize the nitrite released from the degradation of RDX as a nitrogen source for growth (Rylott et al. 2006). Plants co-expressing XplA and XplB, the partnering reductase for XplA in *Rhodococcus*, exhibited an additional, up to 30-fold, increase in the rate of RDX removal (Jackson et al. 2007; Rylott et al. 2011).

18.2 Explosives

Explosives are materials which, when suitably initiated, result in the rapid release of energy. Detonation of the solid explosive generates expanding hot gases. This expansion creates a shock wave which exerts high pressures on the surroundings, causing an explosion. Explosives generally have high nitrogen and oxygen contents which aid the formation of the gaseous products, typically including carbon dioxide, carbon monoxide, oxygen, nitrogen, and water vapor. The development of explosives has sought to provide both greater power and greater control. Mass production of some of these compounds over the last century has led to extensive contamination of land, which now requires remediation (Seth-Smith 2002).

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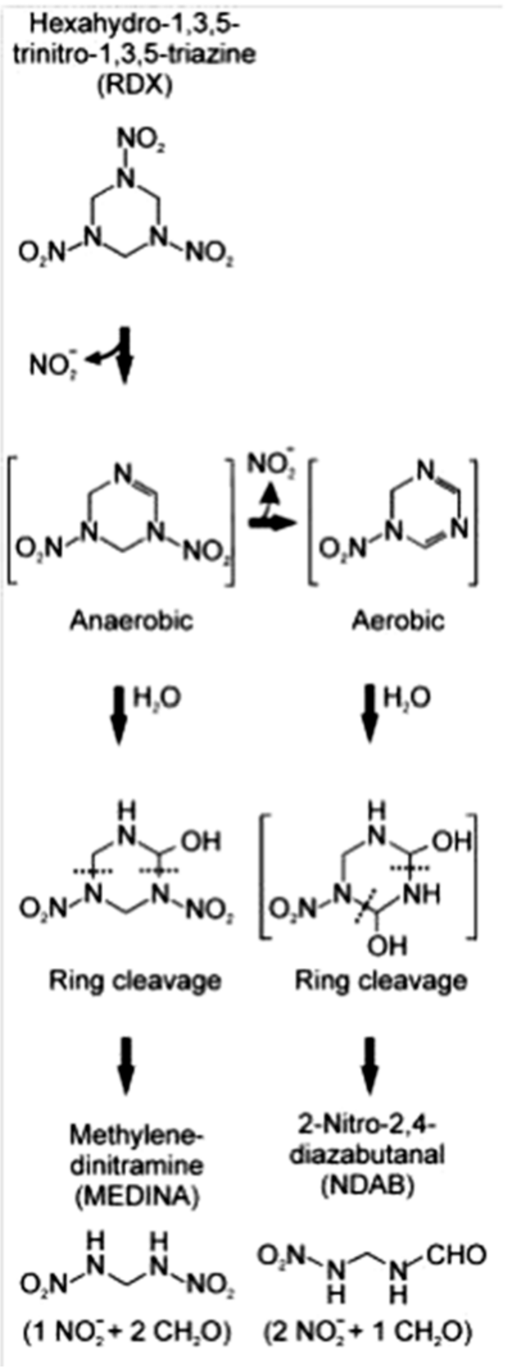

Fig. 18.1 Detoxification pathways of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

18.3 RDX

The first nitramine explosive (characterized by N–NO_2 groups) to be developed was hexahydro-1,3,5-trinitro-1,3,5-triazine, synthesized by Hans Hemming in 1899. In 1920, it was patented as an explosive, and its further development at the War Department in Woolwich, UK, led to its naming as Royal Demolition Explosive or RDX. It is as powerful as PETN and GTN with power index of 159 (Bailey and Murray 2000), but much less sensitive. It is commonly used in explosive mixtures including cyclotol, which comprises 60 % RDX and 40 % TNT, and composition C-4, which comprises 91 % RDX with plasticizers. RDX is currently the most widely used military explosive.

The effects of RDX on mammals are generally characterized by convulsions. Supplying RDX to both dogs and rats results in irritability and convulsions as symptoms of chronic toxicity, and death in the rats was associated with congestion in the gastrointestinal tract and lungs (Burdette et al. 1988; Von Oettingen et al. 1949). Oral LD50 values for RDX in rats are 0.07–0.12 g/kg (Smith-Simon and Goldhaber 1999). RDX toxicity can also cause weight loss associated with a reduction of food intake in rats (Levine et al. 1981). RDX has also been used as a rat poison (Osmon and Klausmeier 1973). There have been several reported cases of RDX toxicity in humans. Workers in RDX factories in Germany, Italy, and the USA have been seen to suffer with symptoms like convulsions, unconsciousness, vertigo, and vomiting after exposure, usually through the inhalation of RDX powder (Kaplan et al. 1965). A study on a child who ingested plasticized RDX and developed seizures found that RDX can transport easily into the central nervous system (Woody et al. 1986). Various reports of men purposefully chewing the plastic explosives C-4 or Semtex, which contain high levels of RDX, show them to develop grand mal seizures with associated headaches or amnesia (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995). Recovery from these episodes is complete, and no recurrence of symptoms is seen in the absence of further exposure. Tests using freshwater invertebrates, green algae, fathead minnow, earthworm reproduction, and luminescent bacterium *Vibrio fischeri* have found RDX to be toxic, but less so than TNT (Burton et al. 1994a, b; Drzyzga et al. 1995; Peters et al. 1991; Robidoux et al. 2000). Although studies testing RDX on both *Salmonella* and mammalian cell lines have shown that it is not mutagenic (George et al. 2001; Lachance et al. 1999), it is designated as a class C carcinogen (Rosenblatt et al. 1991).

18.4 Biodegradation of RDX

RDX is much more amenable to biodegradation than its co-contaminating explosive, TNT. With no aromaticity, it appears to be able to undergo several types of reaction. Under anaerobic conditions, reduction of the nitro groups forms nitroso intermediates which subsequently break down further (McCormick et al. 1981). Under aerobic conditions, nitrite has been seen to accumulate (Coleman et al. 1998; Fournier et al. 2002). Unlike the nitrate ester and nitroaromatic explosives, there has been no identification of enzymes responsible for any of the reactions that RDX undergoes. This interesting and fundamental area remains to be investigated.

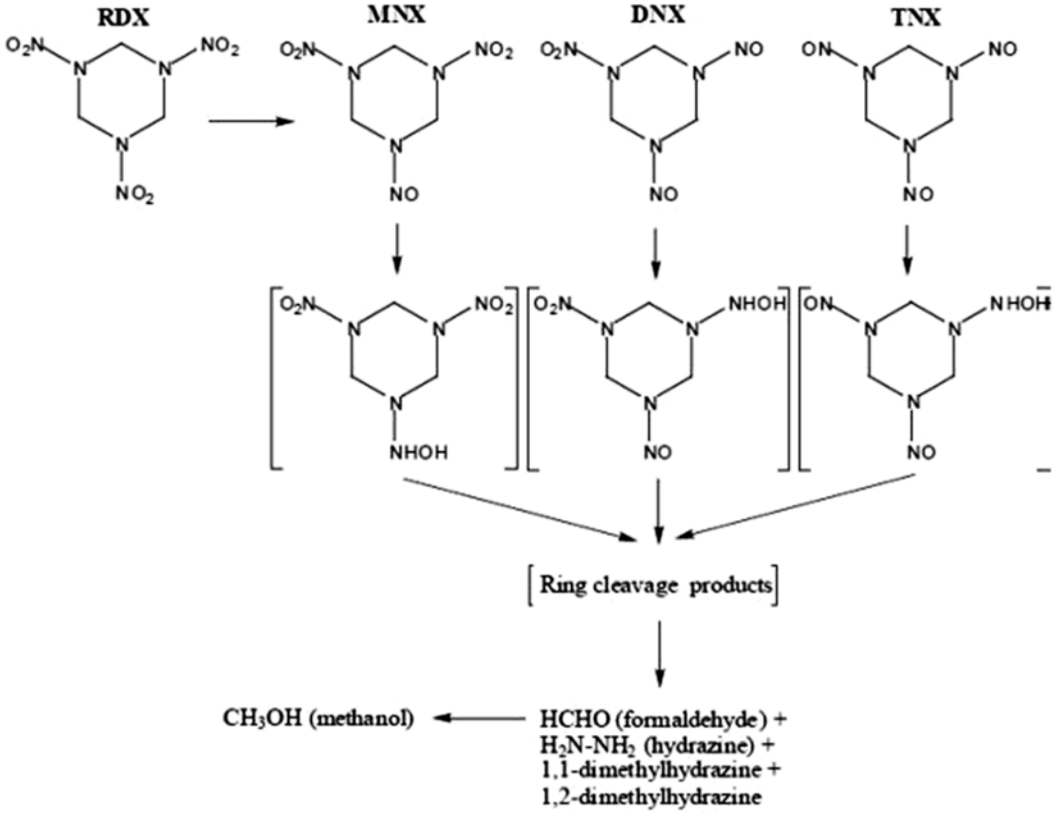

Fig. 18.2 Putative pathway for the anaerobic biodegradation of RDX via nitroso intermediates. Compounds identified include the three nitroso derivatives, formaldehyde, methanol, and hydrazine, and the two dimethylhydrazines. The hydroxylamino derivatives and ring cleavage products are hypothetical intermediates and are shown in *brackets*

18.4.1 Anaerobic Biodegradation of RDX

Biodegradation of RDX was initially studied under anaerobic conditions, and it was thought for a long time that RDX removal could only occur anaerobically (McCormick et al. 1981). RDX removal from culture was first observed in 1973 using a system containing purple photosynthetic bacteria (Soli 1973); the anaerobic photosynthetic activity was thought to be responsible for a possible reduction of the compound. Since then, anaerobic RDX degradation has been observed using microbial consortia from contaminated material and sewage sludge (Funk et al. 1993; Hawari et al. 2000; McCormick et al. 1981; Shen et al. 1998, 2001; Toze and Zappia 1999) and has also been performed under nitrate-reducing (Freedman and Sutherland 1998) and sulfate-reducing (Boopathy et al. 1998a, b) conditions. These cultures generally take between 1 week and 2 months to degrade RDX, when supplied at concentrations ranging from 0.015 to 0.17 mM. The most rapid degradation of RDX using anaerobic sludge reported 90 % removal of 0.27 mM RDX within 2 days (Hawari et al. 2000). In addition to this use of mixed cultures, the vast majority being uncharacterized in terms of the microbes present, some investigations have concentrated on anaerobic pure cultures. *Clostridium bifermentans* was the first pure strain capable of the anaerobic degradation of RDX to be isolated (Regan and Crawford 1994). It was purified from an anaerobic consortium and found to be able to remove 0.23 mM RDX to 25 % of its original concentration within 24 h. *Morganella morganii*, which fully removed 0.33 mM RDX within 27 days, was chosen as the most efficient isolate of the three from the family Enterobacteriaceae, which were found to transform RDX under oxygen-depleted conditions (Kitts et al. 1994). Several strains which could biotransform RDX anaerobically were isolated from horse manure, the most effective being *Serratia marcescens* which removed 0.23 mM RDX over 10 days (Young et al. 1997). During this work on RDX-degrading anaerobes, several intermediates and products have been identified, from which pathways of RDX degradation have been put forward. Using sewage sludge as a source of microbes, 0.23 mM RDX was removed from anaerobically incubated nutrient broth over a period of 7 days (McCormick et al. 1981). Analysis of the compounds formed led to the proposal of a pathway involving the production of nitroso intermediates from RDX through sequential reductions of the nitro groups (Fig. 18.2). MNX is produced first, followed by DNX and TNX, all of which were isolated by high-performance liquid chromatography (HPLC) and identified using gas chromatography-mass spectrometry

(GC-MS). Further reduction is hypothesized to create hydroxylamino-substituted derivatives, with subsequent ring cleavage resulting in the observed products: formaldehyde, methanol (CH_3OH), hydrazine ($H_2N–NH_2$), 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine. The hydrazines are known mutagens, but were present only in very low quantities and have not been identified since in anaerobic systems (Hawari et al. 2000). There have also been queries as to whether the hydrazines were correctly identified, and subsequent studies have found them to be unstable (Hawari 2000). The nitroso intermediates have been identified in several other studies during anaerobic RDX degradation (Freedman and Sutherland 1998; Hawari et al. 2000; Kitts et al. 1994; Young et al. 1997). The enzyme responsible may be similar to a type I nitroreductase from *Enterobacter cloacae*, which has been found to oxidize NADPH in the presence of RDX, albeit at a very low rate (68.7 nmol/min/mg protein), indicating that the RDX is being reduced (Kitts et al. 2000).

Further elucidation of intermediates produced from anaerobic RDX degradation with sewage sludge has led to the proposal of a second mechanism of RDX breakdown, which may occur in parallel with the reductive pathway (Hawari et al. 2000). This involves cleavage of the ring directly to methylene dinitramine and bis(hydroxymethyl) nitramine, which break down to formaldehyde and nitramine ($NO_2–NH_2$). Bacteria present in the sludge are then thought to convert these to the end products carbon dioxide, methane (CH_4), and nitrous oxide (Fig. 18.3).

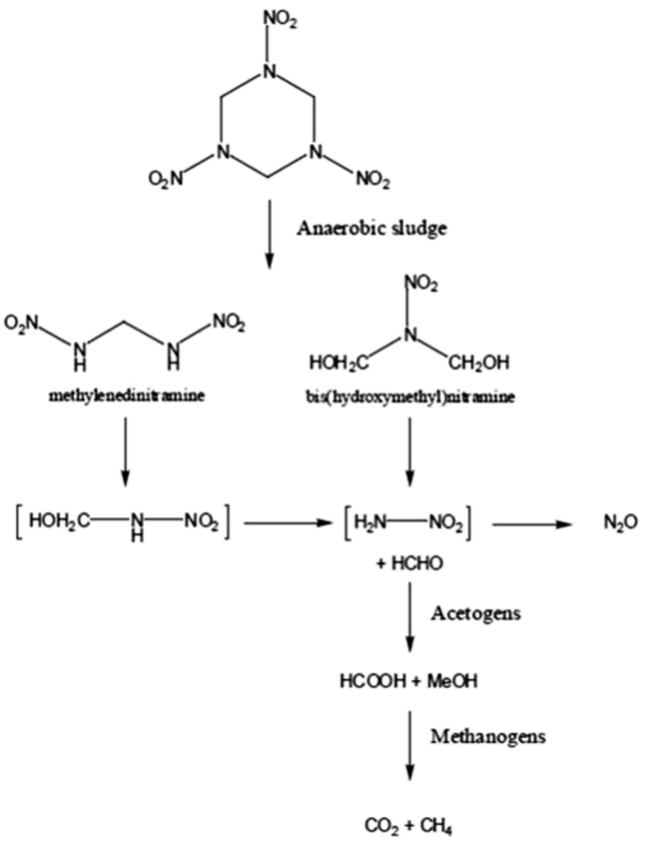

Fig. 18.3 Second putative pathway for RDX degradation by anaerobic sludge. Compounds identified include methylene dinitramine, bis (hydroxymethyl)nitramine, formaldehyde, formate, methanol, nitrous oxide, methane, and carbon dioxide. Two hypothetical intermediates are also shown in *brackets*. Traces of nitrogen gas and nitrite were also detected, along with some soluble, non-extractable degradation products

18.4.2 Fungal Degradation of RDX

Investigations into the fungal biodegradation of RDX have focused on the white rot fungus *Phanerochaete chrysosporium*, which is known to degrade many organopollutants through its nonspecific lignin degrading system. This fungus removed 96 % of 0.125 μM RDX from aerobic liquid culture over 30 days, when the RDX was provided as a sole nitrogen source (Fernando and Aust 1991). Carbon dioxide was liberated from the ring system of RDX throughout the process (determined using ring-labeled 14C-RDX), indicating substantial breakdown of the compound, and possible mineralization. More recent work with *P. chrysosporium* showed that 0.28 mM RDX could be fully degraded over 50 days, with metabolites including nitrous oxide and traces of MNX and methanol (Shermata and Hawari 2000).

18.4.3 Aerobic Biodegradation of RDX

The first reported aerobic degradation of RDX was published in 1983 and identified three pure strains of *Corynebacterium* capable of growing on RDX as a sole nitrogen source (Yang et al. 1983). The fastest of these strains removed 0.18 mM RDX from culture over 32 h. Since then, a consortium of bacteria from contaminated soil was reported to degrade 38 % of 100 mM RDX over 5 days (Thiboutot et al. 1994), and a pure aerobic bacterial strain, also isolated from contaminated soil, was found to be able to remove 0.23 mM RDX from culture over 40 h (Jones et al. 1995). Using this pure strain, the accumulation of an unidentified metabolite was detected using HPLC (Jones et al. 1995), and it has since been tentatively identified as NO_2–NH–NH–CHO, which would indicate that ring cleavage had occurred (Hawari 2000). *Stenotrophomonas maltophilia* strain PB1 removed 0.27 mM RDX over 7 days (Binks et al. 1995), during which two metabolites were identified: C3H9N3O5 and methylene dinitramine (Hawari 2000). The activity was reported to be inducible and to require reducing power provided by sugars (Binks et al. 1995). All these strains grew on RDX when it was supplied as a sole source of nitrogen, and none of them were able to use RDX as a source of carbon. The most thoroughly described aerobic

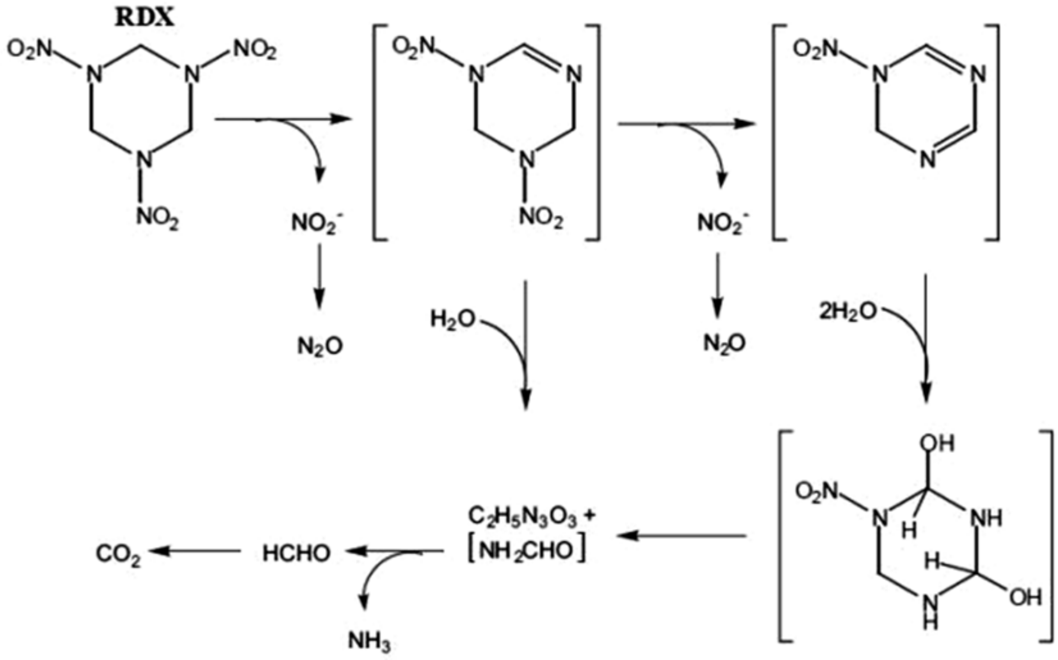

Fig. 18.4 Proposed mechanism of RDX biodegradation by *Rhodococcus* sp. strain DN22. Denitration as an enzymatic first step creates unstable intermediates which undergo ring cleavage. No$_2$, N$_2$O, NH$_3$, HCHO, and CO$_2$ were identified as products of RDX degradation, as well as the dead-end product $C_2H_5N_3O_3$. Hypothetical components of the pathway are shown in *brackets*

RDX-degrading bacterium is *Rhodococcus* sp., strain DN22 (Coleman et al. 1998). This strain was isolated from explosive-contaminated soil and uses RDX as sole nitrogen source, degrading 0.16 mM within 20 h. The activity is repressed by growth on ammonium as a nitrogen source and is thought to be plasmid borne (Coleman et al. 2002). Characterization of the metabolites produced from RDX by this strain has been performed, leading to the proposal of the first pathway for aerobic RDX biodegradation (Fournier et al. 2002). No nitroso intermediates were identified, strongly suggesting that aerobic RDX biodegradation follows a different path to anaerobic biodegradation. The mechanism proposed (Fig. 18.4) involves denitration as a first step to form a hypothetical intermediate identical to that postulated in the alkaline hydrolysis pathway. After a series of hypothetical intermediates, two compounds are formed: one, the hypothetical NH_2CHO, which breaks down further to ammonium and formaldehyde resulting in the liberation of carbon dioxide, and, the second, $C_2H_5N_3O_3$, which accumulates as a dead-end product.

It is apparent that the biodegradation of RDX generally occurs more rapidly using aerobic bacteria than anaerobic microorganisms. In addition, no toxic compounds such as the nitroso derivatives or proposed hydrazines found during anaerobic RDX degradation but have been identified during aerobic degradation, indicating that this may prove a safer method for remediating RDX. As yet, no determinant underlying the ability to degrade RDX has been identified from either aerobic or anaerobic sources, at either the genetic or biochemical level. In order to realize the potential of microbial-based bioremediation, further investigation into aerobic RDX-degrading organisms, and the genetic basis for their ability, is necessary (Seth-Smith 2002).

18.5 Products of RDX Breakdown

A limited set of final breakdown products appear to be produced from RDX degradation, regardless of the mechanism responsible. These are nitrite, nitrate, ammonium (or ammonia), nitrogen, nitrous oxide, nitric oxide, carbon dioxide, carbon monoxide, formate, formaldehyde, water, hydrogen, and hydrogen cyanide. The first action on the RDX molecule includes the following: proton abstraction liberating nitrite, N–N bond scission, concerted breakdown to monomer, reduction of nitro groups to nitroso, and C–N bond cleavage. Some of the intermediates from some of the pathways have been determined, and the end products identified which indicates that the molecule is eventually mineralized after the breakage of any bond. Competing chemical or biological reactions appear to be responsible for the diversity of products seen (Seth-Smith 2002).

18.6 Applications of Explosive-Degrading Enzymes

Once the enzyme responsible for a particular reaction has been identified and purified, it is possible to characterize the mechanism by which the enzyme performs that reaction, without the complications of side reactions that occur using whole cells or cell extract. The products of the reaction can be determined which, for the purposes of bioremediation, should be nontoxic and preferably indicate the mineralization of the compound. Once characterized, possible further uses for an enzyme can be investigated. There is considerable interest in phytoremediation, using plants to decontaminate soil and groundwater. In particular, plants engineered with bacterial enzymes have generated a great deal of interest. Bacteria have the metabolic capabilities to break down xenobiotic compounds, such as explosives, where plants generate large amounts of biomass, are penetrating deep into soil, are self-sustaining, and have the potential to encourage public acceptance in the area of bioremediation (Seth-Smith 2002).

18.7 Phytoremediation

In the mid-1990s phytoremediation emerged as a potential low-cost alternative for ameliorating explosive-contaminated soil and water (Cunningham and Ow 1996). The term "phytoremediation" combines the Greek word "phyton" (plant) with the Latin word "remediare" (to remedy) to describe the process by which aquatic or terrestrial plants, and the microbial communities associated with their rhizospheres, degrade, extract, contain, or immobilize contaminants from both soil and water. The term was first introduced in 1991 to describe the use of plants to accumulate metals from soil and water, but in 1995, the definition was expanded to include the breakdown of organic chemicals (Schnoor et al. 1995). Many plant biochemical processes do not distinguish between contaminants and nutrient sources, leading to the direct translocation or transformation of pollutants. However, attenuation mechanisms involved in phytoremediation are not limited to the direct metabolism of contaminants (Burken et al. 2000). Indirect attenuation mechanisms include the modification of the physical and chemical properties of the soil, increases in organic soil carbon by the release of root exudates, increased soil aeration and porosity, and reversal of the hydraulic gradient by extraction of available water, thereby decreasing vertical and lateral migration of pollutants to groundwater (Chang and Corapcioglu 1998). There are several processes involved in plant-assisted transformation of contaminants. Phytoaccumulation, also termed phytoextraction or hyperaccumulation, utilizes cation pumps and sorption to withdraw metals, salts, and organic compounds from soil via uptake of plant available water (Schnoor et al. 1995). In the process of phytostabilization, plants temporarily control soil properties such as gas exchange and redoximorphic conditions. Phytostabilization may be able to control the movement of heavy metals, phenols, and chlorinated solvents (Cunningham et al. 1995). Volatile metals such as mercury (Hg) and selenium (Se), as well as chlorinated solvents, can be taken up and transpired by a process termed phytovolatilization (Cunningham et al. 1996). Polyaromatic hydrocarbons, polychlorinated biphenyls, and BTEX compounds can be transformed or degraded by rhizosphere bioremediation. This type of bioremediation utilizes enzymatic activity of mycorrhizal fungi and other microorganisms to degrade the contaminant (Cunningham et al. 1995). Phytotransformation, as the name implies, involves the uptake and metabolism of organic compounds to secondary and tertiary transformation products, which may be harmless in some cases. This process is effective in the transformation of some munitions, chlorinated solvents, and phosphorus- and chlorine-based pesticides (Schnoor et al. 1995). The metabolic processes involved in phytotransformation resemble human metabolism of xenobiotics (Burken et al. 2000). For this reason, a "green liver" model is often used to describe the mechanisms involved with phytotransformation (Sanderman 1994). In contrast to microorganisms, plants use photosynthesis as an energy supply and do not need to metabolize organic compounds for an energy source. Similarly, plants detoxify foreign contaminants, much like humans metabolize xenobiotics. During this detoxification process, xenobiotics are transformed, conjugated, and sequestered. Initially, transformation occurs as a result of enzymes, which catalyze oxidation, reduction, and hydrolysis reactions. Then, the secondary product undergoes conjugation with an organic molecule within the plant. This conjugation process generally leads to a reduction in toxicity to the plant (Coleman et al. 1997; Bhadra et al. 1999). Several sequestration processes can follow conjugation. These can include storage in cell vacuoles or covalent bonding, which results in conjugates being incorporated into lignin (Coleman et al. 1997). The latter case is characterized by unextractable or bound residues (Burken et al. 2000). Phytoremediation has many characteristics that make it a desirable form of contamination removal. Many methods of site remediation such as excavation and "pump and treat" involve expensive, laborious procedures which only transfer the pollutant from one medium to another (Mitsch 1993; Fox 1997). Phytoremediation is a more affordable alternative to conventional cleanup methods. Cleaning the top 15 cm of petroleum-contaminated soil with phytoremediation costs between $2,500 and $15,000 per hectare, compared to $7,500–$20,000 per hectare for on-site microbial remediation. In addition, the use of plants for detoxification also leads to a more aesthetically pleasing appearance. It has been suggested that phytoremediation is suitable for use at large-scale field sites, sites with low concentrations of contaminants, and in conjunction with other methodologies where vegetation is used as a final cap and closure of the site (Schnoor et al. 1995). There are, however, several limitations to this new technology. It is only effective in treating shallow soils, groundwater, and surface water. Plants can only effectively remediate contaminants near their root zone (Schnoor et al. 1995). Phytotoxicity is also a limitation of this approach. Many plants are slow growing, difficult to establish, or cannot survive in areas with high concentrations of contaminants. This limits some applications to areas with low concentrations surrounding the primary site of contamination (McCutcheon 1998). Finally, the secondary and tertiary transformation products of many compounds are also toxic and need to be disposed of properly. In some instances, the characteristics of by-products are not known; therefore, remediation to this point may not always be acceptable. Several ammunition manufacturing and testing sites have significant surface soil and groundwater contamination from energetic material, such as RDX and TNT (Spain 2000). Contamination can also be found at ammunition manufacturing sites that are no longer in use (Spain 2000; Pennington and Brannon 2002). These materials have been documented as toxic to a broad range of organisms (Etnier 1989; Robidoux et al. 2002; Lachance et al. 1999; Thompson et al. 1998). Development of an inexpensive and effective process to remove energetic materials from soil and groundwater could reduce the environmental and economic burden caused by this contamination. Significant research has been done on the remediation of energetic materials from soil and groundwater. Phytoremediation, as well as bioremediation, are potential methods to help reduce contamination (Burken et al. 2000; Hannink et al. 2002). Since significant contamination occurs in surface soil, phytoremediation may become an ideal treatment for energetic contamination (Burken et al. 2000; Shanks et al. 2003; Subramanian and Shanks 2003). Phytoremediation studies have shown that plants have the potential to remove energetic materials. RDX can be taken up by plants, but little is known about the transformation pathway (Pennington and Brannon 2002; Shanks et al. 2003; Subramanian and Shanks 2003; Bhadra et al. 2001; Rollo et al. 2004; Thompson et al. 1999; Just and Schnoor 2004; Van Aken et al. 2004b; Best et al. 1999a, b). RDX has also been shown to accumulate in plant tissue as a parent compound (Pennington and Brannon 2002; Bhadra et al. 2001; Thompson et al. 1999; Best et al. 1999a). The phytophotolysis of RDX has been studied by Schnoor and co-workers and may be responsible for the first step in the degradation of RDX (Just and Schnoor 2004).

18.8 Plant Metabolism of RDX

Plant metabolism of xenobiotics involves three phases: activation (transformation), conjugation, and compartmentation (elimination, Sandermann 1992; Ishikawa 1992; Ishikawa et al. 1997; Rea et al. 1998; Coleman et al. 1997; Schaffner et al. 2002). Activation generally involves oxidation or hydrolysis or reduction type of reactions, where functional groups such as hydroxyl (–OH) and carboxyl (–COOH) are added to the contaminant with the enzymatic involvement of cytochrome P450 monooxygenases, esterases, reductases, dehalogenases, and dehydrogenases. The products of phase I (activation) are more hydrophilic and sometimes more toxic than the parent compound. In phase II (conjugation), the activated contaminant undergoes deactivation by the formation of covalent linkages with endogenous hydrophilic molecules, such as glucose, malonate, glutathione (GSH), or carboxylic acids using glucosyltransferase, glutathione-S-transferase, and acyltransferase-mediated reactions that result in water-soluble conjugates that are less toxic compared to the parent compound. Phase III (compartmentation) involves exporting conjugates to either the vacuole or apoplast using ABC transporters or multidrug and toxic compound extrusion (MATE) transporters (Sandermann 1992; Ishikawa 1992; Ishikawa et al. 1997; Rea et al. 1998; Coleman et al. 1997; Schaffner et al. 2002). Several genes induced by RDX treatment in the study done by Rao et al. (2009) suggest RDX detoxification via the three phases (Table 18.1). Functional categorization by loci of the genes upregulated in this study revealed that several genes had transferase activity and transporter activity, further supporting the notion of potential RDX detoxification in *Arabidopsis*. Also, there were nine expressed genes with unknown function from two-color experiment (greater than 1.5-fold upregulation) and 20 from Affymetrix experiment (greater than 2.0-fold upregulation) identified, some of which might be involved in RDX metabolism. There is no earlier report on whole genome expression studies in response to RDX except a serial analysis of gene expression (SAGE) study (Ekman et al. 2005), where gene expression in *Arabidopsis* roots was characterized. These authors reported three cytochrome P450s (At1g16400, At3g20940, At4g13310), induced greater than fivefold in their study, to be possibly involved in phase I transformation of RDX in *Arabidopsis*. They also speculated about a putative peroxidase (At1g49570) and an alpha-hydroxynitrile lyase-like protein (At5g10300) to be involved in RDX metabolism. Incongruence of lists of differentially upregulated genes between Rao et al. (2009) and Ekman et al. (2005) can possibly be attributed to organs used in the respective studies: roots (Ekman et al. 2005) vs. whole plants (Rao et al. 2009). As also suggested by

Table 18.1 List of potential genes suggesting RDX metabolism via three phases of detoxification for RDX (two color and Affymetrix) microarray experiments along with their fold change in linear scale

	RDX (two color and Affymetrix)		
Phases of detoxification	AGI gene ID	Gene name	Fc
Phase I (transformation): Oxidation or hydrolysis or reduction type of reactions (cytochrome P450, esterases, oxidoreductases)	At4g16690	Esterase/lipase/thioesterase family protein	1.54
	At2g48080	Oxidoreductase, 2CG-Fe(II) oxygenase family protein	1.50
	At3g11180	Oxidoreductase, 2CG-Fe(II) oxygenase family protein	1.45
	At5g05600	Oxidoreductase, 2CG-Fe(II) oxygenase family protein	2.80
	At5g22500	Acyl-CoA reductase, putative/male-sterility protein, putative	2.15
	At5g09970	Cytochrome P450 family protein	2.08
	Atlg64590	Short-chain dehydrogenase reductase (SDR) family protein	2.04
	At2g12190/	Cytochrome P450, putative	2.00
	Atlg64950/		
	Atlg64940/		
	Atlg64930		
Phase II (conjugation): Deactivation by the formation of covalent linkages with endogenous hydrophilic molecules such as glucose, malonate, glutathione, or carboxylic acids (glucosyltransferase, glutathione S-transferase, acyl transferase)	At3g29590	Transferase family protein	1.56
	Atlg78270	UDP-glucose glucosyltransferase, putative	1.48
	At5g49690	UDP-glucuronosyl/UDP-glucosyltransferase family protein	4.16
	At5g39050	Transferase family protein	2.68
	At2g39980	Transferase family protein	2.45
	At5g01210	Transferase family protein	2.15
	At3g19710	Branched-chain amino acid Aminotransferase, putative	2.07
Phase II (compartmentation): Exportation of conjugates to either the vacuole of apoplast (via transporters)	At4g36670	Mannitol transporter, putative	1.78
	At1g73220	Sugar transporter family protein	1.57
	At2g39350	ABC transporter family protein	4.82
	At1g73220	Sugar transporter family protein	3.88
	At3g05400	Sugar transporter, putative	2.46
	At1g16370	Transporter related	2.18
	At4g29140	MATE efflux protein related	2.09

Ekman et al. (2005), since RDX is readily translocated and accumulated in leaf tissues (Best et al. 1999; Harvey et al. 1991; Thompson et al. 1999), gene expression in shoots is highly relevant. Rao et al. (2009) analyzed gene expression in whole plants to sample all differentially regulated genes, which is more appropriate and consistent with their objective of phytosensor engineering and phytoremediation of RDX. In another study, Mezzari et al. (2005) used semiquantitative reverse transcription PCR to study the expression of three glutathione S-transferases (GSTs: At4g02520, At1g17170, and At2g29490) and two isoforms of 12-oxophytodienoate reductases (OPR1 and OPR2) from *Arabidopsis*. These authors reported relatively low induction for all the five genes in response to RDX, and their in vivo conjugation reactions with glutathione ruled out GST-catalyzed GSH (reduced glutathione) conjugation with RDX. None of these genes were found to be upregulated in Rao's study. This inconsistency can also be attributed to the difference in duration of exposure and the concentrations between studies. Tanaka et al. (2007) used real-time RT-PCR to study few TNT-inducible genes (chosen from the *Arabidopsis* TNT SAGE study by Ekman et al. 2003) in poplar that was exposed to RDX. Although none of these genes were found to be upregulated in our RDX study, Tanaka et al. (2007) found that all of the selected genes were upregulated in leaf tissues, whereas only one gene was upregulated in the roots. Unlike in *Arabidopsis*, which deals with RDX and TNT differently (consistent with the study of Rao et al. 2009 and also as reported by Ekman et al. 2005), it is interesting to see the upregulation of TNT-inducible genes in RDX-exposed poplar (Tanaka et al. 2007), although a thorough gene expression analysis in poplar in response to RDX and TNT would be necessary to validate this finding. Another interesting upregulated gene found in RDX study was lipoxygenase (LOX2; At3g45140). This gene was strongly upregulated by RDX as shown by both microarray platforms and confirmed by real-time RT-PCR. Lipoxygenases are known to be versatile catalysts (Feussner and Wasternack 2002), and there are several studies indicating that mammalian lipoxygenases are involved in the metabolism of xenobiotics and endobiotics (Kulkarni 2001). Given this background, although it might be tempting to say that LOX2 might be involved in RDX metabolism, further investigation is warranted in this regard. Finally, for the RDX study, the moderate correlation observed between

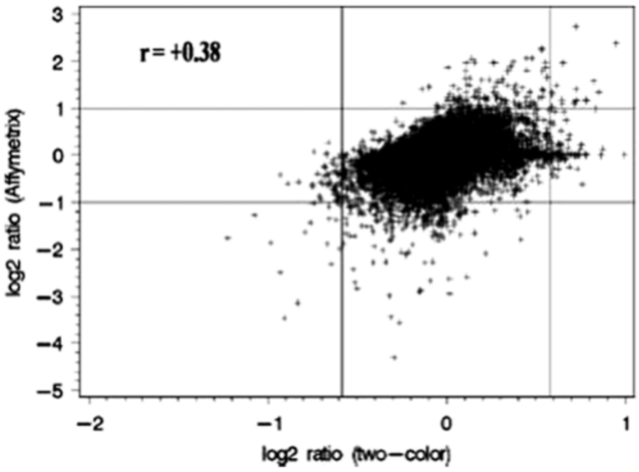

Fig. 18.5 Log2 ratios of signal intensities for RDX from the two platforms (two color and Affymetrix) plotted against each other. On the *y*-axis are the log2 ratios from Affymetrix and on the *x*-axis are the log2 ratios from two color. The *horizontal lines* on the *y*-axis at values +1 and −1 represent the cutoff value of +2 and −2.0 linear fold change, respectively, for Affymetrix, and the *vertical lines* at values +0.58 and −0.58 on the *x*-axis represent the cutoff values of +1.5 and −1.5 linear fold change, respectively, for two color. The value for the Pearson correlation coefficient is represented as *r* on the graph

platforms can be attributed to several factors, including array design, RNA amplification, labeling (single vs. double), hybridization, array scanning, image processing, and normalization techniques (Pylatuik and Fobert 2005). In another study, considerable differences were found to exist across three commercially available platforms (Agilent, Amersham, and Affymetrix) (Tan et al. 2003). Additionally, a lack of agreement was reported between Atlas nucleotide arrays (Clontech) and Affymetrix arrays (Rogojina et al. 2003). However, there are also studies showing reproducibility among platforms (Larkin et al. 2005; Petersen et al. 2005; Shi et al. 2006). Despite only a moderate correlation between the two platforms, here, several significant genes were commonly upregulated and downregulated between the two platforms. In no case did one platform indicate a gene was upregulated, while the other indicated downregulation as evident by the lack of data points in the upper left and lower right corners of Fig. 18.5. Therefore, Rao et al. (2009) conclude that either platform is adequate for screening gene candidates, while the two-color platform might be considered to be somewhat less robust.

18.9 Fate of RDX in Plants

Only a few published studies focus on the fate of RDX in plants. The first examination of RDX fate in plant systems used bush beans (*Phaseolus vulgaris*), grown from seed (Harvey et al. 1991). The plants were initially grown in hydroponic nutrient solutions for 21–26 days. After the seedlings were established, the plants were transferred to nutrient solutions amended with 10 mg/L containing 5.6 μ Ci/500 mL uniformly labeled RDX. Plants were exposed to the RDX solution for either 1 or 7 days. At each harvest, plants were separated into roots, stems, and leaves and then prepared for chemical analysis. Sampling the hydroponic solutions at harvest time established plant uptake of RDX. The initial mass of RDX, 5.14 ± 0.02 mg, decreased to 4.53 ± 0.04 mg after 1 day and 1.80 ± 0.73 mg after 7 days. Transformation products of RDX were not found in the hydroponic solutions, suggesting a lack of microbial and rhizosphere degradation. After 1 day of exposure, RDX concentrations in leaf, stem, and root tissues were 19 mg/kg, 11 mg/kg, and 9 mg/kg, respectively. Based on the extraction process and the recovery of radiolabel, it was determined that RDX was not metabolized. Evidence of bioaccumulation was seen in the 7-day exposures. Leaf, stem, and root tissues contained 97 mg/kg, 11 mg/kg, and 6 mg/kg RDX, respectively. Limited metabolism of RDX was suggested based on the non-extractable fractions of the radiolabel. Emission of $^{14}CO_2$ and volatile organics did not reach detectable levels. Hybrid poplar trees (*Populus deltoides* × nigra, DN34) have also been evaluated for their ability to transform RDX (Thompson et al. 1999). Using hydroponic solutions containing radiolabeled RDX, uptake was monitored by sampling the hydroponic solution for approximately 2 days. As in previous studies, the mass of RDX decreased with time but no metabolites were detected. Plants remained in the system for up to 7 days. Plant extractions resulted in mass balances that averaged 79.7 + 7.1 % recovery. Approximately 60 % of the absorbed RDX was translocated to the leaves, allowing 15–20 % to remain in root and stem tissue. The author suggested that the bound fraction, which was approximately 15 % of the radiolabel, may be an RDX transformation product. As in previous studies, plant respiration of $^{14}CO_2$ and volatile organics was negligible. In contrast to TNT, there has been little documentation of RDX transformation within plants. However, a vegetation survey conducted at the Iowa Army Ammunition Plant (IAAP) produced results that suggest RDX transformation may occur within plants (Schneider 1995). RDX accumulated in the leaf tissues of black locust (*Robinia pseudoacacia*), red cedar (*Juniperus virginiana*), bromegrass (*Bromus inermis*), pigweed (*Amaranthus* spp.), reed canary grass (*Phalaris arundinacea*), Canadian goldenrod (*Solidago canadensis*), and ragweed (*Ambrosia artemisiifolia*). The highest RDX concentration found in the leaf tissues was 38.6 mg/kg although soil RDX concentrations were as high as 114 mg/kg. These tissue concentrations are low when compared to previous laboratory studies. Laboratory studies that implemented significantly lower RDX concentrations in hydroponic media resulted in high leaf tissue concentrations.

The low RDX concentrations found in leaf tissues collected in the field suggest that RDX metabolism may occur over a longer period of time than what has been previously tested in the laboratory. Based on the research reviewed, it is obvious that many important aspects of RDX uptake and transformation by plants are not well characterized. Previous studies were conducted for only short time periods. Also, previous work studied plants subjected to only one explosive at a time, whereas most contaminated sites contain more than one explosive compound within the same media. The rates of uptake and transformation may differ when plants are grown in an environment containing TNT, RDX, and HMX. Further work needs to focus on the long-term fate of explosives in plants, specifically terrestrial species, identification of transformation products in plant tissues, and explosive uptake from aged-contaminated soil (Sealock 2002).

18.10 Phytoremediation of RDX

18.10.1 "Green Liver" Model

Previous research indicates plants are able to uptake several contaminants, such as metals, organic compounds, and explosives from soil and groundwater. The "Green Liver" model is a concept developed to describe the transformation process of xenobiotic pollutants once they are taken up from the soil by plants. It is proposed that the process of how plants deal with contaminants is similar to how the human liver metabolizes toxicants. The three steps proposed in this model include initial transformation or "activation" of contaminant by several reactions such as oxidation, reduction, or hydrolysis, followed by conjugation of activated compounds with plant molecules such as D-glucose, glutathione, or amino acids to produce soluble or insoluble substances, which are subsequently sequestered in cellular compartments of the plant for storage and compartmentalization (Schnoor et al. 2006; Yoon et al. 2005). The soluble compounds are stored in vacuoles or as cell wall material, whereas the insoluble compounds are likely incorporated into the cell wall material (Yoon et al. 2005). Several plant enzymes are responsible for these processes (Doskey 2012).

18.11 Enzymes Involved in RDX Degradation

Nitroreductases are responsible for catalyzing the reduction of the nitro groups in RDX into compounds that may be easier to degrade by the plants and could be more or less toxic in general (Schnoor et al. 2006). These enzymes have also been indicated in the reduction of nitro groups in both HMX and TNT (Schnoor et al. 2006). Other enzymes that might be involved in the activation step of metabolism and/or degradation of RDX include cytochrome P450 monooxygenases and peroxidases, which are responsible for the catalysis of oxidation in HMX, RDX, and TNT and also the catalysis of oxidation in the reduced derivatives (Schnoor et al. 2006). Glutathione S-transferases are involved in the conjugation step of explosive contaminant metabolism, as they catalyze the conjugation of the activated derivatives of these explosives to forms that are much less toxic (Schnoor et al. 2006). In this most recent effort, Bruce and colleagues apply the *xplA* gene from *Rhodococcus rhodochrous* to the degradation of RDX. This gene and *xplA* genes from related bacteria encode a bizarre fusion protein composed of an N-terminal flavodoxin domain and a C-terminal P450 domain (Seth-Smith 2002), a structure never seen before. The authors show that the purified bacterial XplA enzyme can carry out anaerobic NADPH-dependent degradation of RDX to 4-nitro-2,4-diazabutanal, nitrite, and formaldehyde (Fournier et al. 2002) without added ferredoxin as required by standard P450s. Biochemical data suggest that the P450 domain and the flavodoxin domain cooperate in a novel fashion in this catabolic process. Bruce and colleagues go on to demonstrate that when the XplA transgene is expressed in the small model plant *Arabidopsis thaliana*, the plants are highly resistant to the toxic effects of RDX in soil at concentrations that retard the growth of wild-type plants. In fact, the *xplA* transgenic roots generally grow much better on RDX-contaminated soil than either wild-type or *xplA* transgenic roots do on uncontaminated soil. The strong suggestion is that these plants can now use RDX-derived nitrogen (e.g., nitrite) to enhance their growth. Although the authors do not present data on the decay products of RDX in plants, it is clear that transgenic plant shoots contain significantly less RDX than the wild-type plants, a finding consistent with accelerated decay being enhanced by the XplA enzyme. The observed enhancement in plant growth requires that other plant enzymes complement XplA by acting on the products of RDX degradation. This is a major step toward applying phytoremediation to clean up toxic munitions and follows on a string of successes using bacterial enzymes to enhance the natural capabilities of plants to clean up toxic pollutants (Meagher 2006). Rylott et al. (2011) demonstrated the first successful use of transgenic plants to withstand the toxicity of TNT and remediate RDX, two chemically diverse organic compounds. The levels of TNT and RDX contamination tested here also reflect the concentrations found on contaminated training ranges (Talmage et al. 1999; Jenkins et al. 2006) and indicate that this technology is capable of remediating concentrations of RDX found in situ. In addition to the obvious benefits of remediating RDX and TNT from soil and groundwater, these studies show that the levels of RDX in the shoot tissue of transgenic plants expressing XplA were dramatically lower (34- to 94-fold less) than in wild-type, untransformed tissue. This would reduce the availability of this toxin for herbivory and subsequent bioaccumulation in the food chain (Sarrazin et al. 2009; Zhang et al. 2009). Transgenic *Arabidopsis* plants expressing both *xplA* and *xplB* were generated by Jackson et al. (2007). They were able to remove saturating levels of RDX from liquid culture and soil leachate at rates significantly faster than those of untransformed plants and *xplA*-only transgenic lines, demonstrating the applicability of this system for the phytoremediation of RDX-contaminated sites. These studies were performed in *Arabidopsis*, an annual plant species with a relatively small root system penetrating only the top few centimeters of soil and unsuitable for phytoremediation application. Perennial grass species, such as wheatgrass species (*Pascopyrum smithii*, *Elymus trachycaulus*, and *Agropyron fragile*), which are native to military training ranges in temperate regions and produce dense root systems extending over a meter below the soil surface (Frank and Bauer 1991), would be suitable. In addition, these species are low growing, fire resistant, and capable of withstanding and recovering rapidly from disruption by heavy equipment (Asay et al. 2001; Palazzo et al. 2005), all traits advantageous for the phytoremediation of explosives from military training ranges. With so much recent progress in engineered phytoremediation, what impedes its application? At the level of technology, the problems of moving to the field for the remediation of organic pollutants appear almost trivial and should be solvable in a few years. For example, most RDX-contaminated sites also contain toxic levels of TNT, so fast-growing, deep-rooted plants like cottonwood might have to be engineered to express the bacterial nitroreductase as well as XplA to resist and degrade both toxic explosives.

18.12 Conclusions and Future Prospects

Phytoremediation is a relatively new technology that offers clear advantages over traditional methods for site cleanup. Some of its applications have only been assayed at the laboratory or greenhouse level, but others have been field tested sufficiently to allow full-scale operation. Basic research is still lacking in order to exploit efficiently the immense possibilities offered by these technologies. In this regard, the integration of new molecular tools with previous knowledge on the genetics, physiology, and biochemistry of plants is expected to advance significantly our understanding of the relevant mechanisms for explosive degradation. This information will be used to create superior varieties via genetic engineering, an approach that has already proven feasible. Selecting appropriate species is also a key issue for the success of this technology. Poplar trees have attracted considerable attention lately not only for their inherent characteristics like swift growth, low-input cultivation, etc. (Jansson and Douglas 2007), but also for the possibilities offered by recent genome sequencing, the development of systematic molecular tools, and the ease of genetic transformation. Poplars are already the most common choice to phytoremediate organic pollutants (Pilon-Smits 2005; Campos et al. 2008).

Phytoremediation is a slow process and may be limited by the toxicity of energetic materials to plants. The pathway and the fate of RDX by plants are unknown. Even the toxicity of RDX and its degradation products to plants is limited. The environmental fate of RDX and its transformation products, as well as their toxicity, must be known before phytoremediation will be the most successful. Knowledge about the transformation pathways of RDX and other energetic materials would greatly increase our understanding of how phytoremediation occurs. With the understanding of the transformation pathways, native plants could be selected by how well RDX is transformed, while mutant plants could be generated to enhance transformation. A well-defined pathway would also help identify what end products are formed by the remediation of RDX. In order to make the phytoremediation process more effective, a knowledge base of important genetic sequences and biochemical reaction pathways must be formed (Rollo et al. 2004).

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Phytoremediation of Hydrocarbon-Contaminated Soil Using Sedge Species

19

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19.1 Introduction

Crude oil is a homogenous but complex mixture of hundreds of different hydrocarbon compounds that widely vary in their characteristics. Elevated levels of total petroleum hydrocarbons (TPHs) are added to soil during exploration, production, transportation, refining, seepage, and accidental release causing accumulation in land, water bodies, as well as air. Sites with heavy petroleum hydrocarbons (PHCs) contamination lack vegetation and are more prone to erosion and leaching, causing surface spreading of contaminants. Phytoremediation or plant-enhanced bioremediation consists of using the symbiotic relationship between plant and microorganisms, along with soil amendments and proper management practices to remediate contaminated soils in situ (Cunningham and Ow 1996; Cunningham et al. 1997; Kulakow et al. 2000). It is a cost-effective process where plants are used to remediate contaminated matrices such as soil, sediment, surface, and groundwater (Alvarez-Bernal et al. 2007). Native and indigenous plant is more effective than any other exotic plant species for the phytoremediation of hydrocarbon-contaminated soil for ecological and economic reasons, as they may not require long-term maintenance and they are better adapted to the environment.

Sedge species, namely, *Cyperus rotundus* (Linn.), *Cyperus brevifolius* (Rottb.) Hassk, *Cyperus odoratus* L., and *Cyperus laevigatus* L. are native to India which showed significant degradation of hydrocarbon in our study. Our findings suggested that these plants could be effectively used as pioneer of plant vegetation and decontamination of hydrocarbon pollutants in the oil-contaminated areas. After these plants are established in a field, the plants should be continuously monitored to control their spread to another field. A more general notion of a weed is that of "a plant growing in a place where it is not wanted" (Angle and Linacre 2005). Growing these plant species in contaminated areas to remediate hydrocarbon could be considered a positive attribute. Spread of sedge can be easily controlled with proper monitoring.

They propagate by rhizome and seeds; grow in wet, disturbed, and altered areas; and can be vegetatively established in contaminated soil. They have fibrous, perennial root systems with vigorous deep-root rhizomes which can control soil erosion and surface spreading of hydrocarbon contaminants (Basumatary et al. 2013). After successful remediation of hydrocarbon contaminants, these plants can be easily destroyed in the field by cultural, chemical, or biological methods, and vegetation of other plants can be established. The best ways especially are repeated summer tillage to destroy rhizomes and tubers (USDA 2006) and harvesting plants before seeds are produced (Basumatary et al. 2013). Seeds stored in the soil seed bank may be managed by using an on-site volunteer management. This article reviews our findings on sedge species, viz., *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus* for the remediation of hydrocarbon-contaminated soil.

19.2 Effect of Hydrocarbon Contamination on Plant

Plant and root growth were found to decrease for sedge species in our study. The average yields were significantly ($p < 0.05$) higher in uncontaminated soil than in contaminated

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soil regardless of the fertilizer in the contaminated soil compensating for the higher C/N ratio. High rates of plant mortality and reduction in height and biomass are typical reactions caused by oil contamination (Lin and Mendelssohn 1998). The root structure of sedge species was reduced by the toxicity of crude oil. *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus* are herbaceous with extensive fibrous root systems and can propagate by vegetative as well as sexual mode. Despite these characteristics, growth of plant was inhibited by the toxic effect of hydrocarbon during the initial stage of growth. After several days, the species showed adaptability to the toxic environment, as shown by the high rates of tillering and biomass production. However, the plant species were healthy and produced good biomass during the study. There are reports on large differences of PHC tolerance among plants growing in contaminated soil (Radwan et al. 1998; Brandt et al. 2006).

19.3 Hydrocarbon Degradation in Soil and Accumulation in Plant Parts

C. rotundus, *C. brevifolius*, *C. odoratus*, and *C. laevigatus* showed significant ($p<0.05$) degradation of hydrocarbon in soil in our study when they were grown in 8 % TPH-contaminated soil. Degradation is due to the capability of the plant species to tolerate the toxicity of hydrocarbons and degrade. Besides, these plant species have extensive fibrous root system with vigorous deep-root rhizomes which produce more specific root length (Basumatary et al. 2012a–c, 2013; Bordoloi et al. 2012) and large number of microbes in the rhizosphere. In the laboratory scale study, *C. rotundus* showed 50 % degradation of total oil and grease (TOG) during the study of 180 days (Basumatary et al. 2012c), and *C. brevifolius* showed 86 % TOG during the study of 360 days (Basumatary et al. 2012a). In the field experiment of 360 days (Basumatary et al. 2013), *C. rotundus* showed comparatively more degradation of TPH (75 %) in soil than *C. brevifolius* (64 %). In the control treatments the degradation was very low. In the laboratory scale experiment, the degradation of TPH by *C. odoratus* was 78 % and by *C. laevigatus* was 73 % (Basumatary et al. 2012b). Plants provide root exudates of carbon, energy, nutrients, enzymes, and sometimes oxygen to microbial population in the rhizosphere (Campbell 1985; Cunningham et al. 1996; Vance 1996).

These exudates provide sufficient carbon and energy to support large number of microbes in the rhizosphere (Erickson et al. 1995). This plant-induced enhancement of the microbial population is called rhizosphere effect (Atlas and Bartha 1998) and is believed to result in enhanced degradation of PHC contaminants in the rhizosphere. With sufficient oxygen, soil moisture, and an acclimated population of microorganisms, the soil column acts as a natural biofilter within which PHC vapors are degraded at sufficient fast rates (USEPA 2011). As an additional compartment, plant roots can interact with both microbes and organic pollutants (Bossert and Bartha 1984). Root proliferation of the plant can support a flourishing microbial consortium, thus accelerating biodegradation of PHCs (Cai et al. 2010). In turn, the healthier microbial consortium contributes to the better growth of the plant, thus improving phytoremediation efficiency and characteristic of soil. However, due to the complexity of rhizosphere, further investigation is required about relationships between sedge-microbial interactions in respect of hydrocarbon degradation.

Our study revealed that degradation of hydrocarbon in unplanted soil was slow and lesser which can be attributed to its lack of plants. Volatilization of lighter fractions of crude oil might have contributed to the rapid degradation. In addition, microbial degradation might have also contributed to reduction in crude oil in soil. Both these actions constitute part of the natural attenuation phenomenon (Margesin and Schinner 2001; Pichtel and Liskanen 2001; Bento et al. 2005; Chaîneau et al. 2005; Sarkar et al. 2005; Scow and Hicks 2005; Atagana 2010). However, vegetated treatments show significantly ($p<0.05$) higher hydrocarbon degradation than the unvegetated treatments.

Measurement of TPH in plant roots and shoots showed the presence of significant ($p<0.05$) amounts of the hydrocarbons. The accumulation of hydrocarbons in plant parts, on fertilized soils, was greater than in plants grown on unfertilized soils in our study with sedge species. In the field experiment, *C. rotundus* showed significantly more accumulation of TPH in fertilized treatments (587 ppm in root and 786 ppm in shoot) than in unfertilized treatments (402 ppm in root and 480 ppm in shoot) during the study of 360 days. In the case of *C. brevifolius* also, the accumulation of TPH in the plant parts grown in fertilized soil was comparatively higher (450 ppm in root and 655 ppm in shoot) than in the plant parts grown in unfertilized soil (338 ppm in root and 366 ppm in shoot) during the study of 360 days in the field condition (Basumatary et al. 2013). In the laboratory scale study, *C. odoratus* showed significantly more accumulation of TPH in fertilized treatments (650 ppm in root and 508 ppm in shoot) than in unfertilized treatments (480 ppm in root and 610 ppm in shoot) during the study of 360 days. In the case of *C. laevigatus*, the accumulation of TPH in plant parts in fertilized treatments (300 ppm in root and 380 ppm in shoot) were higher than that in unfertilized treatments (260 ppm in root and 320 ppm in shoot) (Basumatary et al. 2012b) during the study of 360 days. Differences of TPH accumulation in the roots and shoots were significant. However, *C. rotundus* showed more accumulation of TPH in roots and shoots than *C. brevifolius* in the field experiment.

The presence of high levels of TPH in shoots and roots is attributable to the accumulation capability of *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus*. The process of phytodegradation or transformation is usually mediated by enzymes, which may be secreted in exudates into the soil, in which the transformation is mediated outside the plant before the uptake of the resultant compound or enzymes within the plant tissues, in which case the compound is transformed within the plant tissue (Atagana 2011). Fibrous root systems with large surface area creating large rhizosphere volume are favorable for phytoremediation (Siciliano and Germida 1998). The roots of *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus* are fibrous which produce more specific root length. This might have positive effect in the degradation of TPH in the soil and accumulation in plant parts. Significant correlation was observed among specific root length, TPH degradation in soil and accumulation in roots and shoots ($p < 0.05$) in our study with these plants.

19.4 Influence of Microorganisms

Microorganisms have significant influence on the growth and development of plants. The microbe-assisted phytoremediation includes the types of microorganisms involved in the degradation of petroleum products, differences in degradation by various microorganisms, characteristics of microbial communities involved in degradation, and the role microorganisms play in reducing phytotoxicity to plants (Basumatary et al. 2013). In our work, plant-promoted degradation of hydrocarbon using sedge species might be due to the complexity of plant and petroleum-degrading bacterial interactions. Cunningham and Ow (1996), Cunningham et al. (1997), and Kulakow et al. (2000) also found enhanced degradation of PHCs by using the symbiotic relationship between plant and microorganisms, along with soil amendments and proper management practices.

Our findings showed variation in the degrading bacterial population during the study. The vegetated treatments showed a significant ($p < 0.05$) increase in petroleum-degrading bacterial population at the end of the experiment in relation to the initial population. Slow and gradual adaptation of the microbial population to crude oil was observed in the fertilized treatments which are attributable to the beneficial effect of adding inorganic fertilizer, resulting in a greater reduction of TPH (Basumatary et al. 2012b). The plant-promoted degradation of TPH observed in *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus* might be due to the plant–microorganism interaction. In the field experiment, *C. rotundus*-planted soil showed the highest number of microbial population (Log CFU per gm of soil) in the hydrocarbon-contaminated soil (6.55 in fertilized soil and 3.10 in unfertilized soil) than *C. brevifolius*-planted soil (5.85 in fertilized soil and 2.99 in unfertilized soil) (Basumatary et al. 2013) during the study of 360 days. *C. odoratus*-planted soil showed comparatively more microbial population (log CFU.g^{-1} soil) (6.80 in fertilized soil and 3.82 in unfertilized soil) than *C. laevigatus* (5.21 in fertilized soil and 3.50 in unfertilized soil) (Basumatary et al. 2012b) during the study of 360 days. Increase of microbial population enhanced degradation of significant amount of TPH in soil and accumulation in roots and shoots.

19.5 Influence of Fertilizer in Hydrocarbon Degradation

Fertilizers increase plant growth in oil-polluted soils in case of nutrient deficiency (Lin and Mendelssohn 1998; Hutchinson et al. 2001). However, overfertilizing usually leads to yield depressions (Brandt et al. 2006). Optimum use of fertilizer significantly impacts on plant growth, increase in bacterial population, and hydrocarbon degradation in soil. Concerning oil degradation, the fertilized soil showed significant amount of TPH degradation in comparison to unfertilized soil. Hydrocarbon-contaminated soil usually has low fertilizer (e.g., NPK) which is insufficient to meet the nutrient demands of plants. In such case, addition of NPK can compensate the nutrient demands of plants for phytoremediation. Hutchinson et al. (2001) observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments. Joner et al. (2002) reported improved degradation of three- and four-ringed PAHs with the addition of N/P but diminished degradation of five- and six-ringed PAHs. In addition, Merkl et al. (2005) found enhanced degradation of crude oil by using *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf in NPK-fertilized soil in comparison to control. Addition of optimum dose of fertilizer helps in the gradual adaptation of the microbial population to the PHC-contaminated soil which results in the higher reduction of TPH (Basumatary et al. 2012a–c, 2013). The increased microbial numbers are primarily due to the presence of plant exudates and sloughed tissue, which serve as sources of energy, carbon, nitrogen, or growth factors (Lee and Banks 1993).

19.6 Conclusion

Sedge species, namely, *C. rotundus*, *C. brevifolius*, *C. odoratus*, and *C. laevigatus*, can tolerate high concentrations of hydrocarbon contamination in soil. These plant species showed significant growth and biomass production and supported the presence of increased microbial population in hydrocarbon-contaminated soil. Significant degradation of hydrocarbon in soil and accumulation of hydrocarbon in roots and shoots were observed in experiments using these plant species. These plant species are important candidate species for the phytoremediation of hydrocarbon-contaminated soil.

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Phytoremediation of BTEX by Plants

20

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20.1 Introduction

Phytoremediation is recognized as eco-friendly and sustainable technology for indoor air pollution control. The use of plants to treat organic pollutants has been widely studied and developed. BTEX (benzene, toluene, ethyl benzene, xylene), a group of highly toxic organic compounds, was also studied to be remediated by plants. High efficiency of gaseous BTEX removal occurred in many species of plant, and complete BTEX-degradation mechanism has been reported (Kvesitadze and Kvesitadze 2009; Wolverton et al. 1989; Orwell et al. 2004; Liu et al. 2007; Treesubsuntorn and Thiravetyan 2012). The BTEX-degradation mechanism suggests that plants could uptake and transform these compounds and use them as carbon and energy sources (Ugrekhelidze et al. 1997; Kvesitadze and Kvesitadze 2009). In gaseous BTEX uptake, the logarithm of the octanol-water partitioning coefficient (log K_{ow}) was considered to be an important factor for the transpiration stream concentration factor (TSCF), an indirect measure of uptake efficiency (Briggs et al. 1982; Kamath et al. 2004).

Enzyme P450 monooxygenase and dioxygenase, the key enzyme for BTEX compounds transformation, has been investigated (Kvesitadze and Kvesitadze 2009). The factors affecting gaseous BTEX removal such as light and dark conditions, number of stomata, photosystem II activity, quantity and composition of wax, and pollutant molecular size were reported (Treesubsuntorn and Thiravetyan 2012). The enhancing of BTEX phytoremediation was also investigated by both plant-microbial association and transgenic plants (Barac et al. 2004; Taghavi et al. 2005; James et al. 2008; Doty 2008; Germaine et al. 2009; Van Aken et al. 2004; James and Strand 2009; Weyens et al. 2009; Yee et al. 1998; Huang et al. 2004). This emerging technology should be precipitated and applied to fieldwork. Our knowledge in BTEX phytoremediation, BTEX uptake and transformation mechanism, factors affecting, case study of phytoremediation of gaseous BTEX, and enhancing technologies have been reviewed and updated.

20.2 BTEX Uptake and Translocation in Plant

Gaseous BTEX can be commonly taken up by both cuticular wax and stomata of plants (Fig. 20.1) (Ugrekhelidze et al. 1997; Ugrekhelidze and Durmishidze 1980; Kvesitadze and Kvesitadze 2009). The uptake of gaseous BTEX by stomata of most plants was found to be more important than uptake by cuticular wax (Ugrekhelidze et al. 1997). However the ratio between stomata and cuticular wax uptake depended on the growing conditions, quantity and composition of wax, and species of plant. In growing conditions, most of the plants show the closing of stomata under dark conditions. However in other plants, the opening of stomata under dark conditions occurred (Ugrekhelidze et al. 1997; Kvesitadze and Kvesitadze 2009). This result suggests that the type of plant such as C_3 and CAM affects the BTEX uptake in different conditions. Although the same plant was applied for BTEX removal, in different conditions, the changing of the ratio between stomata and cuticular wax BTEX uptake pathway might occur. The different plant species show different quantities and compositions of wax that could affect BTEX uptake. In our experiment, a high quantity of cuticular wax showed the increasing of BTEX uptake tendency; however, some plants with low quantities of wax show high BTEX removal efficiency. In addition, plants of different ages show different benzene accumulations on the leaves despite being similar plant species (Ugrekhelidze et al. 1997), so the composition of wax might be a more effective factor than quantity of wax (Topp et al. 1986).

The plant leaf material that shows high C18 fatty acid as a high ratio composition of whole fatty acid was found

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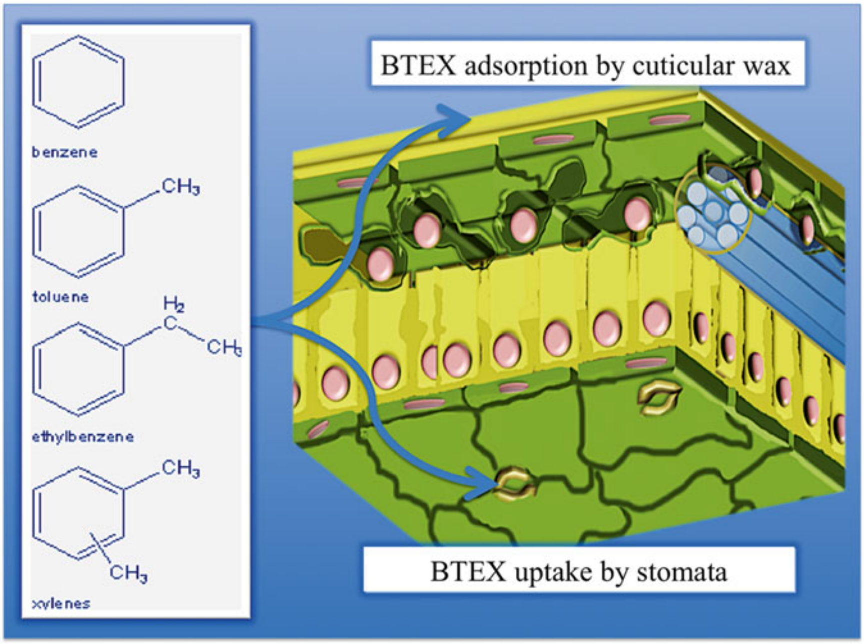


Fig. 20.1 Plant-BTEX uptake pathways: stomata and cuticular wax

to have high benzene uptake efficiency in our previous study. The result suggested that C18 fatty acid might be an important composition for benzene uptake, and a similar result was also found in xylene. Moreover, the logarithm of octanol-water partitioning coefficient (log K_{ow}), which was calculated from the logarithm of the ratio of interested compound concentration in n-octanol and water at equilibrium time, could be applied to present hydrophobicity of the compounds (U.S. Environmental Protection Agency 2009; Pontolillo and Eganhouse 2001; Lyman et al. 1990). The suitable water solubility of xenobiotics affecting the translocation of the compounds from plant root to shoot was widely investigated (Briggs et al. 1982; Kleier 1988; Kenaga and Goring 1980; Francis et al. 1990). For example, the different log K_{ow} compounds in the range of 0.96–5.3 were exposed to the plants by chamber technique, and the concentration of each compound both in the system and in the xylem at equilibrium time was measured. The concentration ratio of each compound in the xylem plant and chamber was reported as the transpiration stream concentration factor (TSCF). The relation of log K_{ow} and TSCF was purposed, and the result suggested that the plant could uptake well and translocate the molecules that have log K_{ow} in the range of 1.5–3.5 (Briggs et al. 1982; Kamath et al. 2004; Francis et al. 1990). BTEX compounds which show log K_{ow} in this range should be easily taken up and translocated in the plant. Passive diffusion was considered the BTEX intracellular translocation mechanism because small hydrophobic properties were found in these molecules (Kvesitadze and Kvesitadze 2009). In addition, we have recently found that the physicochemical properties of each BTEX might affect its removal ability (Sriprapat and Thiravetyan 2013). At the same initial concentration of each BTEX under the control of temperature and pressure experiments showed that the difference in the flux of each BTEX is closely related with the diffusion coefficient of each BTEX. This coefficient is related to the physical and chemical properties of the compound including molecular weight (Little et al. 1994; Bodalal et al. 2000). Therefore, benzene, a smaller molecule, is taken up by plants faster than toluene, ethylbenzene, and xylene.

After intracellular translocation, BTEX transformation occurred, and the metabolite of compounds was collected in the cellular organelle such as nuclei, cell wall, chloroplasts, mitochondria, microsomes, and cytosol (Fig. 20.1) (Ugrekhelidze et al. 1997). In [C-14] benzene translocation study, the intensity of radioactive benzene metabolites was observed and the benzene translocation ratio was calculated in cellular organelles. The result suggests that benzene metabolite translocation depends on plant species. Most of benzene metabolites were translocated to cytosol and chloroplasts (about 70–80 % of whole benzene metabolites) (Ugrekhelidze et al. 1997). Complete benzene uptake and translocation was reported in some plants, but other BTEX compound translocation has not been reported.

20.3 BTEX-Degradation Mechanisms in Plants

The organic pollutant degradation and detoxification mechanism by plants is commonly divided into three phases which are phase I, transformation; phase II, conjugation; and phase III, localization (Fig. 20.2) (Sandermann 1994; Kvesitadze et al. 2006; Kvesitadze and Kvesitadze 2009). The hydrophobic

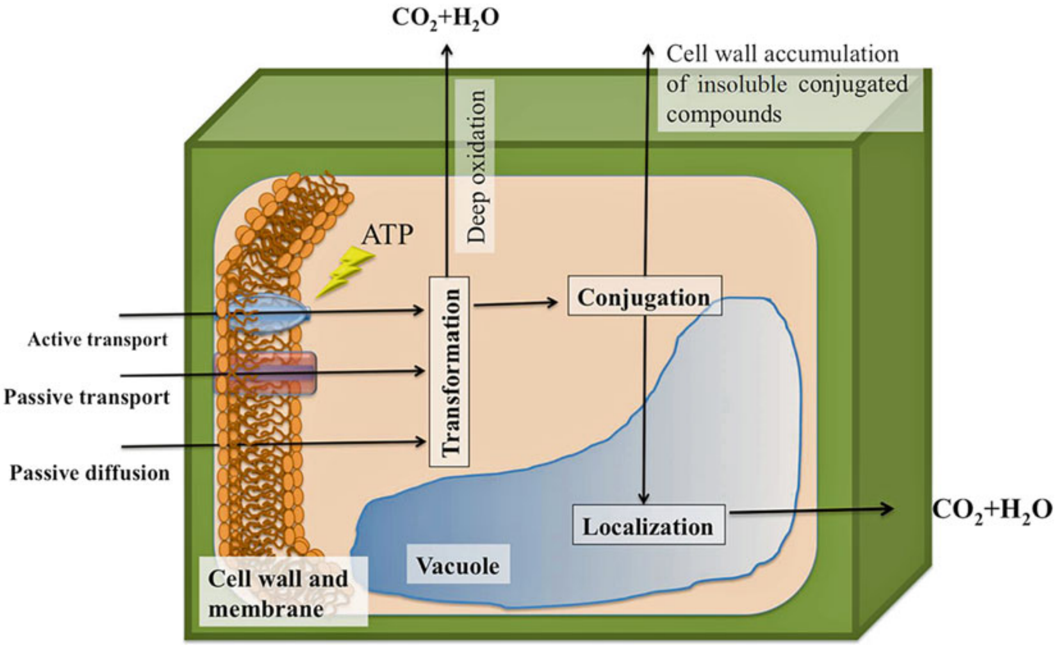


Fig. 20.2 Principle of organic pollutants metabolism in plants "green liver model"

compounds that have high oil solubility and water-insoluble properties had higher polarity than the molecules by plant enzymes in phase I of metabolism. The polarity increased by the addition of hydroxyl, carboxyl, amino, and others, which also increased water solubility. Then, the water-soluble toxic metabolites were conjugated with intracellular compounds, for example, proteins, peptides, organic acids, saccharides, lignin, etc. After phase II metabolism, the combined toxic pollutants with intracellular compounds were translocated to target organelles and accumulated for a long period of time (Kvesitadze and Kvesitadze 2009). However, for some of the pollutants, especially hydrocarbon compounds, only the transformation phase was required. BTEX, one of the hydrocarbon compounds, could be also completely degraded by the use of the transformation phase.

In 1996, benzene degradation mechanism in spinach leaves was studied for initial investigation. Gaseous benzene of 1.76 MBq/mmol was penetrated into the leaf of the spinach for 72 h of exposure time at 22–26 °C. Benzene with radioactivity of [1–6^{14}C] and its metabolites were measured. The result suggests that most of the benzene degrading compound is a low molecular weight compound such as amino acid and organic acid, about 77 % of total benzene metabolites. High molecular weight compounds and CO$_2$ were found to be 18 % and 5 %, respectively. In whole low molecular weight compounds, 84 % of low molecular weight is organic acid and 16 % is amino acid. In organic acid composition, muconic acid and fumalic acid were found to be the largest ingredients, about 37 % and 24 % of whole organic acid, respectively. Tyrosine and phenylalanine, the main amino acid components in benzene degradation, were found to be 34 % and 25 % of the whole amino acid composition, respectively. Toluene was also studied. In toluene detoxification by spinach, a similar tendency with benzene degradation was found, and it was reported that most of toluene metabolites were investigated as low molecular weight compounds, about 83 % of total toluene metabolites. Only 3.5 % of total toluene metabolites were completely degraded to CO$_2$. 79 % and 21 % of low molecular weight toluene metabolites were organic acid and amino acid, respectively. These results suggest that plants can use BTEX as carbon and energy sources, and most of BTEX-degrading metabolites would be low molecular weight molecules such as organic and amino acid (Ugrekhelidze et al. 1997).

With the addition of NADH and NADPH to the exposed spinach with [1–6^{14}C] benzene, the increasing of benzene metabolites was found, suggesting that NADH and NADPH can enhance the benzene degradation in plants (Ugrekhelidze et al. 1997; Kvesitadze and Kvesitadze 2009). The copper- and iron-containing enzymes were reported to be the main BTEX oxidation enzymes (Kvesitadze and Kvesitadze 2009). Cytochrome P450 monooxygenase and dioxygenase, some iron-containing enzymes, showed the role of BTEX oxidation (Fig. 20.3) (Kvesitadze and Kvesitadze 2009; Robineau et al. 1998). This enzyme was found on the membrane of endoplasmic reticulum, and high-affinity nonpolar molecules and inducing by NADH and NADPH were found to be the important properties of the enzyme (Kvesitadze and Kvesitadze 2009). Using NADH and NADPH as electron donors for BTEX oxidation, the free electron was carried to reductase

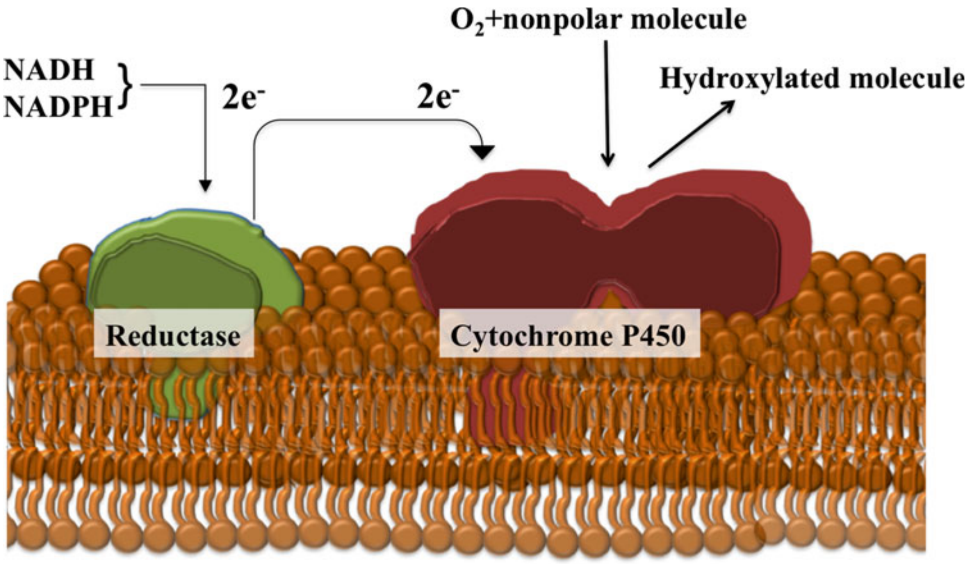


Fig. 20.3 Electron transporting system in P450 monooxygenase

enzymes. The electron acceptation by b_5 intermediate was found. Finally, cytochrome P450 accepts the carried electron from cytochrome b_5 reductase and activates the oxygen molecule. The BTEX was changed to a hydroxylated product by the addition of the hydroxyl group on BTEX molecules by the activated oxygen molecule (Hansikova et al. 1994; Schuler 1996). In the benzene oxidation mechanism, the addition of the hydroxyl group on the molecule by monooxygenase transformed benzene to phenol. A secondary hydroxyl group was added to this molecule of phenol to transform to catechol. This catechol molecule was transformed to o-quinone, a non-stable molecule, and the aromatic ring was cleavaged. Cis-cis-muconic acid was found and purposed as a main and initial compound in benzene metabolism. This organic compound could be a general compound for cell survival and could transform to CO_2 by the TCA cycle. Not only in P450 monooxygenase but also in dioxygenase, the potential for BTEX transformation was found (Kvesitadze and Kvesitadze 2009). In addition, although BTEX is not a substrate for phenol oxidase, the activity of this enzyme generates reactive oxygen species such as hydroxyl radical and superoxide anion radical. The transformation of BTEX by the addition of hydroxyl group was also apparent from this enzyme (Guillén et al. 1997, 2000; Martinova 1993; Kvesitadze and Kvesitadze 2009).

Phytoremediation technology shows the high BTEX removal potential. Absorption and transformation of BTEX was widely studied. Cuticular wax and stomata of plant were considered as important pathways for benzene uptake. In addition, the uptake of BTEX depends on the quantity and composition of the wax. In BTEX transformation, cytochrome P450 monooxygenase and dioxygenase showed the important role of aromatic hydrocarbon oxidation and ring cleavages. For the benzene degradation, phenol was reported to be the initial benzene metabolite and was added to the secondary hydroxyl group to change as catechol. Then, o-quinone was generated by the catechol transformation. Cis-cis-muconic acid was found to be the first organic acid of benzene metabolite. The result showed not only benzene but also that toluene could be degraded to organic acid. From the radioactivity of benzene and toluene distribution in plant experiments, most of benzene and toluene metabolites are organic acid, and in a few CO_2 from these compounds, catabolism was found (Durmishidze et al. 1969, 1979). This evidence suggests that the use of plants for removal BTEX compounds is a suitable, eco-friendly, and sustainable technology. Finally, secondary waste disposal was not required.

20.4 Factors Affecting BTEX-Contaminated Air Removal by Plant

The study of BTEX removal by plants was of interest because it has high potential and low cost and is sustainable and eco-friendly. The study on phytoremediation for BTEX started in 1989. Although phytoremediation has been proposed as an effective method, the low activity in pollutant removal of plants is often found in the practiced applications. This might be because of the surrounding environmental factors including temperature, light, and humidity. Therefore, factors affecting BTEX removal by plants such as toxic tolerance, growing conditions, number of stomata, and quantity and composition of cuticular wax should be the focus.

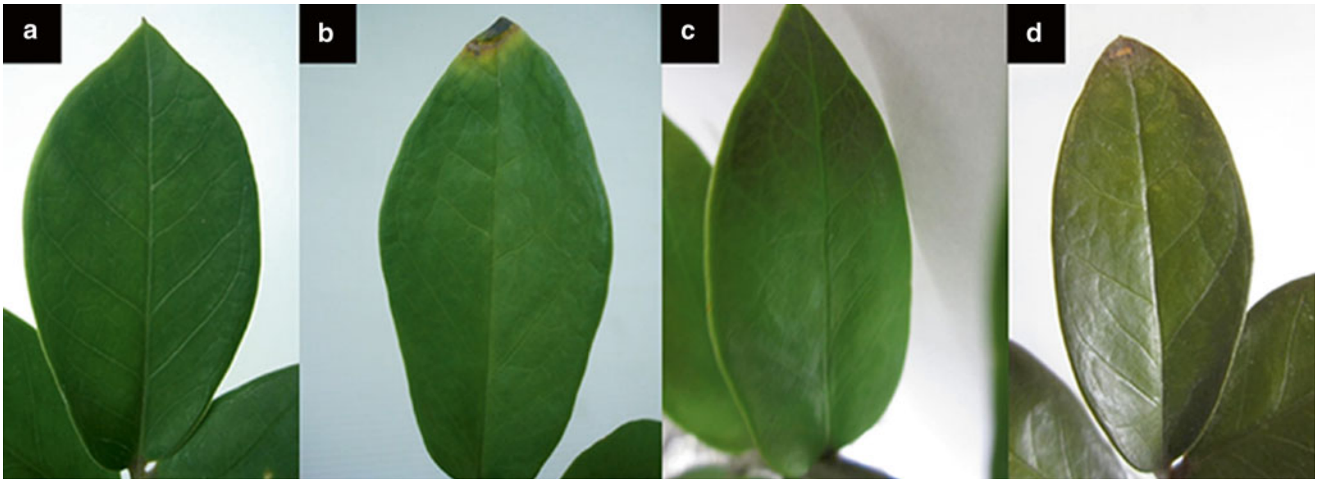


Fig. 20.4 Appearance of toxicity symptoms on *Z. zamiifolia* leaves after 7 days of exposure to different concentrations of xylene. (**a**) Control, (**b**) 2,000 ppm, (**c**) 6,000 ppm, and (**d**) 20,000 ppm of xylene-treated plants (Reprinted from Sriprapat et al. 2014, with permission of Elsevier)

20.4.1 Toxicity of BTEX on Plants

In order to apply a plant to improve indoor air quality, an important factor to be considered is the tolerance of the plant to toxic vapors at different levels of concentrations. Therefore, the details about the response of plants when exposed to the toxic gases should be accounted for.

The toxicity observation found that the xylene-treated plants showed signs of stress, including leaf tips turning yellow and holonecrosis and hydrosis after exposure to xylene. Increasing the xylene concentration also resulted in increased damage symptoms (Fig. 20.4). Currently, a dose-response relationship between BTEX toxicity and plants has been reported. The research indicates the inhibition of toluene on the photosynthesis of plants (Jen et al. 1995). Furthermore, we found the effects of ethylbenzene on the photosynthesis of plants. The ethylbenzene-treated plants had decreased chlorophyll fluorescence parameters (value termed the variable fluorescence (Fv)/maximal fluorescence (Fm); Fv/Fm) when compared with the control plant. In addition, in xylene-treated plants, we found that the increasing of xylene concentration could be the cause of a decrease in the Fv/Fm ratio of treated plants. Therefore, there may be a relationship between the chlorophyll fluorescence parameters and VOC uptake by plants.

Fv/Fm analysis is also related to photosystem II (PSII) in a dark adapted stage. This was used to determine the effects of stress factors on photosynthesis when the plant is exposed to adverse conditions (Maxwell and Johnson 2000). In addition, PSII is located in the thylakoid membrane of the chloroplast of higher plants. Thus, the reduction values of the Fv/Fm ratio might be associated with damage to photosynthetic equipment (Guo et al. 2006; Cassana et al. 2010). It has been shown that the benzene-treated leaves were changed in their cells' ultrastructure especially in the photosynthetic apparatus (Korte et al. 2000; Sadunishvili et al. 2009). In addition, high concentration of xylene exposure resulted to damage chloroplasts of the plant. A high concentration caused chloroplasts to become swollen and damaged (Fig. 20.5).

Chloroplasts appear to play an important role in the detoxification process of organic contaminants in plants (Ugrekhelidze et al. 1997). Therefore, damage to photosynthetic might reduce the degradation efficiency of organic contaminants by plants. It is indicated that the Fv/Fm ratio can give insight into the ability of plants to tolerate air pollution. Based on the aforementioned appearance symptoms of plants, it is indicated that the photosynthetic performance of plants is an essential factor that requires further study.

20.4.2 Physiological and Environmental Factors of Stomata Association

There are currently experimental data obviously demonstrating that stomata and cuticles of plants are important pathways for VOC uptake (Keymeulen et al. 1993; Kvesitadze et al. 2009; Treesubsuntorn and Thiravetyan 2012). Stomata usually open or close also according to environmental factors including light, humidity, and temperature. A study of the effect of light when plants were grown under 24 h dark and 24 h light conditions and the benzene removal under these two conditions were observed. The result showed that benzene removal under light conditions was more effective than under dark conditions (Fig. 20.6) (Treesubsuntorn and Thiravetyan 2012; Sriprapat and Thiravetyan 2013). Light and dark conditions might be related to photosynthesis rate, and from gaseous BTEX uptake by plant, stomata were considered as the main structure to uptake these compounds,

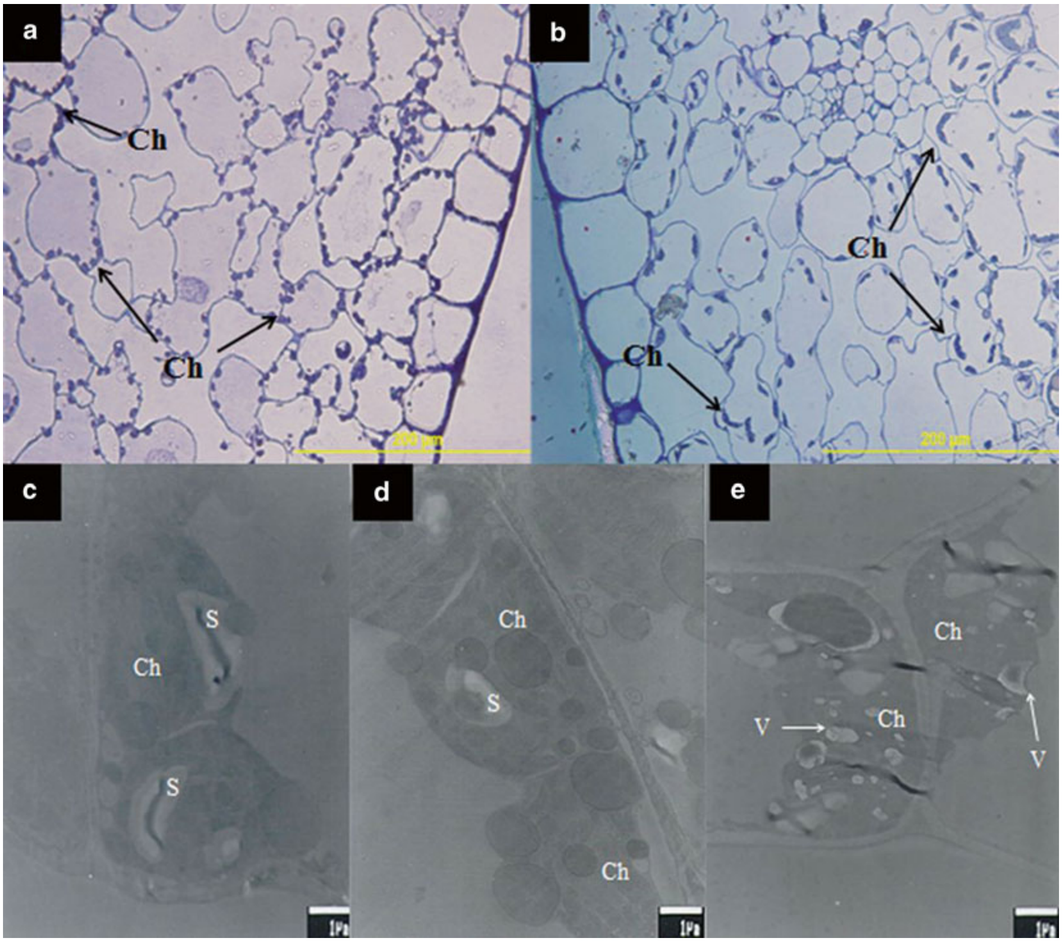


Fig. 20.5 Light microscopy images of xylene on *Z. zamiifolia* after exposure to xylene for 7 days, (**a**) control plant (×40) and (**b**) 20,000 ppm of xylene (×40), and transmission electron microscopy images of xylene on *Z. zamiifolia* after exposure to xylene for 7 days, (**c**) control plant (×6,000), (**d**) 20 ppm (×6,000), and (**e**) 20,000 ppm of xylene (×6,000). *Ch* chloroplast, *S* starch grains, *V* vacuole-like structures (Reprinted from Sriprapat et al. 2014, with permission of Elsevier)

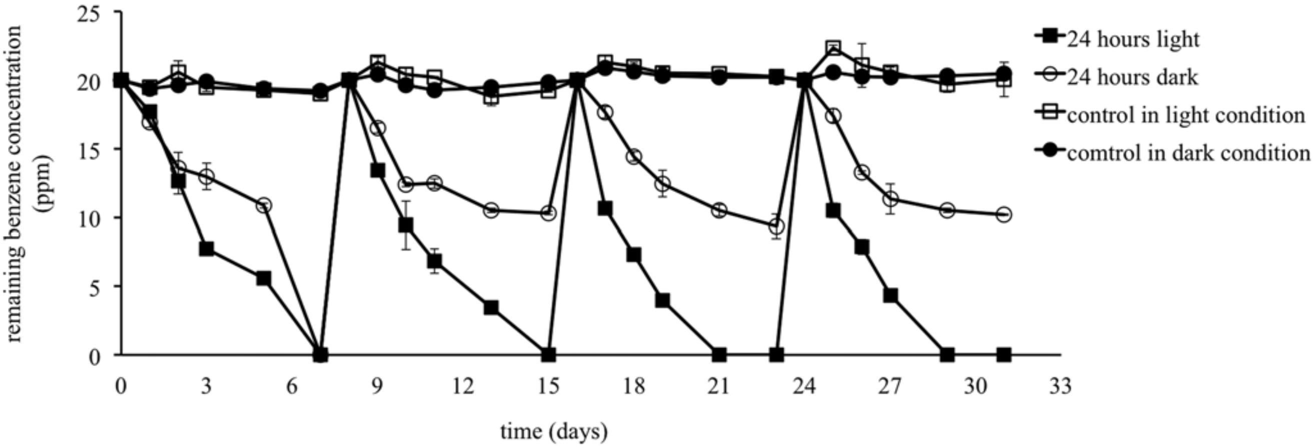


Fig. 20.6 Remaining benzene in 4-cycle experimental system observation of benzene exposure plant growth under 24 h of light and 24 h of dark conditions (Reprinted from Treesubsuntorn and Thiravetyan 2012 with permission from Elsevier)

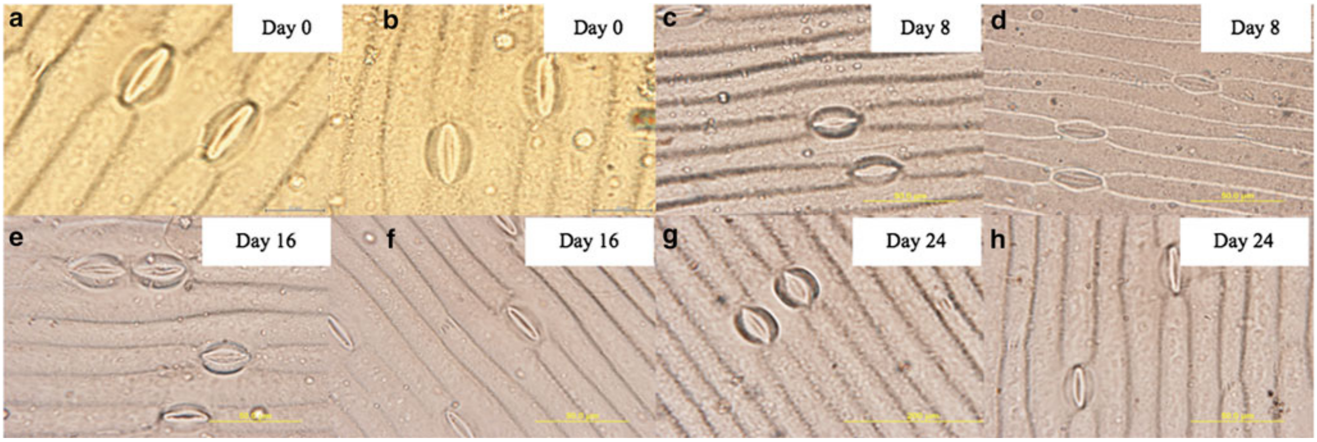


Fig. 20.7 Stomata observation on the leaf of *D. sanderiana* under 24 h light conditions (**a**, **c**, **e**, **g**) and 24 h dark conditions (**b**, **d**, **f**, **h**) when exposure to initial benzene concentration of 20 ppm

so the closing and opening of stomata could also affect benzene uptake. From the stomata observation, the closing of stomata was clearly found in the 24-h dark condition experiment (Fig. 20.7). The result confirms that plants grown under 24 h of dark conditions are shown to have lower BTEX uptake efficiency than plants grown under 24 h of light conditions.

Although stomata were reported as an important pathway for benzene uptake by plants (Treesubsuntorn and Thiravetyan 2012), an unclear relation between the number of stomata and benzene removal efficiency was found from our previous study (Treesubsuntorn and Thiravetyan 2012). This might involve the fact that the closing and opening of stomata was different in different species of plant. A literature review found that the screened plant species could be classified into several different photosynthetic types, including C_3, CAM (crassulacean acid metabolism), facultative CAM, and C_4. Furthermore, it has also been suggested that stomata are important pathways for VOC uptake (Keymeulen et al. 1993). Stomata usually open when leaves are transferred from darkness to light. However, reverse-phase stomata opening in succulent plants or CAM plants has been observed (Lee 2010). Therefore, the differences among these photosynthetic types for CO_2 uptake might affect VOC uptake through stomata under various light conditions. Our experiment found the effect of photosynthetic types on xylene removal. The results suggest that a mixture of facultative CAM, CAM, and C_3 plants is the most suitable system for xylene removal (Sriprapat et al. 2014). Therefore, multiple species are needed for maximum improvement in removing xylene volatile compounds under various conditions.

Furthermore, the weak crassulacean acid metabolism (CAM) characteristic of plants that are unregulated in response to water stress also has an effect on VOC uptake through stomata pathway under various conditions. The BTEX removal efficiency by well-watered plants was involved with day stomata opening and night closing, while the BTEX removal efficiency by a water-stressed plant can occur both day and night at a slightly lower rate than well-watered plants (Fig. 20.8) (Sriprapat and Thiravetyan 2013).

The effect of temperature on the BTEX removal demonstrated that cold temperature can cause low toluene and ethylbenzene removal efficiency. Therefore, the physiological and environmental factors of each plant species which are associated with stomata function including light and temperature should be taken into account for the maximum improvement of BTEX from contaminated air.

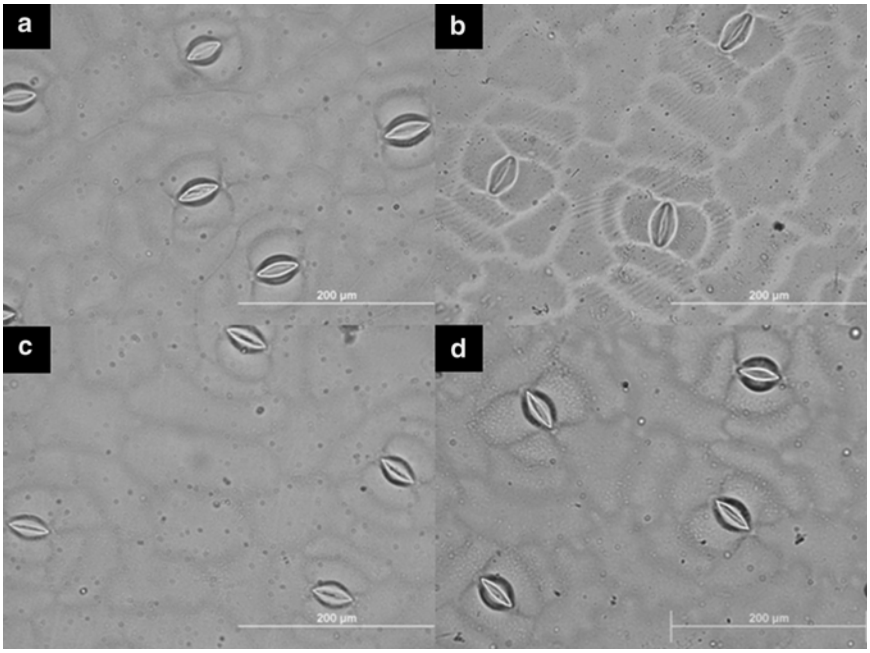
20.4.3 Effect of Cuticular Wax

Obviously, not only stomata number but also quantity and composition of cuticular wax were reported to affect benzene uptake in plants (Ugrekhelidze et al. 1997; Kvesitadze and Kvesitadze 2009; Topp et al. 1986). Cuticular wax was extracted and used directly to treat BTEX in our experiment. The result suggested that cuticular wax could adsorb and accumulate these compounds (Fig. 20.9).

Nevertheless, when the number of stomata and amount of wax were sufficient to remove xylene from the system, a small leaf area might be as efficient as a large leaf area in the treatment of lower dosages of VOCs. It is possible to calculate how much leaf area is needed to achieve the maximum of VOC removal ability (Wood et al. 2006; Sriprapat and Thiravetyan 2013).

Several food safety researches reported also the accumulation of benzene in the wax of plants (Gorna-Binkul et al. 1996; Environment Agency 2009; Slaski et al. 2000; Collins et al. 2000; Riederer 1990; Kylin et al. 1994). In 1996, the benzene contamination on orange shells and parsley was found (Gorna-Binkul et al. 1996), and in blackberry and

Fig. 20.8 Stomata of well-watered *Z. zamiifolia* under 24 h of light conditions (**a**) and 24 h of dark conditions (**b**) and stomata of water-stressed *Z. zamiifolia* under 24 h of light conditions (**c**) and 24 h of dark conditions (**d**) (Reprinted from Sriprapat and Thiravetyan 2013, with permission from Elsevier)



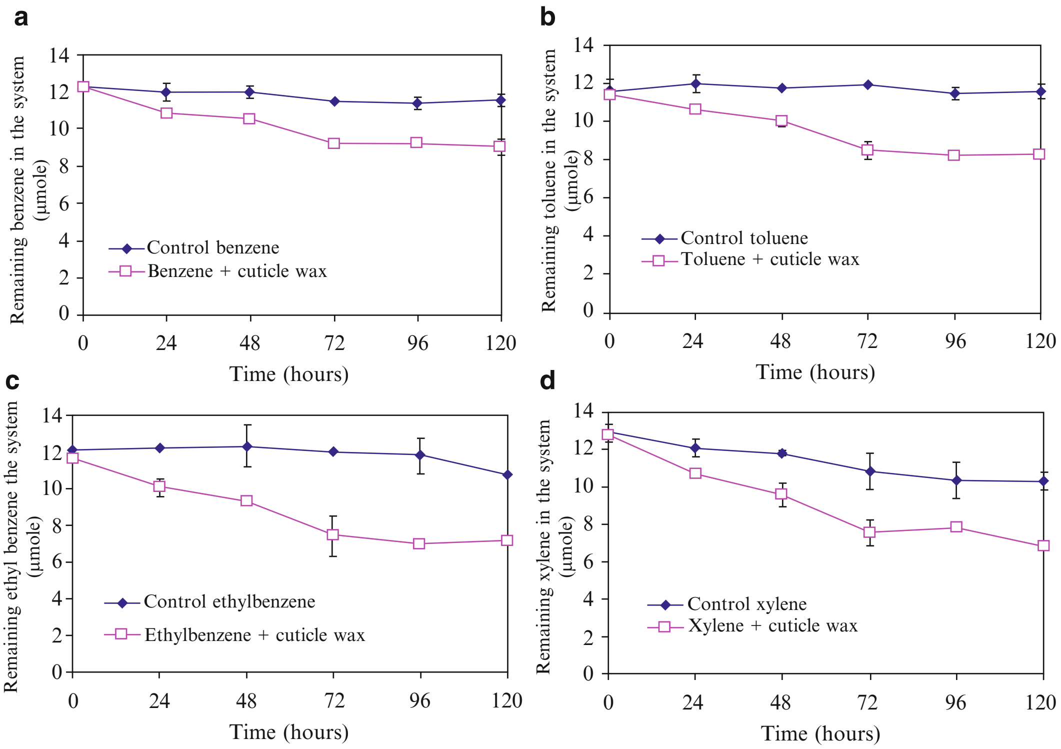


Fig. 20.9 Remaining benzene (**a**), toluene (**b**), ethylbenzene (**c**), and xylene (**d**) concentrations in the system after being treated with crude wax of *Z. zamiifolia* for a 120-h period. Values are means ± standard deviations (Reprinted from Sriprapat and Thiravetyan 2013, with permission from Elsevier)

apple leaf, benzene accumulation was also found (Collins et al. 2000). However, different plant species showed different benzene adsorption efficiencies (Gorna-Binkul et al. 1996; Collins et al. 2000; Ugrekhelidze et al. 1997). The effect of quantity and composition of wax on benzene removal was reported and needed to be studied. The effect of quantity of wax on benzene uptake wax was analyzed. The results showed that although high benzene uptake was found in some high quantity of wax species, some high quantity of wax species showed low benzene removal efficiency

(Treesubsuntorn and Thiravetyan 2012). The quantity and composition of wax was interesting to investigate, and the same results with plant leaf experiments in the relation of quantity of wax and benzene uptake were found in dry plant leaf materials. An unclear relation between quantity of wax and benzene uptake was also found. Although low quantity of wax was found, high benzene removal efficiency was found in *M. paradisiaca* leaf material. In contrast, *C. siamea* leaf material showed low benzene removal despite high wax quantity was found. In previous literature reviews, the composition of wax was reported to be a more effective factor than quantity of wax (Topp et al. 1986). In our research, this experiment was investigated. The wax of plant leaf material was extracted, and the composition was analyzed by GC-MS. C18 fatty acid such as alpha-linolenic acid and octadecanoic acid was found to be a high composition in *M. paradisiaca* leaf material. On the other hand, alpha-linolenic acid was not found in *C. siamea* leaf material, which had low benzene removal despite high wax quantities were found. The increasing number of carbon on the fatty acid molecule can decrease the polar fatty acids and might increase the solubility in benzene (Hoerr and Balston 1994). However, in our study, although octadecanoic acid and alpha-linoleic acid had the same amounts of carbon, the results suggested that only linoleic acid could be a factor in increasing benzene adsorption. In addition, benzene adsorption by pure fatty acids would be further studied for benzene adsorption.

Benzene desorption from plant leaf material by hexane was rapidly found and completed. The results suggested that physical adsorption was shown as the adsorption mechanism. The result was confirmed by FT-IR. The functional groups on the surface of the plant leaf material before and after the treatment showed the same pattern that recognized nonchemical bonding in this adsorption mechanism.

20.5 Phytoremediation-Enhancing Technology

Phytoremediation has been proposed to be an effective method for metabolizing a variety of organic pollutants. Nevertheless, the low activity in pollutant removal of plants is often found in the practiced applications. Based on these findings, the plants' P450 genetic modification was proposed as an interesting field for improving the BTEX phytoremediation efficiency (Doty et al. 2007; James et al. 2008). The efficacy of plants can be enhanced by introducing the genes of other organisms including microbial and humans which are known to be involved in metabolizing BTEX pollutants. Many engineered plants also showed high metabolism of benzene and toluene compared to the wild type (James et al. 2008). Furthermore, early studies of air detoxification by household plants found that the microorganisms in the rhizosphere and phyllosphere could contribute to the VOC removal. Several studies of plant-soil microcosms have shown that benzene or toluene can be metabolized by benzene-degrading or toluene-degrading microbes in the soil or on leaf surfaces (Wood et al. 2001; Orwell et al. 2004; De Kempeneer et al. 2004).

20.6 Conclusion

As we can see, the use of plants to treat BTEX contaminated air is a possible method which has been widely studied. The plants could uptake and transform these compounds by cytochrome P450 monooxygenase and dioxygenase enzymes. These enzymes degraded BTEX to low molecular weight molecules such as organic acids and amino acids. While phytoremediation has been proposed as an effective method, the low activity in pollutant removal of plants is often found in the practiced applications. This might be because of the surrounding environmental factors including temperature, light, and humidity. Therefore, factors affecting BTEX removal by plants should be taken into account. The factors affecting BTEX-contaminated air removal which involve stomata, cuticular wax, pollutant molecular size, plant species, toxic tolerance, and growing conditions were studied. However, for the maximum improvement of BTEX from contaminant air, the other factors should be accounted for. In addition, epiphytic bacteria on the plant leaf might also be involved in BTEX removal and need further studies.

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Phytoremediation of PAH-Contaminated Areas

21

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21.1 Introduction

The polycyclic aromatic hydrocarbons (PAHs) include more than 200 compounds with two or more fused benzene rings, e.g., phenanthrene, anthracene, pyrene, fluoranthene, chrysene, benzo(a)pyrene, and benzo(a)anthracene. It has been found that 16 PAHs are particularly dangerous, especially highly carcinogenic benzo(a)pyrene. Due to urbanization and industrial development, the PAHs are among the most common persistent organic pollutants of the environment with relatively low acute toxicity, but very distinct chronic toxicity, because of mutagenic, carcinogenic, and teratogenic effects on organisms. The toxicity of PAHs is structure dependent. The human body with food takes in 3–4 mg of PAHs, and the permissible concentration in water is 0.2 mg dm^{-3}. PAHs can penetrate through plant membranes, not only causing inhibition of plant growth and morphological changes, e.g., chlorosis, but also initiating oxidative stress with consequent metabolic changes.

21.2 Sources of PAHs in Soils

PAHs in soils originate from the following sources: natural biosynthesis, geochemical reactions, as well as from anthropogenic ones. PAHs may be formed during changes of humic compounds into peat or lignite (a type of brown coal) carried out by the soil microorganisms. The processes in the environment of PAH synthesis by plants and selected algae are regarded as natural sources of these compounds. Precursors for formation of polycyclic aromatic hydrocarbons are terpenes (cyclic hydrocarbons), widely distributed in plants. However, the participation of natural processes in PAH introduction into the environment is minor in comparison to anthropogenic sources (Korte 1999; Nam et al. 2003). The concentrations of aromatic compounds occurring in natural biosynthetic processes are low, and the formed compounds have a lower molecular weight structure than those emitted into the environment as a result of human activity. The anthropogenic sources derive from the combustion of organic matter, and the most significant are coke production, petroleum processing, fuel combustion in car engines, abrasion of road surfaces and tires, combustion of liquid fossil fuels, and incineration and burning of biomass. PAHs may migrate through air due to the high ability of sorption on suspended particle matter. They are present in the soil as a result of dry deposition from polluted air, together with rainfall and surface runoff (Kluska and Kroszczyński 2000; Khodadoust et al. 2000; Maliszewska-Kordybach 2005; Nam et al. 2003). Furthermore, the presence of PAHs in soil is the result of uncontrolled fuel leakage from petrol stations and air bases. The use of agricultural sewage sludges and composts also brings the hazard of contamination of soils and waters with PAHs. At present, the aforementioned way of sewage sludge usage is limited in legislation by the level of contamination of sewage sludges with heavy metals and pathogens. So far, there are no permissible PAH concentrations in sewage sludges applied in agriculture. In EU countries, there are some planned corrections with respect to the sludge directive (86/278/EWG) concerning toxic organic compounds such as PAHs, establishing the permissible total concentration of 11 PAHs at the level of 6 mg/kg d.m. (Directive EU 86/278/EEC; Gromiec 2001).

21.3 PAH Transformation in Soils

PAHs present in the environment undergo continuous changes, both biological and without the presence of microorganisms (Brown et al. 2003; Doong 2003). The processes

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of volatilization, leaching, sorption, photodegradation, chemical oxidation, and reactions with other compounds are thought to be changes without the participation of microorganisms (Enell et al. 2004; Rasmussen and Olsen 2004). The processes of bioaccumulation and biodegradation (metabolism and co-metabolism) are regarded as biological degradation (Giraud et al. 2001). Therefore, the concentration of PAHs in soils results from their accumulation in the soil and continuously occurring degradation. Among the mentioned abiotic processes, sorption on organic matter particles is of high importance. However, under certain conditions, e.g., low hydration, the sorption (reversible or nonreversible) may refer to mineral soil particles. Interactions between xenobiotics and the soil complex may result in formation of so-called bound residues as a result of covalent and ion bonds, which hamper both extraction and their determination. Sorption may also occur as a surface process and then compounds are weakly bound with the soil matrix (hydrogen bonds, van der Waals bonds). The mechanism of immobilization of persistent compounds in soil is complex and depends on the following factors: particle size, content of organic compounds (humic compounds), sorption capacity, time of exposure to pollution, and soil pH (Conte et al. 2001; Kluska 2004; Maliszewska-Kordybach 2005; Zbytniewski and Buszewski 2000). The sorption of hydrocarbons onto solid particles can be restricted by the presence of other compounds that are competitive in the sorption process such as trihalomethanes (THMs), although they are persistent to the sorption process (Traczewska 2003; Xia et al. 2009). During the volatilization and leaching processes, PAHs enter the air or the water. However, the volatilization concerns only compounds with a high value of Henry constant such as hydrocarbons of low molecular weight. Leaching is a result of surface runoff and/or infiltration of rainwater that together with the fine particles contributes a certain load of hydrocarbons to the surface and groundwaters (Enell et al. 2004; Khodadoust et al. 2000; Mulder et al. 2001).

Chemical oxidation as well as photodegradation are the changes when PAHs undergo destruction (Cuypers et al. 2000; Feilberg and Nielsen 2001; Little et al. 2002; Zeng et al. 2000). During reactions of attachment and replacement of hydrocarbons with other compounds of the matrix, the PAH derivatives are formed, whereas catalytic hydrogenation as well as photochemical chlorination are reactions of attachment. The range and the rate of degradation processes of PAH in soil depend on the properties of hydrocarbons and on the soil properties. Abiotic degradation depends on natural factors such as seasonal and geographic conditions as well as physical–chemical soil properties that determine the growth of microflora (e.g., temperature, humidity, pH, organic matter composition) (Maliszewska-Kordybach 2009; Smreczak and Maliszewska-Kordybach 2003). The activity of microorganisms depends on the presence of catalytic factors, e.g., heavy metals and other toxic organic compounds (Baran et al. 2003). Photochemical decomposition is a significant process in the degradation of PAHs in the surface soil layer (up to 3 mm). The PAHs are sensitive to the presence of oxygen and other oxidative compounds. Chemical oxidation leads to the removal of PAHs, but the derivatives occur at the same time as the products of oxidation, of which the identification is fragmentary at present. Depending on the oxidation, chlorine derivatives (chlorine–epoxy–quinones) or volatilized halogen compounds are formed. UV radiation causes the formation of oxygen diols, quinones, and aldehydes. The individual hydrocarbons are not equally susceptible to decomposition. The light persistence decreases independently with the solubility and is as follows: Naph > Phen > Ch > Pyr > Anth > BaP > IP. It was proved that higher intensity of light and higher oxygen concentration increase decomposition of hydrocarbons. It is due to the fact that higher intensity of light may break rings and may cause the degradation of formed derivatives (Little et al. 2002). Oxidation takes place with the use of hydroxyl radical with Fenton reagent, ozone, persulfate permanganate ion, and, in two stages, hydrogen peroxide and UV radiation. The results of investigations indicate the possibility of application of oxidants in in situ and ex situ treatment of sediments and soil (Brown et al. 2003). Oxidation was also applied in the primary process of supporting PAH biodegradation in sediments and soils. The application of oxidant causes changes in the organic matter structure and the desorption of hydrocarbons into the liquid phase. That causes favorable biodegradation conditions as PAHs occurring in high concentrations strongly sorbed onto particles are poorly available for microorganisms. After initial oxidation the level of biodegradation in soil reaches the value of 70 %, whereas in sediments, it reached the value of 90 % (Cuypers et al. 2000).

Biosorption is an important phenomenon with respect to environmental protection. The process is based on the ability of microorganisms to take up, concentrate, and cumulate contaminants, taking place as intracellular accumulation, binding with the wall of the cells or the membrane of the cytoplasm, as well as forming a complex with polymers outside the cells (Traczewska 2003). Sorption in microorganism cells is complex and depends on the solubility and active surface of sorption. Biosorption may have an influence on the further PAH degradation during the remediation of soils originating from contaminated areas. In the investigations into the impact of sorption on the bioavailability and biodegradation in soils coming from polluted areas, a high level of PAH biodegradation was found in soils covered by plants. It is considered that sorption significantly decreases the biodegradation of PAHs in soils in which it was proved that the sorption limited the amount of PAHs available to the biodegradation changes. It is due to the fact that sorbed particles of PAHs are present in micropores that are not big enough to allow the access of microorganisms. The magnitude of desorption and diffusion rates of the aforementioned compounds from the micropores limits the amount of hydrocarbons available for the process of biodegradation.

The degradation of hydrocarbons may be performed by the pure strain bacteria as well as their mixtures in which indirect products of decomposition are used by the others as substrates. Biodegradation can be carried out not only by bacteria but also by fungi; various species of bacteria, e.g., *Actinobacteria*; and some algae, e.g., *Cyanobacteria*, as well. They can be distinguished into two groups with respect to the mechanism of their activity: one group uses the hydrocarbons as the only source of carbon and energy, whereas another group has the capacity for co-metabolic changes (Haritash and Kaushik 2009; Klimiuk and Łebkowska 2003). Although there are many microorganisms able to decompose the PAHs, usually the microorganisms are not ready to use the hydrocarbons in metabolic reactions when the contaminants are entering the environment. It is necessary to produce the proper enzymes and genetic changes leading to the formation of genetic abilities to metabolize aromatic compounds. The time of adoption of the microorganisms in the decomposition of hydrocarbons depends on the composition and the quantity of the population and on environmental conditions. The time is shorter when the microorganisms were formerly exposed to the presence of the hydrocarbons. It should be pointed out that under natural conditions, the oxygen freely diffuses through the layer of soil up to 3 mm. Biological changes under environmental conditions may be hampered by the presence of other chemical compounds, such as surface active agents and pesticides, and determined by the presence of oxygen inorganic compounds, e.g., heavy metals, deficit of nutrients (culture medium) and co-substrates, and the presence of appropriate microorganisms (Chang et al. 2002, 2003; Lazzari et al. 2000). It was proved that there is a linear correlation between the PAH degradation rate and the concentration of the following heavy metals: Zn, Cu, and Pb. The results of the investigations in soil indicated that increasing persistence of PAHs in soils in the presence of Zn, Cu, and Pb is due to the fact of the toxic activity of those metals on the microorganisms participating in PAH biodegradation (Maliszewska-Kordybach 2003). The microorganisms show various possibilities of degradation of individual compounds. The efficiency of hydrocarbon biodegradation depends on the structure of the compound, the level of aromatic concentration, and the physical–chemical properties. Low-ring hydrocarbons undergo decomposition faster however; the microbiological decomposition of hydrocarbons of more sophisticated structure is also possible. The main requirement for the process to take place is the presence of another source of carbon easily available to the microorganisms (co-metabolism) (Giraud et al. 2001; Haritash and Kaushik 2009). The efficiency of PAH biodegradation may vary and depends on the presence of other hydrocarbons. The effect of the interaction with other substrates on the biodegradation rate of PAHs is of prime importance, and it is necessary to recognize the changes in the ecosystem. The intensity of PAH biodegradation under anaerobic conditions is from 10 to 100 times lower than under aerobic ones (Haritash and Kaushik 2009). The interaction between microorganisms and PAHs is not precisely known; however, it should be concluded that the issue is very difficult. The studies of biodegradation of PAHs are usually carried out using distilled water or soil spiked with a known amount of PAHs as a matrix. The process of biodegradation with the application of microorganisms is often carried out using monocultures primarily adapted to the metabolic changes, despite the fact that the mentioned conditions do not occur in the environment (Chang et al. 2002; Doong 2003; Haddox and Cutright 2003; Hallberg and Trepte 2003; Nocentini and Pinelli 2001). Moreover, the biodegradation is conducted at different contents of oxygen: under aerobic and anaerobic conditions as well as under various redox conditions. There are also various parameters such as time of incubation, temperature, and type of added mineral culture medium (Chang et al. 2002). The persistence of PAHs in the environment can be expressed by the half-life. However, in the literature, diverse information can be found as hydrocarbons were determined under different conditions of conducted investigations. The examples of half-life values determined for selected PAHs by different authors are presented in Table 21.1.

21.4 Concentration of PAHs in Soil

The highest concentration of PAHs in the surface soil layer is determined especially in areas of municipal–industrial agglomerations, areas located in the neighborhood of communication routes, fuel processing, steelwork plants, car bases, and petrol stations (Kluska and Kroszczyński 2000). Apart from the distance from the source of emission, an important factor is the type of soil (clay, sandy) and soil properties (grain composition, humic content, sorption capacity, pH, etc.). Moreover, higher concentrations of PAH are determined in the saturation zone than in the aeration zone. Therefore, in the literature, there is divergent concentration of PAHs in soils, and the concentration of individual PAHs varies from the limit of detection up to several hundred μg per kg of soil. In the literature there is available information of the concentration of PAHs up to 30 g kg^{-1} in the neighborhood of gas manufacture sites (Khodadoust et al. 2000). Other investigations regarding soils taken from different areas of Korea indicate that the total concentration of PAHs depends on the location and reached 2.8 mg kg^{-1} in the area of a steelwork, whereas in the area of Seoul, it was three times lower. The concentrations of individual PAHs are included in Table 21.2 and compared with concentration determined in Polish soil.

Table 21.1 Half-life of PAHs in soil days (Korte 1999; Maliszewska-Kordybach 2005; McNally et al. 1999; Northcoot and Jones 2001; Xia et al. 2009)

			Conditions			
			Xia et al. (2009)		McNally et al. (1999)	
PAH	(Maliszewska-Kordybach 2005)	(Korte 1999)	Aerobic	Anaerobic	Absence of fulvic acids	Presence of fulvic acids
Naphthalene	n.d.	766	12	6,192	n.a.	n.a.
Fluorene	7–28	1,168	n.d.	n.d.	0.83	0.75
Phenanthrene	n.d.	2,080	384	9,600	1,15	1.01
Anthracene	18–58	2,883	n.d.	n.d.	n.a	n.a.
Fluoranthene	n.d.	2,847	n.d.	n.d.	0.24	0.36
Pyrene	27–94	3,102	5,040	91,200	1.91	2.04
Benzo(a)anthracene	n.d.	2,956	n.d.	n.d.	n.d.	n.d.
Chrysene	72–976	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(b)fluoranthene	n.d.	3,285	n.d.	n.d.	n.d.	n.d.
Benzo(a)pyrene	n.d.	2,993	n.d.	n.d.	n.d.	n.d.
B(ghi)perylene	n.d.	3,321	n.d.	n.d.	n.d.	n.d.

n.d.—not determined

Table 21.2 Ranges of PAH concentration in soil in selected regions/cities in Polish soil in comparison to soil in Korea (Baran et al. 2004; Kluska 2005; Lisowska 2010; Nam et al. 2003; Włodarczyk-Makuła 2007a)

	Siedlce (Kluska 2005)	Bieszczady (Lisowska 2010)	Dęblin (Baran et al. 2004)	Częstochowa (Włodarczyk-Makuła 2007a)	Korea (Nam et al. 2003)
PAH	(ng kg^{-1} d.m.)	(μg kg^{-1}d.m.)			
Naphthalene	12–125	70–3,180	19–417	84–92	5–157
Acenaphthylene	16–135	170–2,410	49–732	99–108	Up to 41
Acenaphthene	18–123	40–2,730	19–370	36–45	Up to 33
Fluorene	13–115	50–6,260	9–100	302–363	Up to 39
Phenanthrene	10–114	70–3,910	9–449	87–95	1–141
Anthracene	16–117	120–15,500	4–164	146–160	Up to 43
Fluoranthene	13–114	60–13,770	4–775	399–454	2–353
Pyrene	10–113	10–840	14–654	302–344	1–317
Benzo(a)anthracene	10–117	60–2,060	60–794	358–409	Up to 284
Chrysene	8–115	10–130		789–874	Up to 267
Benzo(b)fluoranthene	3–111	10–80	41–645	373–413	Up to 431
Benzo(k)fluoranthene	up to 2.6	20–1,450	11–250	276–299	Up to 138
Benzo(a)pyrene	4–112	20–650	16–464	362–411	Up to 294
Dibenzo(ah)anthracene	n.d.	3,100–194,230	72–369	299–387	Up to 120
Benzo(ghi)perylene	n.d.	2,060–168,950	5–493	401–422	Up to 221
Indeno(1,2,3-cd)pyrene	14–111	690–5,610	3–134	251–348	Up to 248

n.d.—not determined

The total concentration of 16 studied hydrocarbons was in the range of 5.7 mg kg^{-1} d.m. in the area of parking places as well as at petrol stations. In soil taken from the airstrip, the concentrations of PAHs were lower and the total content did not exceed the value of 2.5 mg kg^{-1} d.m (Baran et al. 2003). The content of PAHs in soils originating from incineration of wooden carbon (in Bieszczady Mountains) was much higher and was in the range of 1 g kg^{-1}, of which 96 % were 4-, 5-, and 6-ring hydrocarbons (Lisowska 2010). Much lower concentrations of hydrocarbons were determined in the area of communication routes. In the investigations conducted by Kluska, the total content of PAHs was assessed at the level of 1.5 μg kg^{-1} next to a motorway, but 0.13 μg kg^{-1} at 15 m distance to the motorway.

The contamination of soil and air with PAHs influences their content in plants (trees, grasses, grain) including vegetables and fruits (Liste and Alexander 2000a, b). Taking into account the hazard for humans and animals, it is important that some products are consumed without any processing, ensuring the removal of hydrocarbons. This concerns fruits (e.g., apples) and vegetables (lettuce, cabbage, and tomatoes). In order to establish the aforementioned dependences, Tao et al. published the results of investigations of PAH concentrations in soils, air, and vegetables in China (Tao et al. 2006). The concentrations of 16 PAHs in cabbages were in the range of 65–596 μg kg^{-1} d.m. In the air samples the amount of PAHs was in the range of 0.077–1.32 ng m^{-3} and in soil from the studied area the range of 63.7–1,200 μg kg^{-1} d.m. (Tao et al. 2006).

Similar dependencies were found for vegetables originating from the area of Poland. Vegetables were picked up in the neighborhood of the communication route near Siedlce. Among studied vegetables the highest concentration of total PAHs was found in cabbage and reached 358 ng kg^{-1}. Much lower concentration of PAHs was detected in cucumbers (71.3 ng kg^{-1}), whereas the lowest was found in the root of parsley (29 ng kg^{-1}). High level of PAHs occurred in herbs, which are of prime importance due to the fact of their therapeutic application. The highest total PAH concentration was determined in the dandelion with the amount of 344.2 ng kg^{-1}, and 25 % comprised carcinogenic hydrocarbons. In leaves of coltsfoot, only selected hydrocarbons among those studied were determined, but over 40 % of the total amount were carcinogenic (Kluska 2005). Investigations on PAH transport from soil to wheat, oat, beans, and sunflower were carried out by Maliszewska-Kordybach and Smreczak (Maliszewska-Kordybach 2003; Smreczak and Maliszewska-Kordybach 2003). It was found that transport of PAHs through roots into aboveground parts of the plant is possible mainly for 2- and 3-ring hydrocarbons, whereas 4-, 5-, and 6-ring hydrocarbons are accumulated on the surface of the roots. It was indicated that the dependence of uptake of PAHs by the plants is correlated with their solubility in water, due to the fact that hydrocarbons of high solubility may easily penetrate through roots and they may migrate to the upper parts of the plants. On the other hand, the low soluble compounds of higher capacity for adsorption onto solid particles are not so readily available to the plants (Oleszczuk and Baran 2004).

21.5 Determination of PAHs in Soil and in Plants

Determination of PAHs in environmental samples requires several stages as well as advanced apparatus. The results depend on the precision of conducting the individual stages and applied apparatus. Additional elements limiting analysis are the selection of a representative sample, the presence of organic compounds in the mixture and interfering compounds, and low concentrations of analytes. Reliability of the results can be ensured by the proper preparation procedure and modern techniques allowing determination of quantity and quality of PAHs. The most often applied methods are capillary gas chromatography (CGC) (Method 8270D) with mass spectrometry (MS) or ionization detector GC-FID (Method 8100) and high performance liquid chromatography (HPLC) with fluorescence detector UV (Method 8310). The individual steps in preparation of samples comprise extraction of organic matrix from a sample taken from the environment using a solvent or a mixture of solvents, removal of interferences and isolation of a group of analyzed compounds, and then enrichment and analysis of quality and quantity.

The following extraction methods are applied in order to extract the organic fraction from the environmental samples: liquid–liquid extraction (Method 3510), continuous extraction (Method 3520C), solid phase extraction (Method 3535A), Soxhlet extraction (Method 3540C) and automatic extraction in Soxhlet apparatus (Method 3541), supercritical fluid extraction (SFE) (Method 3561), microwave-assisted extraction (MAE) (Method 3546), and ultrasonic extraction (Method 3550C). The next step in sample preparation is selection of analyzed compounds from the interferences isolated from the samples during extraction. The following processes are applied in order to isolate PAHs from solvent extracts: purification using silica gel (Method 3630C), purification on oxide aluminum (Method 3610B and Method 3611B), gel-permeate GPC purification (Method 36040A), and acid–alkaline purification (Method 3650B). In the case of plant samples, the stage of sample preparation should take into account the presence of chlorophyll and other pigments (carotene), which are hydrophobic and may be extracted together with PAHs. Therefore, gel-permeate purification with the use of chloroform or a mixture of hexane and acetone or a saponification process consisting of the decomposition of plant fats in an alkaline environment is applied. The application of each method is verified by determining the recovery of standard compounds, certified materials, internal standard, or isotope standards (isotopic dilution mass spectrometry, IDMS) as compounds with determined compositions of radionuclide atoms of isotopes ^{13}C and ^{2}H (Mechlińska et al. 2010; Zygmunt et al. 2003).

Due to the variety of methods of sample preparation in soil and plants, scientists apply various procedures of extraction, purification, and identification of quantification of PAHs. Therefore, the results of described investigations are difficult to compare. The second factor affecting the diversity of the results is the heterogeneity of samples obtained from the environment, which is confirmed by the physical–chemical analyses. The results of recoveries of PAHs from studied matrices are often different in comparison to individual compounds. The percentages of recovery of individual compounds from soils and plants are presented in Table 21.3. In investigations on the determination of PAHs in soil, the values of recovery after adding various amounts of standard mixtures of PAHs to samples were determined (Włodarczyk-Makuła 2007b).

The results proved that the amount of added standard (in the studied range) was not significant for the recovery of

Table 21.3 Recovery of PAHs from soil samples (Włodarczyk-Makuła 2007a, b; Dugay et al. 2002)

	Amount of standard mixture added to the soil		Addition of standard during preparation of plant samples (lettuce)		
	2	4	Before		
PAHs	mg kg^{-1} d.m.		Sonification	Saponification	Purification
Naphthalene	20 ± 2.6	22 ± 3.2	14 ± 3	16 ± 2	17 ± 2
Acenaphthylene	22 ± 4.1	19 ± 2.1	16 ± 4	13 ± 2	14 ± 1
Acenaphthene	23 ± 2.2	26 ± 2.8	22 ± 6	17 ± 2	18 ± 2
Fluorene	36 ± 2.8	35 ± 6.2	34 ± 5	32 ± 2	31 ± 2
Phenanthrene	77 ± 11	52 ± 13	38 ± 8	30 ± 2	31 ± 2
Anthracene	66 ± 7.1	56 ± 4.5	49 ± 10	44 ± 4	46 ± 3
Fluoranthene	82 ± 4.2	85 ± 8,0	65 ± 8	70 ± 3	71 ± 2
Pyrene	75 ± 10.0	72 ± 7.2	73 ± 5	75 ± 3	79 ± 3
Benzo(a)anthracene	86 ± 32	95 ± 2.9	93 ± 5	110 ± 5	114 ± 4
Chrysene	106 ± 3.2	98 ± 8.2	82 ± 4	102 ± 4	107 ± 3
Benzo(b)fluoranthene	102 ± 5.4	93 ± 6.0	81 ± 7	107 ± 4	103 ± 3
Benzo(k)fluoranthene	82 ± 2.9	85 ± 3.2	81 ± 7	107 ± 4	103 ± 3
Benzo(a)pyrene	95 ± 11.0	86 ± 3.9	98 ± 5	109 ± 5	113 ± 4
Indeno(1,2,3-cd)pyrene	74 ± 2.5	76 ± 3.6	101 ± 7	110 ± 7	106 ± 9
Dibenzo(a,h)anthracene	85 ± 2.5	77 ± 3.8	110 ± 9	113 ± 10	111 ± 4
Benzo(ghi)perylene	95 ± 2.6	86 ± 3.8	107 ± 11	108 ± 11	118 ± 5

±standard deviation

studied hydrocarbons with the exception of BaP and benzo(ghi)perylene. The lowest values of recovery were found for naphthalene, acenaphthene, and acenaphthylene. The investigations conducted by Dugay et al. (2002) indicated that the place of adding standard mixture to the sample did not significantly impact the value of the recovery.

An example of an applied procedure of preparation of soil and plant samples (10 g) in order to determine PAHs is the extraction with hexane (170 ml) in Soxhlet apparatus during 8 h described by Kluska (2005). After solvent evaporation, the purification process was carried out by solid phase extraction (SPE) on silica gel. Elution of PAHs was conducted three times with a mixture of toluene and acetonitrile (1:3, v/v). The determination was carried out using HPLC with a UV detector (Kluska 2005). Baran et al. (2004) applied sonification after adding dichloromethane. In this case, the solvent was replaced with acetonitrile and extracts were purified by SPE technique filled with octadecyl C18 conditioned with methanol and a mixture of 2-propanol and water. Elution of PAHs was carried out with acetonitrile. Liquid chromatography with UV detection was applied in order to identify the PAHs (Baran et al. 2004; Ling et al. 2010). Cyclohexane can also be used as a solvent (Conte et al. 2001) as well as dichloromethane with acetone (Lisowska 2010). It is necessary to exchange the solvent in the case of application of HPLC with a UV detector that may cause losses of PAHs. However, the exchange of solvent is not required in the case of applying GC-MS or GC-FID. The extraction is usually carried out with the use of dichloromethane or hexane in Soxhlet apparatus. After separation of solvent, the extracts are purified on silica gel and then the extracts are concentrated in a stream of free gas (Khodadoust et al. 2000). Determination of PAHs in plant samples is also carried out with the following detection systems: HPLC-UV, HPLC-FID, and GC-MS. Saponification is often required in the primary step of sample preparation. For this purpose, a solution of KOH or NaOH in a mixture of methanol and water is usually applied. In Table 21.3, the recovery of PAHs from plant samples with the added standard mixture in different preparation steps is presented. For example, the procedure of sample preparation of dry lettuce consisted of ultrasonic extraction with toluene, vaporization of toluene, and saponification of remaining dry matter (solid) with KOH in a mixture of methanol and water (Dugay et al. 2002). Lisowska applied extraction with hexane in Soxhlet apparatus over eight hours. After concentration, the extract was purified on silica gel by SPE. The elution of PAHs was carried out with a mixture of toluene and acetonitrile, and then the solvent was replaced with pure acetonitrile and analyzed by HPLC-UV (Lisowska 2010). Maila and Cloete (2002) applied dichloromethane for extraction of PAHs from grass samples, whereas GC-MS was used in order to identify the individual hydrocarbons. In studies on the accumulation of PAHs in cabbage, a mixture of n-hexane with cyclohexane in Soxhlet apparatus was used, whereas sulfonation ensured the removal of lipids. The determination of PAHs was carried out with GC-MS (Tao et al. 2006). The published results of investigations proved the diversity of applied methods of soil and plant sample preparation in order to determine PAHs, which are the result of application of solvents with various polarity, different extraction methods, and determination based on own experience as well as available facilities.

21.6 Application of Plants in Removal of PAHs from Wastewater and from Soil

The term phytoremediation refers to a method known since the seventeenth century consisting of applying plants to soil in order to treat it. Among phytoremediation technologies, the following can be distinguished: phytostabilization, phytoextraction (phytoaccumulation), phytodegradation, and phyto-vaporization. The major advantages of phytoremediation are favorable impact on the landscape and environment, low costs, and simplicity of the technology. The main disadvantage of phytoremediation is the long time of obtaining results, dependence of effectiveness of the method on bioavailability, and the level of pollution of the matrix. An additional disadvantage of phytoremediation is the necessity of utilization of wastes containing accumulated contaminants. Recently, a solution of the aforementioned problem is management of plant mass as an energy source (Gomez et al. 2010; Mendez and Maier 2008). The following species of plants are applied in phytostabilization technologies: *Sinapis alba*, *Brassica juncea*, and the grasses *Lolium perenne*, *Festuca rubra*, and *Poa pratensis*. They have the ability of immobilization of pollutants in the soil by accumulation in the root zone. The following plants are applied in phytoextraction: *Sida hermaphrodita*, *Spartina pectinata*, and *Salix viminalis* (Gana et al. 2009; Kramer 2005).

The available literature regarding application of plants in soil remediation is mainly concentrated on the removal of inorganic contaminants, especially heavy metals. There are many investigations described together with conditions of conducting the aforementioned processes with respect to soils and plants. Taking into account the removal of organic pollutants by plants, the literary data are limited. The data are mainly concentrated on determination of the level of contaminants in soils and in plants originating from those soils (Kluska and Kroszczyński 2000), or they concern natural application of compost and sewage sludges (Oleszczuk and Baran 2004; Włodarczyk-Makuła 2007b). Investigations of PAH concentrations in soils and plants coming from those soils proved the possibility of phytoaccumulation. It was found that the level of contamination of plants (dandelion, cabbage, parsley, carrot, cucumber) depended on the distance from the source of emission and the resulting amount of those compounds in soil (Kluska and Kroszczyński; Ling et al. 2010). Considering natural application of sewage sludges, it should be noted that sewage sludges, apart from being a source of valuable compounds that improve soil fertility, bring a high load of organic micropollutants such as toxic PAHs. Baran and Dugay determined the influence of such factors on soil properties as well as on the level of bioavailability of selected PAHs on microorganisms and plants (Baran et al. 2004; Dugay et al. 2002).

PAHs occur both in the natural environment and in plants. They may also be a biosynthesis product of algae. The plants may metabolize xenobiotics in the process of oxidation and co-metabolic changes, and they may accumulate reaction products in tissues, e.g., roots. The influence of those metabolites on the plant environment is not well known so far (Dugay et al. 2002). Plants have the ability to metabolize the benzene ring by detaching carbon. Using tea as an example, it was proved that after breaking the ring, organic acids are formed and accumulate in plant organs to which benzene has been added (roots, stem). The probable degradation pathway of benzene is as follows: benzene–phenol–pyrocatechol–benzoquinone–muconic acid. The changes are possible if hydrocarbons are available to plant roots and they migrate through the stem. It was found that plants are able to metabolize benzo(a)pyrene after adding it to roots or leaves (Sims and Overcash 1983).

Haritash and Kaushik (2009), describing the studies of other authors, proved that grasses and leguminous plants, e.g., lucerne, may significantly decrease the amount of PAHs in the contaminated soils. In the case of leguminous plants, the intensification of degradation of pyrene and anthracene of 30–40 % was obtained in comparison to soil without plants. Application of a mixture of grasses proved the intensity of decomposition of benzo(a)pyrene, benzo(a)anthracene, dibenzo(ag)anthracene, and chrysene stimulated by the growth of the aforementioned plants. In the literature, there is also information of the application of wild rye, oat, and wheat when decreases of PAHs in soils were noted.

The efficiency of biodegradation is correlated with the solubility of PAHs in water; higher solubility resulted in higher removal of PAHs from soils. Other studies proved a high quantity of microorganisms and their activity in soil covered by plants. This indicated that roots of the plants stimulating development of microflora by the appropriate enzymes intensify the biodegradation of hydrocarbons. Other scientists have proved that plants play an important role in the degradation of hydrocarbons that are compounds of crude oil by the release of enzymes (dehalogenase, nitroreductase, peroxides, and lactase) from roots that are able to transform organic pollutants. In this study, several species of grass such as *Festuca rubra* and *Lolium* were planted on soil contaminated with crude oil or diesel oil. The efficiency of removal in the presence of the aforementioned plants ranged up to 77 % and 92 %, respectively, whereas it reached 60 % and 74 %, respectively, in their absence (Haritash and Kaushik 2009).

Rasmussen and Olsen (2004) investigated the effectiveness of orchard grass (*Dactylis glomerata*) in the phytoremediation of hydrocarbons from sandy soil. They monitored the concentration of PAHs containing creosote, phenols, and heterocyclic hydrocarbons in water. Higher efficiency of removal of studied compounds was achieved in biological processes on sandy filter planted with grass than in filter without plants. The effectiveness depended significantly on time. It was proved that microbiological degradation occurred in co-metabolic changes and it was stimulated by plants. In the process of sorption and microbiological degradation, 3-ring PAHs were successfully removed.

Liste and Alexander (2000a) investigated degradation of pyrene with plants on clay soil of 2.5 % organic carbon. Nine plants were used in the experiment: oat, lupin, rape, fennel, parsley, pepper, and three pine species. After eight weeks, the efficiency of removal of pyrene ranged up to 74 % in soil covered by plants, whereas in soil without plants, it did not exceed 40 %. However, for individual crops and trees, the level of pyrene degradation was heterogeneous. A weak correlation between shoot and root biomass and capacity of plants for degradation of pyrene was observed. Further investigations by those authors were carried out in order to determine PAH concentrations in the rhizosphere of wheat plants cultivated on soil containing phenanthrene and pyrene. The concentration of pyrene was 4–5 times higher in rhizosphere cultivated in the studied soil than in soil without plants. It was concluded that plants may accumulate hydrophobic compounds by transport from the root system due to the fact that the rhizosphere of the studied kind of plants cultivated on soil contained much more phenanthrene and pyrene than the control soils.

Maila and Cloete (2002) determined the influence of the level of soil contamination with PAHs on the germination of *Lepidium sativum*. There was observed a decrease of the level of germination when an increase of PAH concentration was observed. At the concentration of PAH (naphthalene, fluorene, phenanthrene, anthracene, pyrene) equal to 1,000 mg kg^{-1}, the level of germination was lower than 16 %, whereas for 50 mg/kg, the level was higher than 75 %. Similar inhibition was found in the presence of nonionic surfactants. Tao et al. (2006) determined the dependence between the content of 16 PAHs listed by EPA in the air present in cabbage, in air (solid particles, gas), as well as in soil. The level of pollution of studied samples depended on the concentration of PAHs, meaning the place of taking samples; however, the profile of PAH concentrations in studied matrixes was similar. The concentration of PAHs in cabbage was positively correlated with the concentration in the air, whereas dependence of the level of soil contamination was statistically insignificant. There are a limited number of studies concerning the monitoring of efficiency of PAH removal from hydrophyte treatment plants. The studies are focused on the removal of organic compounds and nutrients. Limited data related to individual PAHs are available. Machate et al. (1997) investigated the degradation of phenanthrene in five-stage hydrophytes of vertical–horizontal flow using the following species of plants: *Scirpus lacustris* and *Typha* spp. The level of removal of phenanthrene reached up to 99.9 %. 1-Hydroxy-2-naphthoic acid (HNA) as a product of metabolic changes of phenanthrene was identified. A large decrease of the concentration of phenanthrene was obtained after the first stage of treatment, whereas in the further step of treatment, the concentration of phenanthrene was at the same level. The efficiency of plants in the removal of phenanthrene was found both in the summer and in the winter. A decrease of its concentration depended on the time of wastewater in the treatment plant, hydraulic parameters such as flow rate and porosity of filling, and the size of pilot installation (Machate et al. 1997). Giraud and coauthors (2001) investigated the process of degradation of selected PAHs during wastewater treatment. The studies were carried out on a pilot hydrophyte treatment plant and wastewater was contaminated with anthracene and fluoranthene. The aim of the investigations was to determine the role of fungi present in the mentioned ecosystem on the PAH changes. Forty species of fungi isolated and identified in the filling of the bed (gravel, sludges) were analyzed. The results of the investigations proved the possibility of removal of anthracene and fluoranthene from wastewater. Thirty-three species of fungi were able to degrade fluoranthene, whereas only two species of fungi were able to degrade anthracene. However, the level of PAH degradation of the abovementioned hydrocarbons did not exceed 70 %. The studies discovered species of fungi that were not formerly identified, which gives the possibility to improve the efficiency of PAH removal in hydrophyte systems after inoculating the filling with appropriate fungal species.

21.7 Mycoremediation as an Alternative Biomethod for PAH Removal

A feasible alternative method to clean up soil contaminated with PAHs is mycoremediation using white rot fungi (WRF). In the 1980s the investigation of possibilities of using WRF in the degradation of xenobiotics polluting the environment was begun. The first study demonstrated that 22 different PAHs were oxidized in the range of 70–100 % during 27 days in nitrogen-limited cultures of *Phanerochaete chrysosporium* (Bumpus 1989).White rot fungi produce highly active ligninolytic enzymes able to decompose lignin or both lignin and cellulose in wood. The secreted enzymes with low specificity towards the oxidized substrate also have the ability to break down organic xenobiotics structurally similar to lignin, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), dioxins, pesticides, explosives, dyes, solvents, etc. (Arun et al. 2008; Baldrian 2003; Joshi and Gold 2000). The extracellular ligninolytic enzymatic complex consists of peroxidases [lignin peroxidase (LiP), manganese peroxidase (MnP)] and versatile laccase (Lac) (Bogan and Lamar 1995; Hammel et al. 1986; Moen and Hammel 1994; Sanglard et al. 1986; Zheng and Obbard 2002).

Although both LiP and MnP are ferric iron containing heme proteins requiring peroxide for their function (Zheng and Obbard 2002), their catalytic mechanisms are different. Lignin peroxidases in conjunction with H_2O_2 or other hydroperoxides (e.g., oxalic acid, veratryl alcohol) can remove one electron or a hydrogen atom from a PAH molecule to form an aryl cation radical which is oxidized further to form a quinone with subsequent cleavage reactions (Barr and Aust 1994; Hatakka 1994; Kersten et al. 1985; Mester and Tien 2000; Reddy 1995). The degradation of PAHs by most white rot fungi was via quinone intermediates, but degradation without quinone intermediates, e.g., anthracene degradation by *Trametes trogii*, was also confirmed (Field et al. 1992; Levin et al. 2003). MnP generate Mn^{3+} from Mn^{2+}, which is stabilized by chelators acting as low molecular weight redox mediators (Cavalieri and Rogan 1985). The Mn^{2+} regulates the induction of LiP and MnP (Zheng and Obbard 2002). Activity of LiP is inverse, while MnP activity is a direct function of Mn^{2+} concentration (Bonnarme and Jeffries 1990; Kishi et al. 1984). Laccase is an extracellular copper-containing polyphenol oxidase (Collins et al. 1996; Torres et al. 2003), also called "blue enzymes for green chemistry," because of being friendly to the environment (Riva 2006). Pure laccase can degrade numerous xenobiotics, but its direct use in the environment is limited by high cost (Li et al. 2010). The most effective laccase production and PAH degradation was documented at pH of 4.0 for *Pleurotus ostreatus* (Pozdnyakova et al. 2006) and *Ganoderma lucidum* (Ting et al. 2011). Usually optimum PAH degradation occurs in acidic environments and those rich in organic carbon, but for *Lentinula edodes*, rapid PAH degradation was also confirmed at pH 6.86–7.48 (Gąsecka et al. 2012). PAH oxidation by ligninolytic enzymes is limited by ionization potential (IP); e.g., *Phanerochaete chrysosporium* LiP can oxidize PAH up to IP of 7.55 eV (Hammel et al. 1986) and MnP in manganic acetate–acetic acid systems with IPs of ≤ 7.8 eV (Cavalieri and Rogan 1985). However, the oxidization of phenanthrene by MnP requires high IP (i.e., $IP = 8.03$ eV) (Moen and Hammel 1994).

Phanerochaete chrysosporium was the most intensively studied fungus, but the degradation of PAHs by other fungi secreting ligninolytic enzymes was also confirmed and ranged from a few to nearly 90 % of the initial concentration (Arun et al. 2008; Cajthmal et al. 2002; Valentín et al. 2006). A number of rings in the PAH molecule, species of fungus, and experimental conditions affected oxidation of PAHs. The 2-ring naphthalene was documented to be oxidized from 12 (*Pycnoporus sanguineus*) to nearly 36 % (*Daedalea elegans*), the 3-ring acenaphthene from 2 (*Coriolus versicolor*) to nearly 21 % (*Pleurotus ostreatus*), fluorene (3 rings) from 7 (*Fomitopsis palustris*) to 23 % (*Coriolus versicolor*), anthracene (3 rings) from ~2 (*Daedalea elegans*) up to nearly 32 % (*Fomitopsis palustris*—brown rot fungi secreting ligninolytic enzymes), phenanthrene ~26 % (*Pleurotus* sp.), pyrene from ~4 (*Pycnoporus sanguineus*) to more than 80 % (*Irpex lacteus*, *Phanerochaete sordida*, *Bjerkandera adusta*), fluoranthene (4 rings) 12 % (*Phlebia radiata*) to 71 % (*Pleurotus eryngii*), chrysene (4 rings) ~30 % for most of the mentioned fungi, and 45 % for *Phanerochaete chrysosporium* (Arun et al. 2008, Valentín et al. 2006). Oxidation of more concentrated PAHs (5 and 6 rings) is a very difficult process and ranges from 29 to 42 % (Gramss et al. 1999).

PAH degradation by *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Polyporus ciliatus*, *Stereum hirsutum*, *Pleurotus eryngii*, and *Phlebia radiata* was strongly and negatively dependent on salinity. However, ligninolytic activity of *Irpex lacteus* and *Lentinus tigrinus* was not dependent on salinity at sea water level, while 32 % of salinity inhibited ligninolytic activity of *Bjerkandera adusta* (Valentín et al. 2006).

The biodegradation of PAHs is also limited by their bioavailability due to low aqueous solubility related to strong adsorption to soil particles (Sarthoros et al. 2005; Zheng and Obbard 2002). Surfactants' addition through solubilization and increasing microbial accessibility to insoluble substrate (Tiehm et al. 1997; Volkering et al. 1992; Zheng and Obbard 2000) enhanced PAH bioavailability of all 16 PAHs for *Pleurotus ostreatus* to 86 %, reaching 89 %, 87 %, and 48 % of 3, 4, and 5 rings, respectively (Eggen 1999; Zheng and Obbard 2001).

The oxidation of PAHs can also be enhanced by synergistic interactions between white rot fungi and soil indigenous microorganisms. The oxidation of low molecular weight PAHs (acenaphthene, fluorene, phenanthrene, fluoranthene, and pyrene) was increased up to 43 % for *Phanerochaete chrysosporium* in a soil slurry, while high molecular weight PAHs (chrysene, benzo(a)pyrene, dibenz(ah)anthracene, and benzo(ghi)pyrene degradation) were decreased in the presence of fungi (Zheng and Obbard 2002). However, soil inoculation with *Pleurotus ostreatus* increased oxidation of high molecular weight PAHs with simultaneous inhibition of anthracene and pyrene degradation (In der Wiesche et al. 2003). On the other hand, coculture of *Pleurotus ostreatus* and *Irpex lacteus* with bacteria *Pseudomonas putida* did not improve the efficiency of phenanthrene, anthracene, fluoranthene, pyrene, and benzo(a)pyrene compared to degradation with individual fungi and bacteria (Šašek et al. 2003).

A beneficial solution in remediation of soils contaminated with PAHs is the use of spent mushroom compost/substrate (SMC/SMS) or colonized mushroom substrate (substrate before fruiting). The mushroom industry involves large quantities of SMC/SMS (by-product from commercial mushroom production) with great capacity to decompose a wide range of organic pollutants including PAHs (Eggen 1999; Gąsecka et al. 2012, 2013; Li et al. 2012). Moreover, SMC as an excellent source of enzymes, microorganisms, nutrients, and organic matter functions as a nutritional supplement for crops and a soil conditioner (Ribas et al. 2009; Semple et al. 2001; Zebulun et al. 2011). The SMC can be directly applied to the environment because of its ability to change the physical and chemical soil properties such as pH, moisture, and soil structure and to increase the activity of entering or already present soil microflora (Ribas et al. 2009; Semple et al. 2001; Zebulun et al. 2011). Compost from edible white rot fungi such as *Pleurotus ostreatus*, *Lentinula edodes*, and other *Basidiomycota*—phase II mushroom compost (mature compost from *Agaricus bisporus* cultivation)—was confirmed to degrade a high percentage of PAHs (Eggen 1999; Gąsecka et al. 2012, 2013).

The degradation of selected PAHs by refuse from the *Pleurotus* industry was dependent on initial concentration of PAHs, number of aromatic rings in the PAH molecule, the ratio of contaminated soil to by-product, time of incubation and type of refuse (compost or colonized substrate), and the way of compost application (Eggen 1999; Gąsecka et al. 2013). The degradation of acenaphthene, fluorene, and phenanthrene may reach up to 90 %, anthracene to 58 %, fluorene to 51 %, and pyrene to 48 % (Eggen 1999; Gąsecka et al. 2013). The removal of 4- and 5-ring PAHs was much more inconvenient and the results indicated degradation of 79–90 % (chrysene and fluoranthene) but only 45 % degradation of 5-ring PAHs (Eggen 1999).

The high potential to oxidize PAHs up to 86–87 % of anthracene, 78–79 % of phenanthrene, 40–83 % of fluoranthene, and 63–85 % of pyrene was also confirmed for phase II mushroom from *Agaricus bisporus* and *Lentinula edodes* composts (Gąsecka et al. 2012; Reid et al. 2002).

The satisfactory results were also confirmed in an experiment of degradation of naturally contaminated soil by PAHs from Silesia in Poland. The selected PAH concentrations in Silesia were as follows: 138–142, 357–465, 367–400, and 271–326 μg kg^{-1} d.m. of anthracene, fluoranthene, phenanthrene, and pyrene, respectively. After 12 weeks of incubation with 20 % of compost from *Pleurotus ostreatus* or *Agaricus bisporus*, the degradation was in the range 40–75, 43–68, 38–62, and 45–70 % for anthracene, fluoranthene, phenanthrene, and pyrene, respectively (Gąsecka, unpublished data). Another study on cleaning up PAH-contaminated soil by compost confirmed total degradation of 15 PAHs up to nearly 33 % after 60 days of incubation (Li et al. 2012).

21.8 Biomonitoring of PAH

For nearly two decades, pine needles have been studied for the accumulation of PAHs and employed as passive vegetation samplers in numerous European, American, and Asian countries. Within the *Pinus* genus, numerous species (e.g., *P. sylvestris* L., *P. pinea* L., *P. pinaster* Ait., *P. brutia* Ten., *P. massoniana* Lamb., *P. nigra* Arn., *P. strobus* L.) have proved to serve as cost-effective and easily collectable matrices suitable for long-term monitoring of lower troposphere pollution with PAHs (Hwang et al. 2003; Hwang and Wade 2008; Lang et al. 2000; Lehndorff and Schwark 2004; Piccardo et al. 2005). The high wax content in the cuticle and pore structure of the surface layer of pine needles provides high entrapment efficiency and enables high rates of PAH accumulation from the vapor phase (as well as particle-bound pollutants) over time. Additionally, needles from second- and third-year shoots can be collected simultaneously, since pine trees maintain their needles for even several years of vegetation. Although PAHs (and other semivolatile organic compounds) trapped on pine needles undergo volatilization and phytodegradation processes (Sun et al. 2010; Wang et al. 2005) and low molecular weight PAHs are easily removed from the needle surface with rainfall water, numerous studies have attempted to confirm a quantitative relationship between their accumulation in plants and the level in the environment (air and/or soil) (Augusto et al. 2010; Zhu et al. 2008). In comparison to technical monitoring, passive vegetation dosimeters provide data of the cumulative (time-integrated) concentration of the contaminant and its real impact on vegetation, as well as of the rate and drift of pollution spreading in the troposphere. Biomonitors allow a large number of sampling sites and simultaneous analysis of numerous pollutants (metals, sulfur, radionuclides, dioxins, PCB, pesticides, etc.) in the same sample (Wolterbeek 2002). Furthermore, the impact of specific anthropogenic activities on air pollution and contamination patterns according to the diagnostic PAH ratio indicating petrogenic, pyrogenic, or mixed sources of air pollution may be assessed (Zhang et al. 2005, Tobiszewski and Namieśnik 2012).

Recent studies on accumulation of PAHs in pine needles were conducted by Ratola et al. to monitor the levels and sources in ambient air pollution in Portugal (2010) and the island of Crete (2011). In both cases, the most abundant *Pinus* species were chosen for the studies, and pine needles were collected at industrial, urban, rural, and remote sites throughout the investigated area. In Portuguese continental territory, the 16 PAHs were detected in all the sites within the range from 76 up to 1,944 ng g^{-1} d.w. for remote and urban sites, respectively. Among the total PAHs, 3- and 4-ring hydrocarbons were dominant, and Phe reached the maximum level due to relatively high photostability in atmospheric air conditions (~30 % on average of the total 16 PAH concentration). In contrast, Naph occurred at lower concentrations due to its volatilization, relatively high water solubility, and high rate of removal from needle surfaces with rainwater. Studies also revealed species-specific sorption abilities towards the 16 PAHs with stronger entrapment efficiency observed for *P. pinaster* versus *P. pinea*. Among weather conditions, only wind speed significantly influenced the accumulation of PAHs in pine needles. In Crete, second- and third-year pine needles accumulated from 36 and 89 ng g^{-1} up to 2.6 and 2.9 μg g^{-1} d.w. at remote and urban sites, respectively, with the highest level observed in Heraklion (the capital of Crete). Similar as in Portugal, the Cretan study revealed a species-specific entrapment efficiency towards the 16 PAHs, i.e., *P. brutia* yielded two to three times higher contamination level than *P. pinea* in the case of highly polluted (urban) sites. In addition, the PAH load in second-year needles was lower than after three years of exposure for both species investigated. In contrast to pine needles, similar PAH accumulation was observed in -, 2-, and 3-year-old leaves of *Quercus ilex* L. sampled in urban and remote sites of Campania region in Southern Italy (De Nicola et al. 2011). The level of PAHs in leaves collected in urban sites was up to 30 times higher than in remote (control) ones. In addition, the sorption of PAHs was not influenced by the content of hexane-extractable lipids in leaves. Thus, the authors suggest that all the age classes of *Quercus* leaves can be employed in biomonitoring of air pollution and PAH source diagnostics. In addition, other tree species have been studied for their feasibility in PAH biomonitoring. Among roadside tree species investigated within the City of Tokyo, *Ginkgo biloba* L. accumulated the greatest amounts of PAHs from air near heavily trafficked streets and was considered to monitor the impact of car traffic on airborne contaminants in urban areas (Murakami et al. 2012). The level of PAH accumulation was not correlated with the lipid content of leaves but rather with the leaf structure, i.e., the wavy-walled surface which enlarges the active sorption area. Most of the 16 PAHs (~80 % on average) were accumulated within the cuticular wax, and nearly 17 % penetrated to the leaf interior. HMW PAHs were present mainly in water-washed and wax fractions, as a result of their migration (diffusion) from particles to the wax lipids. LMW PAHs were observed essentially in the cuticular wax as a result of the partitioning between gas-phase and leaf surface lipids. Additionally, LMW PAHs were present in the leaf interior, proving the contribution of the stomatal pathway to PAH accumulation. Furthermore, a significant correlation was found for the total 16 PAHs in ginkgo leaves and their content in air collected with a high-volume air sampler. A linear correlation was found between accumulation of PAHs in leaves and their concentration in the gas phase or particles for LMW and HMW PAHs, respectively. Along with PAH biomonitoring with trees, recent studies proved the feasibility of lichens (symbiotic associations of fungi and algae/cyanobacteria) to monitor the atmospheric deposition of numerous contaminants (Augusto et al. 2010; Blasco et al. 2006). Augusto et al. (2010) collected *Parmotrema hypoleucinum* (Steiner) Hale from branches and trunks of *Pinus pinea* L. and *Quercus suber* L. within highly industrialized region of the SW coast of continental Portugal. In comparison to pine needles, the accumulation of 16 PAHs in lichens sampled in heavily polluted areas was on average about twice as high and reached ~874 ng g^{-1} d.w. The average profiles of PAHs in lichens and soil were distinct in background areas, but tended to be similar in urban and industrialized sites where atmospheric deposition is more intense.

21.9 Conclusions

Phytoremediation is an alternative and acceptable method for cleaning up PAH-contaminated soil. There are some limitations of its use, for example, the long time for obtaining the results or appropriate conditions for growth and development of plants used for decontamination of the soil. However, the relatively low cost in comparison to conventional methods and the possibility of its application over extensive areas make it a competitive method for removing PAHs from polluted soil. Additionally, the use of plants, fungi, or SMC not only improves the quality of soil but also has further advantages such as allowing monitoring of lower troposphere pollution with PAHs or utilizing a wide range of residues from the mushroom industry.

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Phytoremediation of Degraded Mine Soils Using Organic Amendments and Metal-Tolerant Plants

22

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Abbreviations

BCF	Bioconcentration factor
CEC	Cation exchange capacity
E	Soil of El Cuadron
E0	Non-amended El Cuadron soil
E30M	El Cuadron soil mixed with 30 t ha^{-1} of manure
E60M	El Cuadron soil mixed with 60 t ha^{-1} of manure
E30P	El Cuadron soil mixed with 30 t ha^{-1} of pine bark
E60P	El Cuadron soil mixed with 60 t ha^{-1} of pine bark
EC	Electrical conductivity
FA	Fulvic acids
G	Soil of Garganta
G0	Non-amended Garganta soil
G30M	Garganta soil mixed with 30 t ha^{-1} of manure
G60M	Garganta soil mixed with 60 t ha^{-1} of manure
G30P	Garganta soil mixed with 30 t ha^{-1} of pine bark
G60P	Garganta soil mixed with 60 t ha^{-1} of pine bark
HA	Humic acids
HI	Humification index
HR	Humification ratio
M	Horse and sheep manure
P	Pine bark
TF	Translocation factor
TOC	Total organic carbon

22.1 Introduction

In the EU there may be up to 3.6 million potentially contaminated sites. In the last 30 years, more than 80,000 sites have been cleaned up, but 250,000 still require urgent attention (EEA 2007). Among these sites, those affected by mining activities are major sources of metal contamination in soils.

Mine soils usually contain high levels of trace metal contamination, even several years after the mine was abandoned (Alloway 2010). This accumulation can be a concern for human and animal health, decrease plant growth and ground cover, and lead to a risk of lixiviation of metals to groundwater (McGrath et al. 1995; Vamerali et al. 2010). Moreover, these soils often show low-fertility conditions and poor physical structure, which limit the establishment of vegetation and intensify erosion by rain and wind (McLaughlin and Singh 1999; Tordoff et al. 2000).

Phytoremediation is a cost-effective and environmentally friendly strategy, which can be employed to remediate these soils. This technique is defined as the use of plants to remove organic and inorganic pollutants from the environment or to render them harmless (Vangronsveld et al. 2009). The different plant-based technologies of phytoremediation, each having a different mechanism of action for the remediation of metal-polluted soils, are classified into: phytoextraction, phytostabilization, phytovolatilization, phytodegradation, and rhizofiltration (Prasad and Freitas 2003).

Phytoextraction is focused on hyperaccumulator plants, which are capable of accumulating extremely high concentrations of metals in their aerial tissues without being damaged but they have slow growth rates and a low biomass production (Wenzel 2009). An alternative approach is the use of non-hyperaccumulator but metal-tolerant and high biomass plants. Several authors do not recommend phytoextraction as a suitable technique in the case of mine soils or other highly metal-polluted soils, and they propose phytostabilization as an alternative to phytoextraction (Dickinson et al. 2009; Mendez and Maier 2008). Phytostabilization

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involves the establishment of a plant cover on the contaminated sites to reduce the mobility of contaminants within the vadose zone through accumulation by roots or immobilization within the rhizosphere. This process includes transpiration and root growth that immobilizes contaminants by reducing leaching, controlling erosion, creating an aerobic environment in the root zone, and adding organic matter to the substrate that binds the contaminant (Bolan et al. 2011).

The availability of metals is another limitation for successful phytoremediation by tolerant plants in mine soils. The addition of organic amendments to the soil modifies metal availability and soil properties that could improve plant growth, enhance metal uptake by plants, and permit the reestablishment of the vegetation cover (Kumpiene et al. 2008; Mench et al. 2006; Park et al. 2011; Vangronsveld et al. 2009). The increasing plant biomass due to the incorporation of organic amendments could increase the accumulation of metals in plant tissues (Lin et al. 2009), either in the shoots, which is advantageous for phytoextraction, or in the roots, which is better for phytostabilization. Reduction of metal bioavailability by organic materials is due to adsorption on solid surfaces and complexation with humic substances. This adsorption process depends upon the particular metal and soil type involved, degree of humification of the organic matter, content of metals and salts, and the effects of organic matter on the redox potential and soil pH (Clemente et al. 2005; Narwal and Singh 1998; Shuman 1999; Walker et al. 2004). Phytoremediation of mine sites can be enhanced by using these organic waste materials as soil amendments that immobilize metals combined with plant species that are tolerant of high levels of contaminants and low-fertility soils or tailings (Bolan et al. 2011; Clemente et al. 2012; Mendez et al. 2007; Pérez-Esteban et al. 2013a, b).

In this work, we gathered and compared the results of our previous studies of the use of organic materials and tolerant plants for the phytoremediation of mine soils (Pérez-Esteban et al. 2012, 2013a, b), as well as other unpublished results. The objectives of those studies were to evaluate the effects of two organic amendments (horse and sheep manure compost and pine bark compost) on the soil conditions, the stabilization of metals, and the phytoremediation capacity of several metal-tolerant plants (*Atriplex halimus*, *Brassica juncea*, and *Thlaspi arvense*) and also to assess the potential of those species as suitable candidates for the phytoremediation of metal-contaminated mine soils.

22.2 Characteristics of Mine Soils

In our studies we examined two mine soils from the Lozoya valley, located in the north of Madrid (Spain), where there was an intense mining activity during the nineteenth and the twentieth century. The site location of one of these soils is the village of Garganta de los Montes (G), which is in close proximity to a copper mine that was abandoned in 1965. The location of the other soil is situated in El Cuadron (E), which contains an old zinc blende mine that was abandoned in 1862. Both soils have been classified as Dystric Cambisols (Food and Agriculture Organization of the United Nations 1990) and Dystroxerepts (Soil Survey Staff 1999). The vegetation of these sites is mainly composed of *Quercus pyrenaica*, *Fraxinus angustifolia*, wet and temperate grasslands, and tilled pastures (Pastor et al. 2007). Soil samples were collected within the top 20 cm from different points around the mine dumps, where the ores were processed, and were composed of natural soil and mine tailings deposited in the soil.

Table 22.1 Properties of the mine soils

Properties[a]	G[b]	E
Clay (%)	3.1	3.3
Sand (%)	78.2	79.4
Silt (%)	18.8	17.3
pH	6.2	5.5
EC (dS m^{-1})	0.08	0.10
TOC (%)	0.88	1.39
Fe oxides (g kg^{-1})	11.9	7.31
CEC ($cmol_c$ kg^{-1})	4.74	4.79
Exch. Ca ($cmol_c$ kg^{-1})	2.31	3.28
Exch. Mg ($cmol_c$ kg^{-1})	0.50	0.61
Exch. K ($cmol_c$ kg^{-1})	0.25	0.66
N (%)	0.04	0.09
Total Cu (mg kg^{-1})[c]	913	248
Total Zn (mg kg^{-1})	203	146
Total Cd (mg kg^{-1})	3.68	1.32
Total Pb (mg kg^{-1})	87	75

[a] Texture by Bouyoucos hydrometer; pH and EC (electrical conductivity) in water extracts (1:2.5 w/w); TOC (total organic carbon) by loss on ignition; Fe oxides by ascorbate-oxalate; CEC (cation exchange capacity) and exchangeable cations (Ca, Mg, and K) by barium chloride; N (total nitrogen) by Kjeldahl; total metal concentrations (Cu, Zn, Cd, and Pb) by microwave-assisted acid digestion with aqua regia

[b] G, Garganta soil; E, El Cuadron soil

[c] European Union limits (mg kg^{-1}) for agricultural soils receiving sewage sludge (pH 6–7): Cu 50–140, Zn 150–300, Cd 1–3, and Pb 50–300 (Council of the European Communities 1986)

The main properties of these soils (G and E) are shown in Table 22.1. Both soils showed characteristics typically found in mine soils: acidic, loamy sand texture, low salinity (EC, electrical conductivity) and cation exchange capacity (CEC), small content of organic matter content (TOC, total organic carbon) and nutrients, and also contained large concentrations of Fe oxides. These soils exhibit pH-dependent surface sorption charges which are dependent on the small clay contents and large concentrations of metal oxides.

The total Cu and Cd concentrations of soil G exceeded the European Union maximum permitted levels for agricultural soils that receive sewage sludge at pH 6–7

(Cu 50–140, Zn 150–300, Cd 1–3, Pb 50–300 mg kg^{-1}) (Council of the European Communities 1986). The total Cu concentration of soil E also exceeded the limits permitted by the EU, although it was lower than in soil G. According to the critical metal concentrations set by Kabata-Pendias and Pendias (2001), the total Cu concentration was greater than the upper critical level (60–125 mg kg^{-1}) in both soils, showing a potential for toxicity in plants. In contrast, Zn, Cd, and Pb levels were below the upper toxic level (Zn 70–400, Cd 3–8, and Pb 100–400 mg kg^{-1}).

These soils present poor fertility conditions and relatively high concentrations of metals that could limit the establishment of plants. Therefore, the reduction of toxic metals and the improvement of soil conditions are necessary for the remediation of these soils.

22.3 Organic Amendments

The incorporation of organic amendments can improve the fertility and physical and chemical properties of mine soils, allowing plant survival and growth. Moreover, organic materials such as manures and composts have proved successful at reducing the mobility of contaminants in metal-polluted soils (Karami et al. 2011).

In our studies, two organic amendments provided by a commercial supplier were used: compost made of horse (50 % v/v) and sheep (50 %) manure (M) and a compost of pine bark (50 % v/v), wood fiber (30 %), and sphagnum peat (20 %) (P). The manure compost is usually applied as soil amendment at 20–40 t ha^{-1} of dry organic matter, and it was composted for 4–5 months in piles with periodical turning in an open-air composting plant located in Segovia (Spain). The pine bark compost is normally used as growing medium in pot culture, and it was composted for 9–10 months in piles under open-air conditions with periodical turning before the composted barks were ground and sieved and mixed with wood fiber and peat.

Table 22.2 shows the main properties of both organic amendments (M and P). The pine bark amendment samples exhibited low pH values, which can increase metal solubility. On the other hand, the manure amendment samples had high pH values, and its application on soils could lower metal availability. The manure amendment also presented a higher salinity and total N content than pine bark. The higher pH and nutrient content of the manure amendment can improve the fertility conditions of mine soils.

The manure amendment had a higher degree of humification (humification ratio, HR), a greater proportion of humic acids (humification index, HI), and a lower C/N ratio, which indicate a higher degree of maturity and stability than the pine bark amendment and could also result in decreasing metal mobility and availability by adsorption and the formation of stable complexes with humic substances (Benito et al. 2009; Clemente et al. 2006; Halim et al. 2003). In contrast, pine bark contained higher C/N ratio, lower degree of humification, and higher fulvic acid (FA) content. This organic fraction is more soluble than humic acids (HA) and could increase metal mobility due to the formation of soluble organometallic complexes.

Table 22.2 Properties of the organic amendments

Properties[a]	M[b]	P
pH	9.4	5.6
EC (dS m^{-1})	4.95	0.66
TOC (%)	27.2	45.8
EXC (%)	5.4	6.2
FA (%)	2.8	4.1
HA (%)	2.6	2.1
HR (%)	19.7	13.4
HI (%)	9.4	4.6
N (%)	1.5	0.7
C/N	19	69
Total Cu (mg kg^{-1})	30.9	1.07
Total Zn (mg kg^{-1})	179	35.7
Total Cd (mg kg^{-1})	<dl[c]	<dl
Total Pb (mg kg^{-1})	69	98

[a] pH and EC (electrical conductivity) in water extracts (1:5 v/v); TOC (total organic carbon) by loss on ignition; EXC (humic extractable organic carbon) by sodium hydroxide and sodium pyrophosphate at pH 13; FA (fulvic acid carbon) measured after precipitation of HA (humic acid carbon) at pH 2 in EXC extract; HR (humification ratio) = (EXC/TOC) × 100; HI (humification index) = (HA/TOC) × 100; N (total nitrogen) by Kjeldahl; total metal concentrations (Cu, Zn, Cd, and Pb) by microwave-assisted acid digestion with aqua regia

[b] M, horse and sheep manure amendment; P, pine bark amendment

[c] <dl, below detention limits (Cd < 0.02 mg L^{-1} in the extracts)

Total metal concentrations were very low in the pine bark compost. However, manure presented higher metal concentrations, especially Zn levels, which are probably due to the use of this metal as a nutritional supplement in the form of a feed additive for intensive animal production (Bolan et al. 2004). Although the application of manure might not help to reduce Zn concentration of soils with low metal levels, this organic material could be a suitable amendment to stabilize metals in contaminated soils.

22.4 Effects of Organic Amendments on Soil Properties and Metal Fractionation

In order to study the effects of the organic amendments on fertility conditions and metal bioavailability of contaminated mine soils, ten different treatments were prepared with mixtures of each soil and one of the amendments and their chemical properties and the metal fractionation were determined

Table 22.3 Properties of the treatments of mine soils with organic amendments

Treatment	pH[a]	EC dS m^{-1}	TOC %	CEC cmol$_c$ kg^{-1}	Exch. Mg cmol$_c$ kg^{-1}	Exch. Ca cmol$_c$ kg^{-1}	Exch. K cmol$_c$ kg^{-1}
G0[b]	6.2	0.08	0.9	4.74	0.50	2.31	0.25
G30M	6.5	0.51	1.1	5.28	0.76	2.73	1.18
G60M	6.8	0.94	1.5	5.99	1.00	3.44	2.24
G30P	5.9	0.11	1.1	5.30	0.59	2.57	0.28
G60P	5.6	0.17	1.5	5.68	0.63	2.62	0.30
E0	5.6	0.10	1.4	4.79	0.61	3.28	0.66
E30M	5.8	0.58	1.6	5.67	0.93	4.00	2.00
E60M	6.1	0.99	1.9	6.16	1.09	4.09	3.08
E30P	5.4	0.16	1.6	4.87	0.64	3.16	0.72
E60P	5.3	0.16	1.7	5.89	0.64	3.38	0.74

[a] pH and EC (electrical conductivity) in water extracts (1:2.5 w/w); TOC (total organic carbon) by loss on ignition; CEC (cation exchange capacity) and exchangeable cations (Ca, Mg, and K) by barium chloride

[b] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

Table 22.4 Concentrations of Cu (mg kg^{-1}) among the different operationally defined fractions in the treatments of mine soils with organic amendments by a sequential extraction procedure

Treatment	Water-soluble	Exchang.	Acid-soluble	Fe–Mn oxide	Organic	Residual
G0[a]	3.1	27	90	397	192	127
G30M	4.5	19	80	356	276	129
G60M	4.8	16	64	335	262	120
G30P	4.7	34	86	367	232	145
G60P	4.1	34	81	352	248	130
E0	2.0	10	27	66	49	56
E30M	1.9	4.6	19	57	63	54
E60M	2.2	1.3	11	46	83	58
E30P	1.4	7.6	22	59	60	51
E60P	1.5	10	22	58	60	62

[a] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

(Pérez-Esteban et al. 2012). The applied doses were 0, 30, and 60 t ha^{-1} of dry organic matter. Thus, treatments with G soil were the following: non-amended soil (G0), soil with 30 t ha^{-1} of manure compost (G30M), soil with 60 t ha^{-1} of manure (G60M), soil with 30 t ha^{-1} of pine bark compost (G30P), and soil with 60 t ha^{-1} of manure (G60P). Treatments with E soils followed the same sequence: E0, E30M, E60M, E30P, and E60P.

The characterization of the prepared treatments is shown in Table 22.3. Both amendments increased TOC, EC, and CEC of both soils. Increasing addition of manure raised the pH of both soils, which could reduce metal availability, whereas the low pH of pine bark reduced the pH values. Besides this, manure considerably increased exchangeable cation contents (Ca, Mg, and K), which might improve plant growth, whereas the pine bark amendment did not significantly impact on nutrient levels in these soils. Organic matter provided by manures acts as a nutrient pool, enhances nutrient cycling, increases CEC and buffer capacity, and improves the physical properties of soil (Stewart et al. 2000).

A sequential extraction procedure (Tessier et al. 1979) was carried out in the different treatments with soils and amendments to study the effect of these organic amendments on the chemical fractionation of the metals.

Table 22.4 shows Cu concentrations among the different operationally defined fractions in each treatment. The addition of manure to both soils significantly decreased the concentrations of Cu in the exchangeable, acid-soluble, and Fe–Mn oxide fractions, which reduced the bioavailability of this element. This loss in the more labile fractions was balanced by an increase in the organic-bound Cu. Other authors reported similar results with manure amendments (Liu et al. 2003). The application of pine bark also raised the concentrations of organic-bound Cu, but this increase was mainly associated with a decrease in the acid-soluble and Fe–Mn oxide fractions and not with the exchangeable fraction. Moreover, pine bark increased the concentration of exchangeable Cu in soil G probably due to the low pH of this amendment and therefore increased Cu bioavailability. Other authors have reported that pine bark compost presents a

Table 22.5 Concentrations of Zn (mg kg^{-1}) among the different operationally defined fractions in the treatments of mine soils with organic amendments by a sequential extraction procedure

Treatment	Water-soluble	Exchang.	Acid-soluble	Fe–Mn oxide	Organic	Residual
G0[a]	0.7	6.5	5.4	51	9.1	136
G30M	0.9	8.3	6.1	48	9.3	135
G60M	1.0	7.9	5.9	47	9.1	130
G30P	1.2	8.2	4.7	48	9.8	131
G60P	1.1	8.5	4.9	47	11	132
E0	1.3	19	5.0	29	7.9	59
E30M	1.4	19	6.2	29	8.0	58
E60M	1.2	15	7.5	33	8.9	56
E30P	1.4	18	4.9	27	8.0	59
E60P	1.4	19	4.2	28	8.6	61

[a] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

lower capacity to immobilize metals than other organic materials such as spent mushroom and pruning waste + biosolids composts due to its lower pH, degree of humification, and CEC (Eymar et al. 2009; Tapia et al. 2010). The greater retention of Cu by the organic matter provided by the manure amendment in comparison with the pine bark amendment probably resulted from its greater pH, degree of humification, and proportion of HA.

The pH conditions and the contribution of organic matter by both amendments affected the adsorption sites of these variable-charge soils and changed the Cu distribution. TOC was inversely correlated with Cu concentrations in the acid-soluble and Fe–Mn oxide fractions and was positively correlated with organic Cu concentrations, but it was not correlated with the exchangeable fraction, which was negatively correlated with soil pH. McBride et al. (1997) also suggested that the Cu solubility is mainly controlled by sorption on organic matter.

The Zn fractionation in each treatment is shown in Table 22.5. No significant differences were observed in the Zn distribution between the different treatments in the samples of soil G, but the addition of a high dose of manure amendment in the soil E significantly decreased the Zn concentration in the exchangeable fraction and increased the amount of Zn in the acid-soluble and Fe–Mn oxide fractions. This reduction of Zn availability resulted from the high pH of this amendment, which increased Zn sorption onto Fe and Mn oxides and other soil surfaces. In contrast, the addition of pine bark, with a lower pH, resulted in a slight decrease in the concentration of Zn in the acid-soluble and Fe–Mn oxide fractions. Similar results were obtained by Shuman (1999), who observed that the addition of organic materials with high pH redistributed Zn from the exchangeable fraction to the Fe–Mn oxide fraction. Xian and Shokohifard (1989) found that the proportion of Zn in the exchangeable fraction increased and that Zn in the acid-soluble fraction decreased when soil pH was lowered.

Unlike Cu, the application of organic materials did not significantly increase the amount of Zn associated with the organic fraction. This metal was more affected by pH-dependent sorption processes than by complexation with organic compounds. Positive correlations were found between soil pH and Zn concentrations in the acid-soluble and Fe–Mn oxide fractions, whereas no fractions were correlated with TOC. Narwal and Singh (1998) reported that the organic matter provided by different manure amendments did not affect Zn fractionation in soil.

The application of these organic amendments modified the soil fertility and the metal bioavailability, which could affect plant growth and metal uptake by plant species used for the phytoremediation of these contaminated soils.

22.5 Greenhouse Experiments Using Metal-Tolerant Plants and Organic Amendments

Greenhouse experiments were conducted to evaluate the application of these organic amendments on phytoremediation techniques and to assess the suitability of several tolerant plant species in the studied mine soils.

Plants of *Atriplex halimus* (Pérez-Esteban et al. 2013a), *Brassica juncea* (Pérez-Esteban et al. 2013b), as well as *Thlaspi arvense* were selected. Those plants were grown in pots filled with the mine soils (G and E samples) mixed with 0, 30, and 60 t ha^{-1} of the horse and sheep manure and pine bark amendments.

A. halimus (Mediterranean saltbush) was selected due to its high biomass production and its deep root system, which is able to cope with the poor structure and xeric characteristics of several polluted soils. It is a halophytic species that is present as a natural invading shrub in several mining areas of northern Africa and southern Europe. *Atriplex* species are not hyperaccumulator plants, but they can tolerate and accumulate metals (Lefèvre et al. 2009; Lutts et al. 2004).

On the other hand, *Brassica juncea* L. (Indian mustard) is an oilseed crop tolerant of the Mediterranean climate with relatively high biomass production and capable of substantial metal accumulation in its aboveground parts. Several studies have recognized *B. juncea* as a plant suitable for metal phytoextraction (Blaylock et al. 1997; Kumar et al. 1995), but more recent papers have considered that this species is not such efficient plant for phytoextraction in comparison with others grown in soils with low concentrations of available metals (Chaney et al. 2007; Ishikawa et al. 2006).

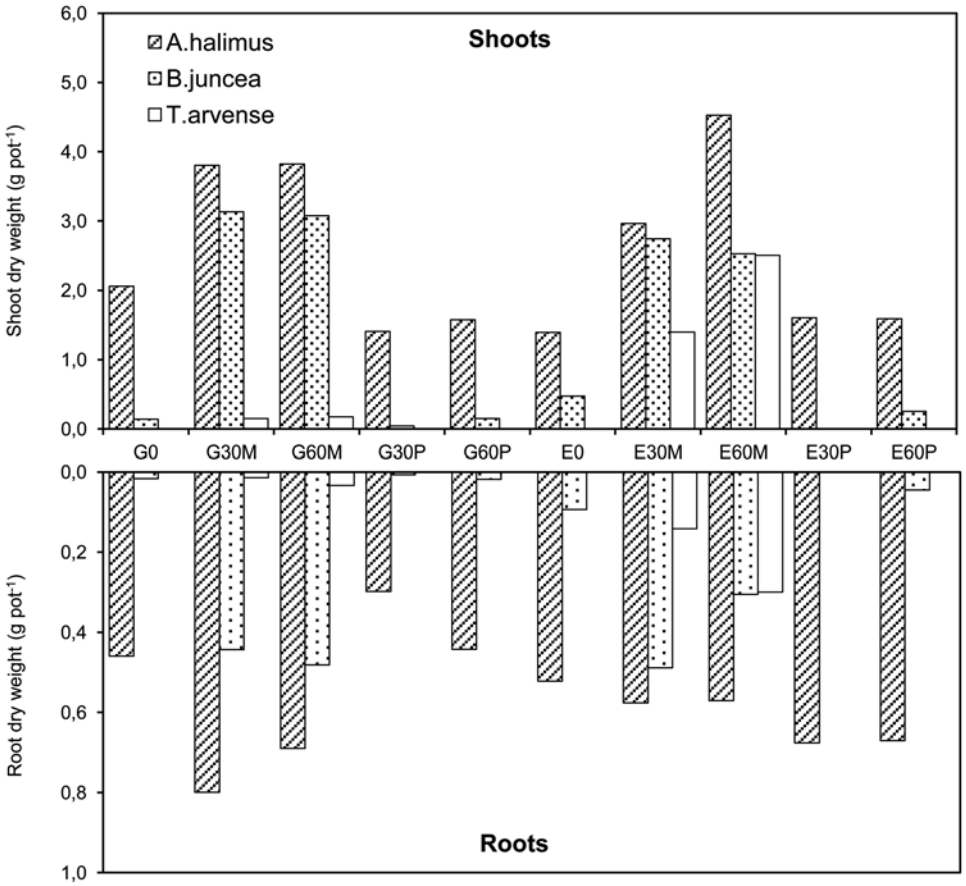


Fig. 22.1 Shoot and root dry weights of the tolerant plant species in the treatments of mine soils with organic amendments. Soils: G, Garganta; E, El Cuadron. Organic amendments: M, manure; P, pine bark. Application rates (t ha^{-1}): 0, 30, and 60

To compare with the previous species, we also used *T. arvense*, which is a closely related non-accumulator species of the well-known hyperaccumulator *T. caerulescens*, which has been shown to accumulate high concentrations of Zn and Cd in its tissues without showing any toxicity symptoms. In contrast, *T. arvense* has only an average capacity for metal uptake and translocation to the shoots (Lasat et al. 2000; Ozturk et al. 2003).

Plant biomass and metal concentrations in shoots and roots of these three species were measured to evaluate their growth, tolerance for metal toxicity, metal accumulation and translocation to aerial organs in relation to metal bioavailability and soil conditions affected by the organic amendments.

22.5.1 Plant Growth

Plant growth presented different responses among the applied organic amendment treatments, as indicated by the shoot and root dry weights (Fig. 22.1).

The biomass of the three species achieved higher yields in manure treatments than those with pine bark or non-amended soils. Moreover, *B. juncea* and *T. arvense* plants grown in manure mixtures presented flowering at the end of the crop growth and did not display chlorosis symptoms. Conversely, plants grown in pine bark mixtures and in non-amended soils yielded lower biomass and in many cases showed critical chlorosis symptoms. In most of these pots, the growth of *B. juncea* and *T. arvense* was even negligible.

The improvement of soil fertility with manure amendments could have contributed to the increased biomass production. Manure amendments not only provided organic matter and a more adequate pH to soil, they also increased CEC and supplied a higher amount of nutrients as exchangeable Ca, Mg, and K than pine bark compost (Table 22.3). In our studies, the concentrations of these nutrients in soil and pH values were strongly and positively correlated with the shoot and root dry weights of these plants. Safari Sinegani and Khalilikhah (2011) also found that sheep manure extracts stimulate shoot and root biomass in *Brassica napus*. Other works have also reported that the addition of compost to mine soils enhances plant growth (Clemente et al. 2012; Mendez et al. 2007).

The low plant biomass and the presence of chlorosis in several samples of pine bark and non-amended treatments were probably due to the low pH, CEC, and nutrient contents, but it might have also been due to the greater metal bioavailability in these treatments and therefore to the higher metal stress suffered by plants, as it was shown by

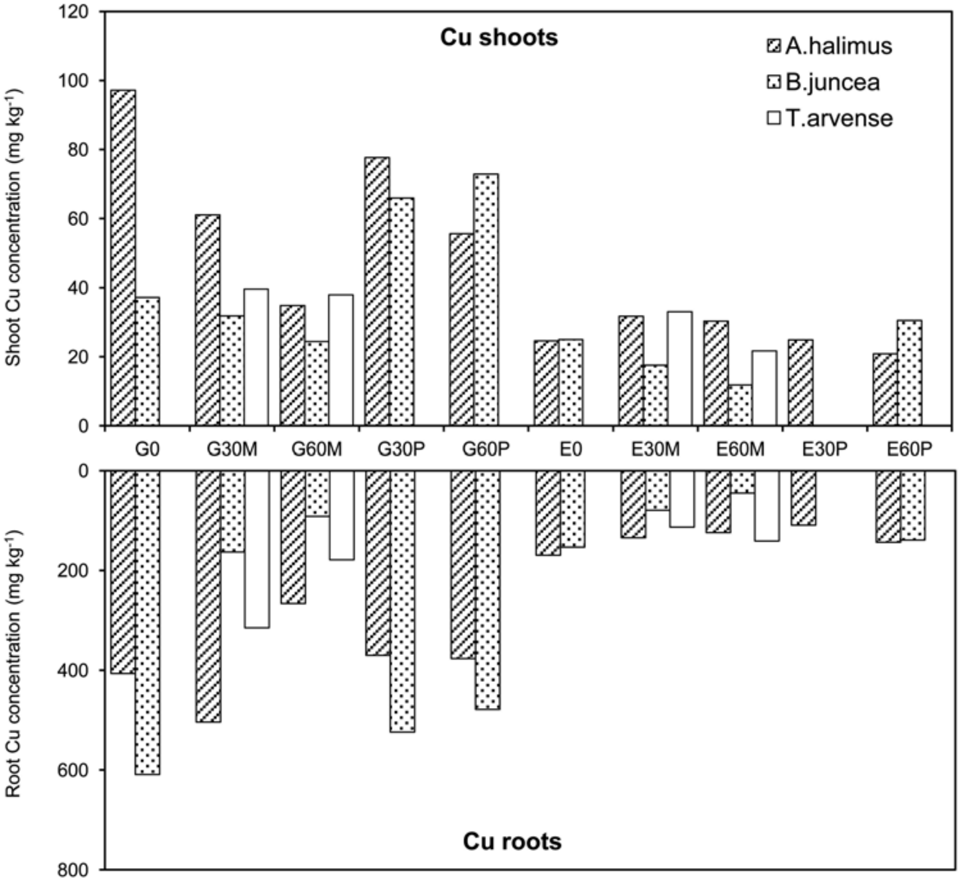


Fig. 22.2 Cu concentrations in shoots and roots of the tolerant plant species in the treatments of mine soils with organic amendments. Soils: G, Garganta; E, El Cuadron. Organic amendments: M, manure; P, pine bark. Application rates (t ha^{-1}): 0, 30, and 60

the negative correlations found between bioavailable metal concentrations in soil with plant dry weight. These negative correlations were stronger for Zn than for Cu in *B. juncea* and *T. arvense* plants, showing a lower tolerance for Zn in these species. Ebbs and Kochian (1997) also reported that exposure to high Zn concentrations in *Brassica* spp. caused chlorosis in young leaves, while Cu exposure had little effect on chlorophyll levels. In contrast, *A. halimus* presented stronger negative correlations with Cu, although this species did not show toxicity symptoms.

If the growth of the three species is compared, *A. halimus* developed more biomass production in all treatments than *B. juncea* and *T. arvense*. The last was the species that presented the lowest growth, especially in soil G treatments, where the high metal content of this soil might have induced stress in this plant.

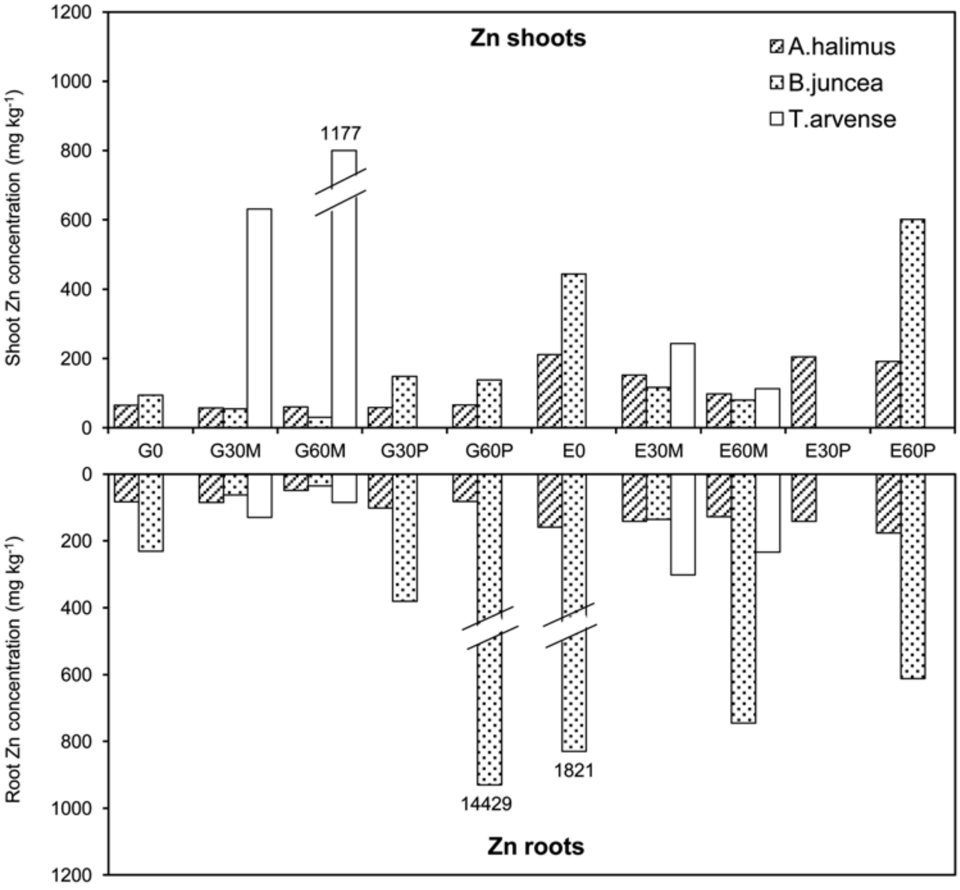
22.5.2 Metal Concentrations in Plant Tissues

Figures 22.2 and 22.3 show Cu and Zn concentrations, respectively, measured in the shoots and roots of the three species.

Changes in metal bioavailability in soil with the application of the different amendments (Tables 22.4 and 22.5) were reflected in the metal concentrations in plant tissues: metal concentrations in plants significantly decreased with the application of manure amendment in the same way as metal bioavailability, whereas the addition of pine bark generally increased metal concentrations. Our studies reported significant and positive relationships between the metal concentrations in plants and the labile metal concentrations in soil, determined as the sum of the first three metal fractions (water-soluble, exchangeable, and acid-soluble fractions; Tessier et al. 1979). Other extraction procedures used for the assessment of metal bioavailability in soil, such as the *Rhizo* method (Feng et al. 2005a, b), produced strong and positive correlations with metal concentrations in plant tissues (Pérez-Esteban et al. 2013a). The total Cu concentration in soil was also positively correlated with Cu concentration in plants, but no correlations were observed between the total Zn in soil and its concentrations in plant tissues, showing that the measurement of total concentrations is not a suitable method for predicting metal bioavailability in soil and its uptake by plants. Xian (1989) also found that metals in the exchangeable and acid-soluble fractions strongly controlled the uptake by plants more than the total metals content in the soil.

The increase in soil organic matter added by the manure amendment and its high pH reduced metal bioavailability and therefore affected metal uptake by plants. Strong and negative correlations were found between TOC and Cu

Fig. 22.3 Zn concentrations in shoots and roots of the tolerant plant species in the treatments of mine soils with organic amendments. Soils: G, Garganta; E, El Cuadron. Organic amendments: M, manure; P, pine bark. Application rates (t ha^{-1}): 0, 30, and 60



concentrations in shoots and roots. Zinc concentrations in plants were not so related to soil organic matter, but were strongly and inversely correlated with pH. These results are in agreement with those reported by other authors (Clemente et al. 2005; Narwal and Singh 1998; Walker et al. 2004), who observed that the addition of manure with a high pH reduced metal uptake by plants.

As was mentioned above, the higher metal bioavailability in pine bark and non-amended treatments increased the metal concentrations in plants, and as a result it could have severely affected plant growth and the presence of chlorosis symptoms. Significant and negative correlations were found between root and shoot dry weight and metal concentrations in tissues of *B. juncea* and *T. arvense*. Conversely, no such correlations were found in *A. halimus* plants, which indicate a possible greater tolerance for these metals. According to Kabata-Pendias and Pendias (2001), shoot Zn concentration was above the upper toxic level (400 mg kg^{-1}) in several samples of *B. juncea* and *T. arvense* plants, where chlorosis symptoms were present. However, Cu concentrations were below toxic levels (100 mg kg^{-1}) in these two species. Previous studies reported that increases in shoot Zn concentration by *B. juncea* and *T. arvense* result in the development of severe leaf chlorosis associated with Zn toxicity (Ebbs and Kochian 1997; Lasat et al. 2000; Ozturk et al. 2003). In *A. halimus* plants, shoot metal concentrations were always below the toxic level established by Kabata-Pendias and Pendias (2001).

In most cases there was a greater Zn concentration in shoots and roots of *B. juncea* and *T. arvense* than those in *A. halimus*, but this last had the highest shoot Cu concentration. Ebbs and Kochian (1997) showed that *Brassica* spp. were more effective at removing Zn from a nutrient solution than Cu. Other authors found that *A. halimus* was tolerant to Zn, but its concentration in aboveground tissues was kept generally at low levels (Lutts et al. 2004; Manousaki and Kalogerakis 2009).

22.5.3 Translocation and Bioconcentration Factors

The values of the translocation factor (TF) and the bioconcentration factor (BCF) among the different treatments and plant species are shown in Tables 22.6 and 22.7, respectively. The TF is defined as the ability of plants to translocate metals

Table 22.6 Translocation factor (TF) of metals of the tolerant plant species in the treatments of mine soils with organic amendments

	Cu			Zn		
Treatment	*A. halimus*	*B. juncea*	*T. arvense*	*A. halimus*	*B. juncea*	*T. arvense*
G0[a]	0.30	0.06	nd[b]	0.66	0.43	nd
G30M	0.13	0.20	0.13	0.85	0.94	4.87
G60M	0.17	0.27	0.21	1.36	0.89	13.55
G30P	0.23	0.13	nd	0.64	0.39	nd
G60P	0.40	0.15	nd	0.95	0.01	nd
E0	0.14	0.17	nd	1.34	0.26	nd
E30M	0.23	0.24	0.29	1.09	0.86	0.78
E60M	0.25	0.26	0.16	0.78	0.11	0.48
E30P	0.24	nd	nd	1.49	nd	nd
E60P	0.15	0.24	nd	1.08	1.09	nd

[a] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

[b] nd, not determined because no plants in these treatments survived until harvest

Table 22.7 Bioconcentration factor (BCF) of metals of the tolerant plant species in the treatments of mine soils with organic amendments

	Cu			Zn		
Treatment	*A. halimus*	*B. juncea*	*T. arvense*	*A. halimus*	*B. juncea*	*T. arvense*
G0[a]	0.10	0.04	nd[b]	0.32	0.44	nd
G30M	0.07	0.04	0.04	0.29	0.27	2.99
G60M	0.04	0.03	0.03	0.30	0.15	3.52
G30P	0.08	0.08	nd	0.28	0.74	nd
G60P	0.06	0.08	nd	0.32	0.70	nd
E0	0.10	0.10	nd	1.61	3.34	nd
E30M	0.14	0.07	0.13	1.18	0.90	1.79
E60M	0.12	0.05	0.08	0.75	0.61	0.86
E30P	0.10	nd	nd	1.67	nd	nd
E60P	0.09	0.12	nd	1.58	4.63	nd

[a] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

[b] nd, not determined because no plants in these treatments survived until harvest

from the roots to the shoots, and it was calculated by dividing the metal concentration in the shoots by the metal concentration in the roots ($TF = [metal]_{shoots}/[metal]_{roots}$). The BCF is defined as the ratio of metal concentration in plant shoots to total metal concentration in soil, as a measure of the ability of a plant to take up and transport metals to the harvestable aerial parts ($BCF = [metal]_{shoots}/[metal]_{soil}$) (Kachout et al. 2011; McGrath and Zhao 2003). Mendez and Maier (2008) suggested that plants which show a $BCF > 1$ and a $TF > 1$ could be suitable for phytoextraction, while plants with BCF and $TF < 1$ should be used for phytostabilization.

Copper TF presented low values and was always less than 1, which may be due to the complexation and sequestration of this metal in the vacuoles of the root cells, making it unavailable for translocation to shoots (Lasat et al. 2000). In contrast, Zn concentrations in shoots were also generally lower than the root concentrations but in some cases were similar or higher, resulting in higher TF values and thereby showing a better translocation of this metal to the shoots. Gupta and Sinha (2007) also reported that the concentrations of metals in shoots of *B. juncea* grown in contaminated soils were lower than in roots, and this shoot to root ratio was higher for Zn than for Cu. Different results were obtained by Clemente et al. (2005), who reported a higher Cu and Zn concentrations in leaves and stems than in roots of *B. juncea* grown in contaminated soils. Lutts et al. (2004) found that *A. halimus* grown in nutrient solution accumulated more Zn in roots than in shoots and suggested that *A. halimus* is a halophytic species capable of precipitate Zn as oxalate salts mainly in stems in order to prevent metal toxicity.

On the other hand, Cu BCF values were also always lesser than 1. However, Zn BCF values were in many cases higher than 1, and they were considerably higher than Cu BCF values.

Regarding the amendments, although there was not a clear tendency in the variation of the TF among the different

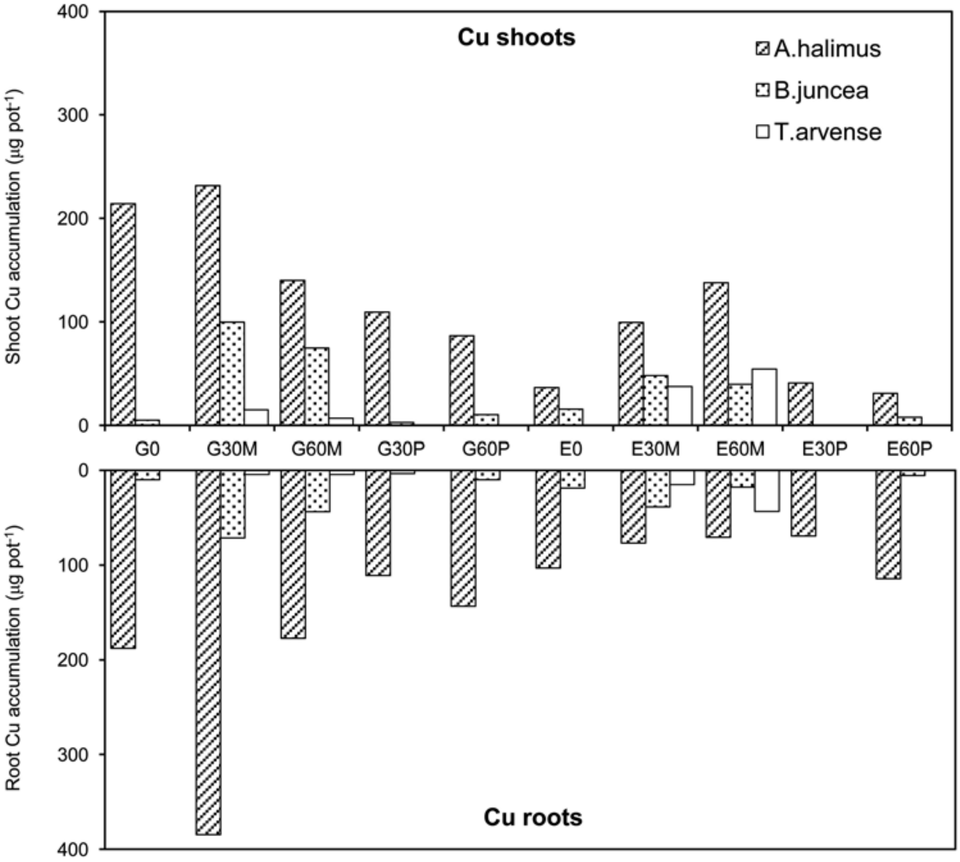


Fig. 22.4 Total Cu extracted per pot and accumulated in shoots and roots of the tolerant plant species in the treatments of mine soils with organic amendments. Soils: G, Garganta; E, El Cuadron. Organic amendments: M, manure; P, pine bark. Application rates (t ha^{-1}): 0, 30, and 60

treatments, Table 22.7 shows that Cu and Zn BCF generally decreased with the addition of manure. The addition of manure amendment not only reduced the concentrations of metals in plant tissues but also reduced the BCF values in these plants and therefore could improve the phytostabilization capacity of this species.

In most cases, the three species presented similar TF and BCF values. However, Zn TF and BCF values of *T. arvense* were extremely higher in soil G treatments. The low translocation of metals and the relatively high metal tolerance of *B. juncea* and *A. halimus* species could make them useful for phytostabilization purposes rather than for phytoextraction ones in sites contaminated with Cu and Zn. These results differed from those reported in previous works with *B. juncea*, which considered this species as a plant suitable for metal phytoextraction (Blaylock et al. 1997; Kumar et al. 1995). Mendez et al. (2007) found that other *Atriplex* species are good candidates for phytostabilization strategies in mine soils. On the other hand, the low tolerance for metal toxicity and the high translocation of Zn in *T. arvense* make this species unsuitable neither for phytoextraction nor for phytostabilization.

22.5.4 Total Metal Accumulation in Plants

Metal concentrations in plants should not be the only parameter used to evaluate the phytoremediation capacity of species because it does not take plant biomass into consideration. It is more informative to determine the total amount of metal removed from the growing media and accumulated in plant tissues, by calculating the product of shoot and root dry weight and their metal concentration (Figs. 22.4 and 22.5).

In spite of the lower concentrations of metals in plants grown in soils with manure amendments, the higher growth reached in these treatments generally resulted in a greater metal accumulation in plant roots and shoots in comparison with pine bark and non-amended treatments. Manure amendments could be useful not only for stabilizing metals in contaminated soils but also for achieving a higher accumulation in roots, thereby enhancing the phytostabilization capacity of plants. Manure also managed to increase metal accumulation in the aboveground organs of plants and to remove metals from the soil, which might also be interesting for phytoextraction purposes.

Although *A. halimus* presented lower Zn concentrations, it was the species that achieved the greatest Cu and Zn accumulation in shoots and roots because of its greater biomass production, making it suitable for phytoremediation techniques in contaminated soils. In contrast, *T. arvense* plants achieved the lower amount of metals removed from soils and accumulated in their tissues due to the low growth reached by this species.

However, metal uptake and translocation to shoots by these species were very low compared to the total metal content in soils. To assess the feasibility of these plants and the amendments for phytoextraction, the number of crop cycles

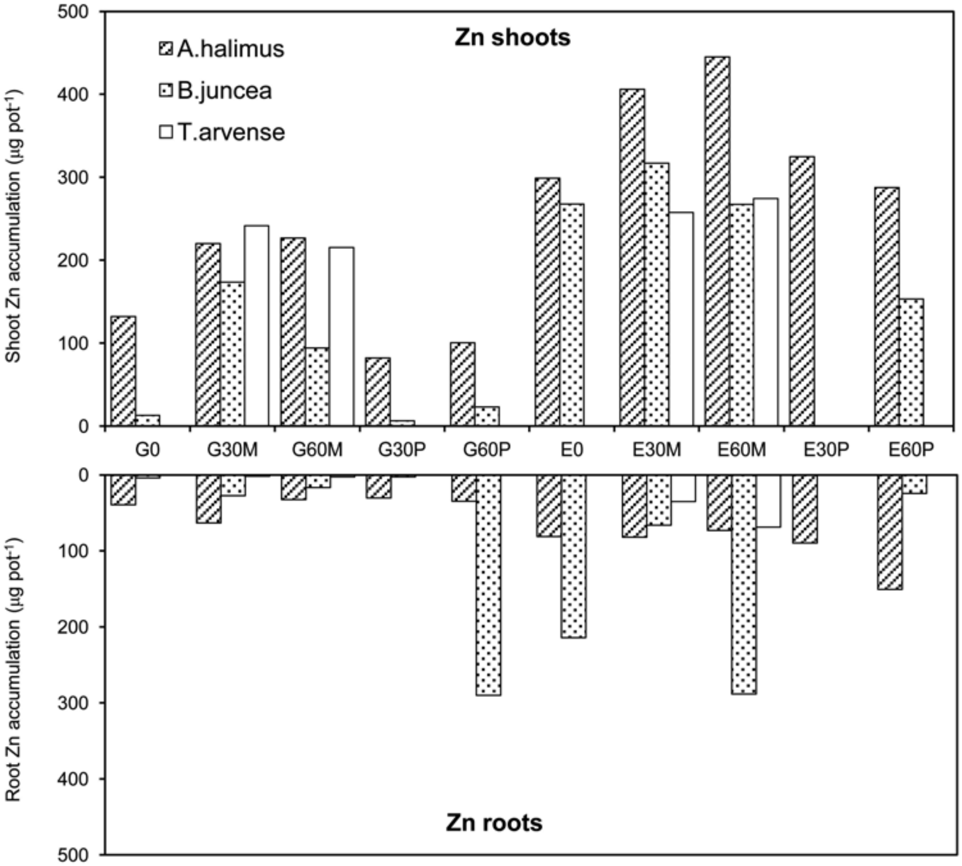


Fig. 22.5 Total Zn extracted per pot and accumulated in shoots and roots of the tolerant plant species in the treatments of mine soils with organic amendments. Soils: G, Garganta; E, El Cuadron. Organic amendments: M, manure; P, pine bark. Application rates (t ha^{-1}): 0, 30, and 60

Table 22.8 Number of crop cycles of the tolerant plant species required to reduce the total metal concentrations of the mine soils to acceptable levels

	Cu			Zn		
Treatment	*A. halimus*	*B. juncea*	*T. arvense*	*A. halimus*	*B. juncea*	*T. arvense*
G0[a]	2,796	119,026	nd[b]	286	3,413	nd
G30M	2,548	5,976	38,589	170	206	176
G60M	3,797	7,734	83,281	152	398	171
G30P	5,666	203,570	nd	502	5,511	nd
G60P	7,080	55,675	nd	419	1,437	nd
E0	3,826	9,048	nd	0	0	nd
E30M	1,293	2,841	3,943	0	0	0
E60M	1,060	3,495	2,930	0	0	0
E30P	3,180	nd	nd	0	nd	nd
E60P	4,010	18,477	nd	0	0	nd

[a] G0, non-amended Garganta soil; G30M and G60M, Garganta soil mixed with 30 and 60 t ha^{-1} of manure, respectively; G30P and G60P, Garganta soil with 30 and 60 t ha^{-1} of pine bark; E0, E30M, E60M, E30P, and E60P, the same sequence for El Cuadron soil

[b] nd, not determined because no plants in these treatments survived until harvest

required to reduce the total metal concentrations of these soils to acceptable levels was calculated as described in Clemente et al. (2005). Thus, the total metal accumulation in shoots per pot and the difference between the total metal soil concentrations and the lower metal limits established by the EU for agricultural soils that receive sewage sludge at pH 6–7 were considered to calculate the required number of crop cycles, which are shown in Table 22.8.

Manure amendments considerably reduced the required number of crop cycles. Besides this, *A. halimus* was the species that would need less cycles. However, a great number of them would be still necessary to reduce metal content in soils to the maximum values permitted by EU. These results make phytoextraction techniques unviable for the remediation of soils with high levels of metals in the short to medium term. These species could have a better potential for phytostabilization strategies.

22.6 Conclusions

The mine soils of our studies presented poor fertility conditions and large levels of metal contamination that could limit the establishment of the vegetation cover and the implementation of phytoremediation techniques. The application of horse and sheep manure as a soil amendment managed to decrease the metal bioavailability of these soils and to improve the soil fertility conditions due to its high pH and nutrient contents and the contribution of organic matter with a high degree of maturity and humification. In contrast, pine bark amendment did not achieve such fertility improvements and even increased metal bioavailability in the soils.

The results of the greenhouse experiments showed that the enhancement of the fertility status of the soils and the reduction of metal toxicity by the manure amendment allowed a higher biomass production of the three tolerant species studied and managed to decrease metals BCF, which might improve the phytostabilization capacity of these plants. Despite the lower metal concentrations achieved in the plants grown in manure treatments in comparison with pine bark and non-amended soils, those plants removed a greater amount of metals from the soils accumulating them in their tissues due to their higher growth. Thus, this amendment not only can effectively stabilize metals in mine soils, it can also decrease the number of crop cycles required to reduce metal concentrations to acceptable levels by accumulating metals in the harvestable parts of the plants, which might be interesting for phytoextraction purposes, and also it can be a useful tool for phytostabilization because of the high metal accumulation achieved in plant roots.

Among the three tolerant species, *A. Halimus* was the plant that developed a higher biomass production and accumulated a greater amount of metals in its tissues without being severely affected by metal toxicity, followed by *B. juncea*. Conversely, *T. arvense* presented limited growth and tolerance for metal toxicity and a low metal accumulation, making it unsuitable for phytoremediation techniques. Although the phytoextraction capacity of these species resulted to be insufficient to remediate soils with high levels of metals in the short to medium term, the use of *A. halimus* and *B. juncea* could be a useful and cost-effective strategy for the phytostabilization of metal-contaminated mine soils in combination with manure amendments.

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Salt Marsh Plants' Potential for the Remediation of Hydrocarbon-Contaminated Environments

23

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23.1 Introduction

23.1.1 Petroleum Hydrocarbon Contamination

The levels of petrochemical products in the environment, particularly in estuarine and coastal areas, have increased in the last decades (Lima et al. 2007). In fact, contamination by these products occurs in a number of ways: from municipal and industrial wastewater discharges, by small leakages and spills from commercial ships and recreational boats, by urban runoff from land-based traffic accidents, by major shipping accidents and from offshore production facilities.

Petroleum contamination can cause serious damage to the ecosystems, and, therefore, effective countermeasures are required to minimise ecological impacts. Conventional techniques used for remediation of hydrocarbon-contaminated sediment/soil include physical and/or chemical treatments, either in situ or ex situ, which are used to destroy (i.e. chemically convert), separate or immobilise the contaminants. These techniques include, for instance, mechanical oil removal, hot water flushing, dispersant addition, chemical oxidation, solidification/stabilisation or thermal desorption. Although ex situ treatments require shorter time periods than in situ treatments, the former may be cumbersome to implement. Indeed, they require increased engineering equipment and costs, creating potential threats to human health and the environment, which can arise during the handling and transporting of hazardous material (Vidali 2001). Depending on the affected ecosystems, those physical and/or chemical treatments can have some drawbacks, associated to the high costs involved and the lack of public acceptance, and can be more harmful than successful. Therefore, there is a need for more economic and environmental friendly remediation approaches, such as natural biological processes.

23.1.2 Bioremediation

Bioremediation is an option that offers the possibility to destroy or reduce to harmless forms various contaminants using the natural biological activity of the affected environment. Bioremediation can also be used as an ex situ treatment technique, involving land farming, composting and biopiles, but intrinsically linked with these technologies are excavation or removal of contaminated soil/sediments, moving the contamination elsewhere. Therefore the main advantage of the in situ bioremediation processes is that they allow soils/sediments to be treated without being excavated and transported, resulting in less disturbance of site activities at a relative low cost.

Bioremediation that occurs without human intervention is called natural attenuation. This natural attenuation relies on

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natural conditions and behaviour of indigenous soil/sediment microorganisms (Mills et al. 2003). In the case of hydrocarbons, intrinsic bioremediation often takes a long time to be completed because the number of indigenous degrading microorganisms before the contamination is normally low (due to resource constrains), their number only increasing a time period after the exposition to that contamination. So, it is important to use approaches that accelerate that natural attenuation.

One approach is biostimulation which improves the degradation potential of the microbial communities capable of the desired degradation already present in the affected environment. This is done by adjusting environmental conditions, for instance, by adding nutrients or adequate electron donors/acceptors that are not available in suitable concentrations in the environment (Nikolopoulou and Kalogerakis 2009). In fact it has been shown that the degradation of petroleum hydrocarbons by a given native microbial population can be favoured by the presence of the required nutrients [in an appropriate ratio (normally C:N:P of 100:10:1)] in the contaminated site (Delille et al. 2004). This strategy promotes biodegradation, especially in environments where nutrients' availability is often limited (Burns et al. 1999).

Another bioremediation approach is bioaugmentation. Bioaugmentation or seeding is the addition of highly concentrated microbial populations (single strains or consortia) capable of degrading the pollutant in the contaminated site (Gentry et al. 2004). A common procedure used for bioaugmentation is the addition of an exogenous pure bacterial strain or consortia known to degrade the pollutant (El Fantroussi and Agathos 2005). Another option is the addition of autochthonous pre-grown microbial communities resistant to the pollutant and capable of degrading it (Hosakawa et al. 2009).

23.1.3 Phytoremediation

Phytoremediation appears also as an approach to increase the pace of bioremediation, compared with natural attenuation. Phytoremediation is a plant-assisted bioremediation technique that uses plants to degrade, stabilise and/or remove contaminants (Gerhardt et al. 2009; Glick 2010). The action of plants includes degradation, adsorption, accumulation and, in some cases, volatilisation of compounds or enhancement of soil rhizosphere activity (Newman and Reynolds 2004). Phytoremediation technology is cost effective, nonintrusive, relatively easy to implement and environmentally friendly, and there is no need for disposal sites avoiding excavation and heavy traffic (Alkorta and Garbisu 2001; Dowling and Doty 2009). It has also potential to treat a diverse range of hazardous materials, and as long as the impacted site can support plant growth, a remediation scheme can be used anywhere (Gerhardt et al. 2009). Phytoremediation is also socially accepted by surrounding communities and regulatory agencies (Alkorta and Garbisu 2001).

Although phytoremediation has been used for several contaminants, it has been mostly applied to metal phytoextraction. Nevertheless, there is a growing interest in broadening its applications to remove/degrade organic contaminants in the environment (Alkorta and Garbisu 2001; Chaudhry et al. 2005), including petroleum hydrocarbons as widespread and recalcitrant pollutants (Schnoor et al. 1995).

The main advantages of phytoremediation in comparison with classical remediation approaches can be summarised as follows: (1) it can be applied in situ and has potential versatility to treat a diverse range of hazardous materials; (2) it is less disruptive to the environment, since it avoids excavation and heavy traffic; (3) it is economically competitive and has favourable public opinion; and (4) it can improve soil quality by preserving the natural structure and texture of the soil and prevents erosion.

23.1.4 Rhizodegradation

There are several types of phytoremediation techniques that are classified based on the contaminant type and fate: phytoextraction, phytotransformation, phytostabilisation, rhizodegradation, rhizofiltration and phytovolatilisation (Vidali 2001). Regarding organic contaminants that have normally low plant uptake, rhizodegradation is normally the process of choice, since the degradation processes take place in the area surrounding the roots of plants.

For instance, Frick et al. (1999) indicated that, in terrestrial environments, the primary loss mechanism for petroleum hydrocarbons is the degradation of these compounds by microorganisms in the rhizosphere of plants.

Transformation of organics by microorganisms may be carried out by their need to reduce toxicity and not only by energy needs (Chaudhry et al. 2005). However, to reduce toxicity microorganisms can suffer an energy deficit with root exudates providing abundant energy for this process (Chaudhry et al. 2005). In addition, plants can also increase nutrient availability to microorganisms. Root exudates cannot only mediate acquisition of minerals by the plants but also modify pH, water flux and availability of oxygen in the root region (Chaudhry et al. 2005). As many of the root exudates, including CO_2 and amino acids, are acidic, inorganic nutrients can be dissolved or weathered in the rhizosphere. Thus, bioavailability to microorganisms can change (Kaksonen et al. 2006). Plants also take advantages from these interactions with microorganisms by the increase of the availability of soil-bound nutrients, enhancing nutrient uptake, and by the reduction of the toxicity of soil contaminants, through removal of phytotoxic soil contaminants (Chaudhry et al. 2005).

The composition of microbial communities in the rhizosphere is known to vary both qualitatively and quantitatively from that in a non-colonised soil (Chaudhry et al. 2005), having also much greater adaptability to grow on different carbon sources (including contaminants) than microflora from non-colonised soil. Microbial communities in the rhizosphere are composed of different and synergistic populations having, consequently, distinct enzymatic capabilities, which probably work in a sequential and synergistic order (Chaudhry et al. 2005). Plants can also supply additional surfaces to microbes that will allow its growth, creating different ecological niches (Kaksonen et al. 2006).

Biological factors that may affect rhizodegradation include contaminant degradation ability of microorganisms associated to roots, plant root architecture, plant growth rate and plant exudate production.

The presence of plants has shown to enhance degradation of certain recalcitrant organic chemicals in soil such as chlorinated pesticides, petroleum hydrocarbons, explosives, dyes and detergents (Chaudhry et al. 2005). However, not all plant species will tolerate the presence of organic compound contamination (Tesar et al. 2002) or be able to effectively enhance remediation of contaminants from the soil (Madsen and Kristensen 1997). Therefore, the selection of suitable plant species for rhizodegradation is an important issue.

A large variety of plant species has been assessed for their phytoremediation potential. For instance, research has shown that plants, like grasses, with highly branched and fibrous root systems are potential candidates for the rhizodegradation of hydrocarbons (Aprill and Sims 1990; Merkl et al. 2006). Fibrous root system covers a larger volume of soil and would support greater rhizosphere–contaminant–microbe interactions (Aprill and Sims 1990; Yateem et al. 2007) owing to the increased surface. However, a lack of knowledge regarding the role of the root system in hydrocarbon rhizoremediation is still evident.

As mentioned, plants with particular morphological characteristics (e.g. fibrous root systems), coupled with growth characteristics (e.g. tolerate hydrocarbon contamination) and physiology (e.g. root exudates that stimulate hydrocarbon-degrading microorganisms), seem to be the ideal for the in situ treatment of hydrocarbon-contaminated soil.

In fact, a key element for an effective phytoremediation approach is related with the use of a plant species that can proliferate in the presence of high concentrations of contaminants (Huang et al. 2005). Plants that are less affected by soil contaminants are healthier, more persistent and will generate healthier root systems and greater top growth (Alkorta and Garbisu 2001). Plants may enhance biodegradation of entrapped contaminants by different ways (Banks et al. 2003). On one hand, fine roots and root hairs penetrate micropores, disrupt soil aggregates concomitantly increasing exposed surface area and transport microorganisms that were attached to the root surface, allowing them to thrive in regions of the soil that were inaccessible without roots (Banks et al. 2003), also increasing oxygen contents in that part of the soil. On the other hand, root exudates may enhance desorption of contaminants from soil as plants have been shown to secrete enzymes and secondary metabolites that also have a surfactant activity, which may increase available contaminant concentration in soil (Chaudhry et al. 2005). Moreover, when design cost-saving rhizoremediation strategies, plant capabilities to foster microbial pollutant degraders in their rhizosphere may be a useful tool (Ribeiro et al. 2011).

Among the plant species that can be useful for rhizoremediation, salt marsh plants can be an option. Salt-tolerant plants such as herbs, grasses or low shrubs are adapted to complete their lifecycle in salty environments (Kennish 2001). In fact, a salt marsh, also known as tidal marsh, is a coastal ecosystem in the intertidal zone between land and coastal salt or brackish water that is regularly flooded by the tides.

Salt marsh plants face serious challenges to cope with the environmental stresses to which they are exposed since they inhabit waterlogged, anoxic and reduced environments as salt marsh sediments (Manousaki and Kalogerakis 2011). For that, salt marsh plant roots stimulate several biogeochemical changes on rhizosediment which are imperative for plants' survival in such environments and may represent pivotal features, among others, that make salt marsh plants a possible and efficient resource for phytoremediation purposes. In addition, these plants can be accustomed with several pollutants since estuarine salt marshes are areas normally subject to high anthropogenic contamination. For these reasons, it has been suggested that salt-tolerant plants may represent a better choice over salt-sensitive plants when dealing with soil/sediment remediation (Manousaki and Kalogerakis 2011). Among these, not only salt marsh plants but also other marsh plants that can adapt and grow on these salty environments, such as *Phragmites australis*, can be an option.

Ribeiro et al. (2013a) reported that the salt marsh plants *Juncus maritimus* and *Triglochin striata* and the salt-tolerant *Phragmites australis*, all found in an estuarine salt marsh, could positively influence the microbial community by increasing total microbial abundance and could promote the development of hydrocarbon-degrading microbial populations on its rhizosphere. This assumption corroborates the "rhizosphere effect" (Olson et al. 2003) described for terrestrial environments. Greater bacterial counts in the rhizosphere including specific organisms capable of metabolising the contaminant of concern are considered significant to plant-fostered rhizodegradation (Liste and Felgentreu 2006). In fact, Ribeiro and collaborators (2011, 2013b) also showed differences in the degradation potentials with higher hydrocarbon degradation rates in rhizosediments (sediments in contact with roots of the mentioned plants) than in uncolonised sediment. Moreover, it was observed that *Juncus maritimus*, *Phragmites* *australis* and *Triglochin striata* may be responsible for the movement of compounds into the rhizosphere, contributing to higher hydrocarbon compound retention at the vicinity of their roots (Ribeiro et al. 2013a). So, salt marsh plant–microorganism associations may contribute actively to hydrocarbon removal and degradation, namely, in estuarine environments affected by petroleum contamination.

A few studies (e.g. Lin and Mendelssohn 2008; 2009) showed also higher hydrocarbon degradation in the rhizosphere region of two salt marsh plants, *Spartina patens* and *Juncus roemerianus*, compared to uncolonised sediments. So, salt marsh vegetation could be a useful technique for the recovery of oil-affected coastal wetlands. Nevertheless, information regarding temperate salt marsh plants that effectively reduce hydrocarbon levels in contaminated sediments is still scarce, that information being essential for a successful restoration and remediation of oil-impacted habitats. In fact, salt marsh plant species around the world can be different (Adam 2002) and can vary particularly with respect to root morphology, root exudation, root decomposition and associated microbial communities (Lee et al. 2008).

It is, therefore, important to evaluate the potential of other salt marsh plants for the rhizodegradation of hydrocarbon-contaminated sediments/soils. In this chapter are reported two studies that were carried out to ascertain the phytoremediation potential of salt marsh plants, or salt-tolerant marsh plants, in near-natural controlled conditions. In the first study, the suitability of *Juncus maritimus* and *Phragmites australis* for petroleum hydrocarbon rhizoremediation in contaminated estuarine sediment, the environment where they grow, was assessed. The second study involved soil contaminated with petrochemical products and evaluated the capability of the salt mash plants *Halimione portulacoides*, *Juncus maritimus* and *Scirpus maritimus* for soil remediation. As mentioned previously due to their capability to cope with the environmental stresses to which they are exposed, salt marsh plants can be also an option to deal with contaminated soils and contribute for its remediation.

23.2 Suitability of *Juncus maritimus* and *Phragmites australis* for Petroleum Hydrocarbon Rhizoremediation in Contaminated Estuarine Sediment

This study aimed to access the suitability of *Juncus maritimus* and *Phragmites australis* (a salt marsh plant and a salt-tolerant marsh plant that can grow in salty environments as estuaries) for petroleum hydrocarbon rhizodegradation in contaminated estuarine sediment. For that a 5-month greenhouse experiment was carried out, the hydrocarbon degradation being evaluated periodically. Sediments and plants were collected in March of 2012 at low tide from a salt marsh located in the Lima River estuary (NW Portugal). *Juncus maritimus* presents a significant rhizome structure, with adventitious roots borne in it, and *Phragmites australis* presents a fibrous and dense root system. Plants were collected together with the sediment involving their roots (rhizosediment) (cubes of approximately 15 cm × 15 cm × 15 cm). Simultaneously, uncolonised sediment, located within 2 m of the colonised sediment, was collected.

At the laboratory, sediments were separated from roots, homogenised and mixed manually with Arabian light crude oil (supplied by an oil refinery) to a concentration of 12 ml $L_{\text{wet sediment}}^{-1}$, simulating an oil spill. The same procedure was carried out for the non-colonised sediment. After 48 h the plants were transplanted to pots containing the respective contaminated sediment (three pots per plant). Pots without and with plants were kept in a greenhouse exposed to natural light and environmental temperature conditions (greenhouse placed outdoors). Throughout the experiment, pots were irrigated with a saline nutrient solution (one quarter-strength modified Hoagland nutrient solution) to maintain nutrients at optimal levels for plants and microorganisms. During the 5-month experiment, sediment samples were periodically collected from all pots. Total petroleum hydrocarbon (TPH) concentrations were determined by Fourier transform infrared spectroscopy (FT/IR), using a previous optimised method (Couto et al. 2014). Gas chromatography with flame ionisation detection (GC/FID) was used to obtain information on the aliphatic hydrocarbon profile in the sediments, in the hydrocarbon range between C_{10} and C_{40} (adapted from Saari et al. 2007). This range is lower than that measured using FT/ IR.

Hydrocarbon degradation percentages are shown in Fig. 23.1. These percentages were calculated attending to the TPH concentrations measured in the initially contaminated sediments and in the sediments collected along the 5-month experiment.

Uncolonised sediments presented the lowest degradation percentages. In fact, around 15 % of degradation was observed in the first 90 days of the experiment, being reduced to ca. 3 % until the end of the experiments. This reduction of the degradation percentage can be related with the analytical techniques used. In fact, the FT/IR technique used for TPH assessment includes the simpler hydrocarbons. Along time, degradation of the more complex hydrocarbons can result in simpler hydrocarbons, which will then be included in the measurements by FT/IT.

Regarding colonised sediment, in *Phragmites australis* rhizosediment, hydrocarbon removal was observed in the first 30 days. After that hydrocarbon degradation percentages did not present a significant variation along time, being of about 20 %. In general this degradation percentage was higher than that observed for non-colonised sediments indicating that the plant had a positive role on the removal of

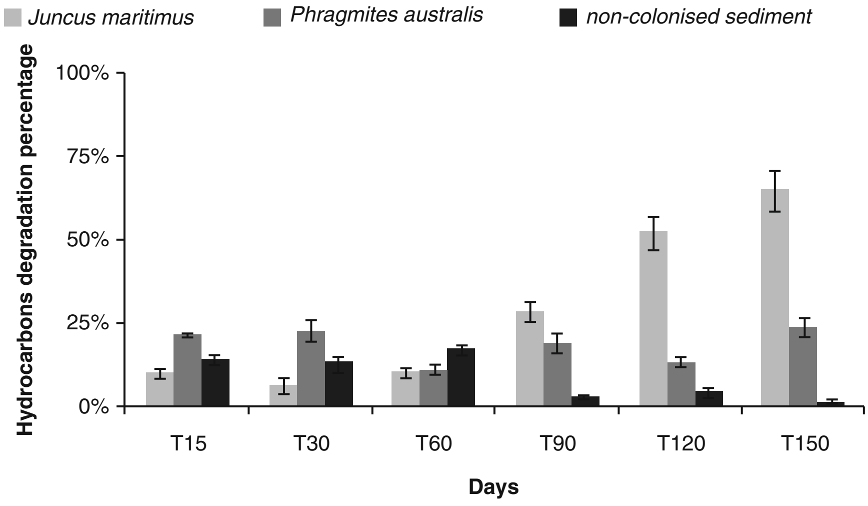

Fig. 23.1 Hydrocarbon degradation percentages observed along the 5 months of experiment, calculated for each time period relatively to the hydrocarbon initial content

hydrocarbons from the contaminated sediments. As for *Juncus maritimus* rhizosediment, hydrocarbon degradation percentages were low until day 60, being in general lower than that observed for non-colonised sediment or for *Phragmites australis* rhizosediment. However, after this exposure period, the percentage significantly ($p<0.05$) increased over the remaining time of the experiment, reaching 65 %. Hydrocarbon removal from colonised sediments seemed to be related with the physiological status of the plants. Both plants seemed to be adversely affected by the transplantation. After 30 days, visual inspection clearly showed that *Juncus maritimus* presented stress symptoms, with a senescent appearance, whereas *Phragmites australis* appeared healthy. By the end of 60 days, *Phragmites australis* stems presented a senescent appearance and no apparent recoveries were noted until the end of the experiment. However*,* *Juncus maritimus* plants started to recover after 60 days, with new stems growing.

So, with time *Juncus maritimus* was more efficient than *Phragmites australis* in the removal of petroleum hydrocarbons from the environment, probably due to the recovery of the plant activity after 60 days of adaptation.

Scrutiny of GC profiles can be useful to gain more insight about the hydrocarbon biodegradation susceptibility in salt marsh sediments (Fig. 23.2). Chromatogram profiles corroborate the degradation rates observed. In fact, in the case of *Juncus maritimus,* after full plant recovery (90 days), a significant ($p<0.05$) degradation of the aliphatic hydrocarbons, with a clear reduction in the measured chromatogram peaks, was observed.

Therefore, the presence of the plants clearly enhanced hydrocarbon degradation compared to the uncolonised sediments. In fact, significant ($p<0.05$) hydrocarbon degradation was found both in *Juncus maritimus* and *Phragmites australis* rhizosediments, pointing to the potential of plants' rhizosphere with associated microorganisms for hydrocarbon removal. Nevertheless, significant differences were observed between the behaviour of the two plants species. The physiological status of the plants seems to be related with hydrocarbon degradation potential. As mentioned these two plants also have completely different root systems which can clearly affect their potential to be used in rhizodegradation technology.

These preliminary results suggest that plants can play an important role in restoration and remediation of oil-impacted salt marsh sediments. However, the species of the plant to be used has to be carefully chosen. In addition, the time factor can be very important for several plant species, as, for instance, in the present study *Juncus maritimus* required a longer period of time to significantly remove the hydrocarbons. So, the time frame should be considered when engaging a revegetation of estuarine hydrocarbon-polluted sites with this plant, which will be linked also with the plant acclimation.

One should be aware that the efficiency of rhizodegradation will depend on the establishment of a dynamic and synergistic relationship between plants and microorganisms (Wenzel 2009) but also on the environmental conditions and plant characteristics. In addition, for rhizodegradation strategies, indigenous plants are preferred as they are well adapted to the environmental conditions and because it should also be intended to preserve native biodiversity. Nevertheless, invasive plants can also have a role in the recovery of impacted ecosystem. Although it is not recommended using such plants for marsh revegetation, when they are already present at the impacted site,they can also contribute for the recovery, as long as their dissemination is controlled.

Therefore, more research on this topic is needed. In addition, experiments should be carried out to assess ways to improve the potential for hydrocarbon removal shown by these plants.

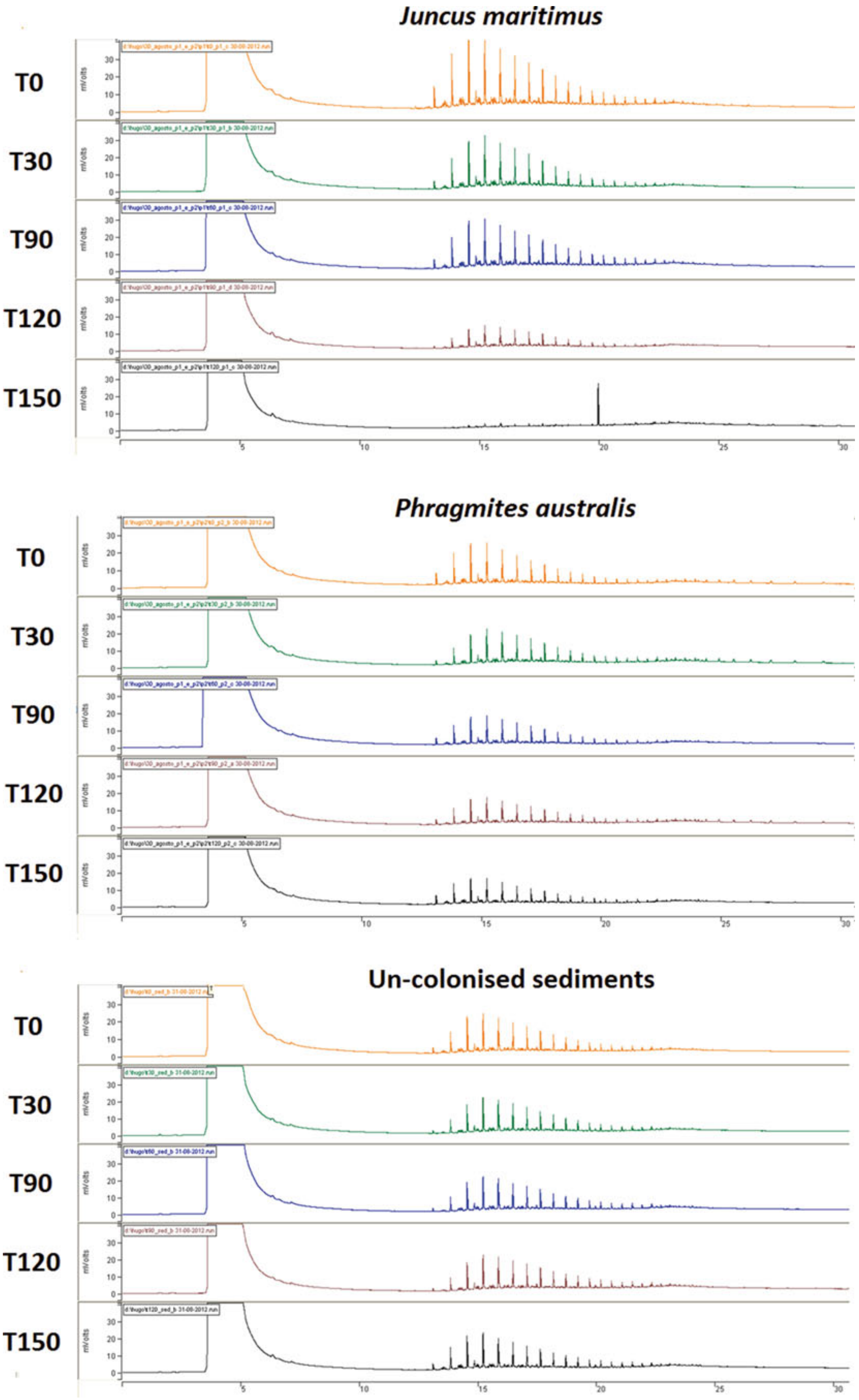

Fig. 23.2 Chromatogram profiles (aliphatic hydrocarbons in the range between C_{10} and C_{40}) of the contaminated sediments along the 5 months of experiment. Data for uncolonised sediment and sediment colonised by *Phragmites australis* and by *Juncus maritimus* at the beginning (T0) and after 30 (T30), 60 (T60), 90 (T90) and 150 (T150) days of experiment is shown

23.3 Evaluation of the Capability of the Salt Marsh Plants *Halimione portulacoides*, *Juncus maritimus* and *Scirpus maritimus* for Soil Remediation

As mentioned previously, the information on suitability of salt marsh plants for hydrocarbon remediation is hard to find in the literature. The few studies published (Lin and Mendelssohn 2008; 2009; Ribeiro et al. 2011) indicated that these plant species can be suitable to remediate hydrocarbon-contaminated sediments, and the study reported in the previous section clearly indicated that some have that potentiality, contributing for the recovering of estuarine impacted sites. However, hydrocarbon contamination affects not only estuarine and coastal areas but also terrestrial nonsaline area, as, for example, those where refinery facilities are implemented. Soils at these facilities can be contaminated due, for instance, to accidental leakages. In addition, petroleum refineries are often installed near coastal areas, and salt marsh plants can be potentially suitable to remediate these soils.

The potential of different salt marsh plants (*Halimione portulacoides, Juncus maritimus and Scirpus maritimus*) to improve degradation of hydrocarbons present in soil from a petroleum refinery was researched. This study is reported in detail in Couto et al. (2011), being summarised here. The different plant species used are very common in salt marsh areas in the Atlantic coast of Europe, being collected at a salt marsh area in the Douro River estuary (NW Portugal).

A 7-month experiment was carried out with soil contaminated with hydrocarbons (Couto et al. 2011). Two types of contamination were present in the soil: an old contamination with crude oil that occurred a few years ago at the refinery site where the soil was collected and a mixture of old and recent contamination with turbine oil simulated at the laboratory. The soil without new contamination and soil after the new contamination (done manually with physical homogenisation) were put in pots, and the different plants were transplanted to them. Pots with old contaminated soil and pots with soil with a mixture of old and recent contamination remained non-colonised to be used as controls (natural attenuation). The pots were placed in the exterior, in a non-covered area. Biweekly, all the pots were supplied with one quarter-strength modified Hoagland nutrient solution. At the end of the experiment, soil samples were collected (in the depth in contact with plant roots) for TPH content analysis, similar to that reported in the study of the previous section.

Results showed that, in the soil with old contamination, at the end of the experiment, significant ($p<0.05$) removal of hydrocarbons was observed when *Scirpus maritimus* was present (TPH concentration lowered 13 %) (Table 23.1). A similar trend was observed for *Halimione portulacoides* (contamination 10 % lower than the initial one). On the other hand, *Juncus maritimus* did not have any measurable effect on hydrocarbon removal, indicating that both the nature of the plant and environmental conditions may condition rhizodegradation ability. In addition, in the uncolonised pots, hydrocarbon removal was not observed, which suggested that most of the bioavailable hydrocarbon fraction of this old contaminated soil had been already degraded by indigenous microorganisms. So, the presence of *Scirpus maritimus* made possible remediation of less available contamination which seemed recalcitrant to be remediated by microorganisms (Couto et al. 2011).

On the other hand, in the soil containing also a new contamination, significant ($p<0.05$) and marked hydrocarbon removal was observed in all pots after 7 months (Table 23.1). In addition, hydrocarbon removal was higher in vegetated pots than in non-colonised ones, indicating that the plants directly or indirectly favoured this hydrocarbon removal. This was particularly notorious in the pots with *Scirpus maritimus* or with *Halimione portulacoides*. A parallel study carried out by the authors (Couto et al. 2012) in mesocosm containers with the same soil (a study carried out in the refinery environment) showed similar results for *Scirpus maritimus*. In that study a chromatographic analysis of the hydrocarbons in the range between C_{10} and C_{40} (analysis carried out as described in the previous section) indicated that *Scirpus maritimus* favoured degradation of both recent

Table 23.1 Total petroleum hydrocarbon (TPH) concentrations in the tested soil at the beginning and after 7 months of experiment (mean and standard deviation in brackets)

	Treatment			
	Scirpus maritimus	*Juncus maritimus*	*Halimione portulacoides*	Non-colonised soil
Old contamination (initial TPHs: 10.4 ± 0.9 mg g^{-1})				
TPHs (mg g^{-1})[a]	9.1 (0.1)	15 (3)	9.4 (1.2)	10 (2)
TPHs removal (%)	13	0	10	3
Mixture of old and recent contamination (initial TPHs: 30 ± 2 mg g^{-1})				
TPHs (mg g^{-1})[a]	6 (2)	16 (1)	11 (2)	17 (2)
TPHs removal (%)	79	48	64	42

The respective mean removal hydrocarbon percentage is also presented (Adapted from Couto et al. (2011) with permission from Elsevier)

[a] Mean values and respective standard deviation (between brackets, $n=3$)

and older contamination, being particularly efficient for the heavier hydrocarbon fraction (in the range C_{22}–C_{40}).

Therefore, this study clearly showed that the salt marsh plants *Scirpus maritimus* and *Halimione portulacoides* have potential to be used in remediation of hydrocarbon contamination in soil (Couto et al. 2011). In the presence of these plant species, the hydrocarbon removal was faster and more extensive than in non-colonised soil. In addition, in only 7 months of exposure, *Scirpus maritimus* (the most efficient plant) was capable of removing not only all the recent contamination but also 40 % of the older contamination which was refractory to natural attenuation (Couto et al. 2011). In contrast, *Juncus maritimus* did not reveal significant capability to remove hydrocarbons when transplanted to soil, a totally different result from that observed in the study reported in the previous section where the plant was transplanted to its natural sediment. These results show that the specificities of the plant species and of the environmental conditions must be taken in consideration when planning and designing rhizodegradation techniques' implementation.

23.4 Conclusions

Biological remediation technologies can be applied all over the world as they have positive impact in public opinion and they do not damage the ecosystem's integrity.

Presented studies clearly indicate the potentiality of some salt marsh plants, or salt-tolerant marsh plants, to remediate not only hydrocarbon-contaminated sediments but also hydrocarbon-contaminated nonsaline soils, indicating that they can have a broader application in different environments. However, future research work is necessary to complement and substantiate the information provided by the present studies.

In any case, at least two items should be well thought out when designing rhizodegradation strategies: (a) the suitability for hydrocarbon remediation of the plant in the contaminated place and (b) the time that the plant requires to act as an efficient remediator of hydrocarbons.

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Phytoremediation in Thailand: A Summary of Selected Research and Case Histories*

24

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24.1 Historical Overview

24.1.1 Collaborative Research on Inorganic Contaminants

Research on the potential use of phytoremediation to remediate or to repair damaged habitat in Thailand and the surrounding region began in 1999. The initial research projects were planned as a collaborative effort between the Department of Biology at Mahidol University in Bangkok and the Environmental Science Program at the University of Massachusetts, Amherst. Collaborating faculty at the partner institutions recognized the many advantages to developing phytoremediation applications in tropical and semitropical ecosystems because of their high diversity of plant species and a favorable growing climate. Funding for the new program came from the Royal Golden Jubilee Scholarship Program of the Thailand Research Fund and the Massachusetts Department of Environmental Management.

The initial goals of the research program focused on studying inorganic contaminants and plants with the potential to remediate or partially remediate damaged habitat by extracting metal and metalloid contaminants from soil, water, and sediments. Collaborative phytoextraction studies done by Thai scientists in the USA used plant species found in both Thailand and the USA.

24.1.2 Phytoextraction and Phytostabilization

Because metal contaminants do not biodegrade, the basic strategy was to use native plants or plant communities to gradually phytoextract or phytostabilize contaminants by successive uptake and plant harvesting. The approach offered a relatively low-cost solar-driven biotechnology that provided the removal of contaminants with minimum disruption to the habitat under remediation. An ideal plant for metal phytoextraction or phytostabilization has to be tolerant to high levels of the metal and must accumulate high metal concentrations in its harvestable parts. Additional favorable traits are fast growth, easy propagation, and a profuse root system (Garbisu and Alkorta 2001; Vassilev et al. 2002). The phytoremediation of soils and sediments contaminated with heavy metals/metalloids basically includes: (1) phytoextraction, which uses metal-accumulating plants to extract metals from soils and concentrate them in the harvestable parts, and (2) phytostabilization, which uses metal-/metalloid-tolerant plants to reduce the mobility of metals/metalloids by accumulating them in their roots, adsorbing them on root surface, and decreasing mobility through changes in soil chemistry, thereby reducing risks of further environmental degradation by leaching metals/metalloids into groundwater (Salt et al. 1995; Vangronsveld et al. 1995; Dahmani-Müller et al. 2000).

Phytoextraction is a long-term remediation effort, requiring many cycles to reduce metal concentrations to acceptable levels, and the approach is far from being considered a mature technology (Luca et al. 2007). Most of data published

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have been extrapolated from experiments performed under conditions that are not adequate to give results applicable for the future cleanup of contaminated areas (Baker and Whiting 2002; McGrath et al. 2006; Luca et al. 2007).

The potential to use phytoextraction and/or phytostabilization as a cleanup technology could be of particular value in mining areas where the soils are heavily contaminated with metals. The normal phytoremediation practice is to choose metal-tolerant, fast-growing plants with high biomass that can grow in metal-contaminated and nutrient-deficient soils. The establishment of a permanent cover of vegetation can fulfill the objectives of stabilization, pollution control, visual improvement, and removal of threats to humans (Wong 2003). The use of metal-tolerant plants in revegetation is not a new concept and was investigated in the 1960s in field trials (Tordoff et al. 2000). Plant species that may be considered suitable for revegetation on mine tailings should have evolved biological mechanisms to resist, tolerate, or thrive on the toxic metalliferous substrates (Whiting et al. 2004). These evolved species could be an ideal choice as pioneer species to remediate damaged habitat. Grasses and legumes are the favorable option because of their adaptation to deficiency of nutrients and fast-growing traits (Li 2006).

In recent years Pb hyperaccumulators (Pb in shoot biomass >1,000 mg kg/1) have been identified. These plants are usually derived from Pb-contaminated areas and have the ability to tolerate high concentrations of Pb in the soil where they are grown. Many of these plants belong to the following families: Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae (Kucharski et al. 2001). There are seven recognized plant species that are considered to be hyperaccumulators of Pb: *Armeria maritima*, *Thlaspi rotundifolium*, *T. alpestre*, *Alyssum wulfenianum*, *Polycarpaea synandra* (Piechalak et al. 2002), *Hemidesmus indicus* (Sekhar et al. 2005), and *Sesbania drummondii* (Barlow et al. 2000; Sahi et al. 2002).

24.2 Selected Examples of Phytoremediation Research

24.2.1 Phytoextraction of Inorganics from Water

Table 24.1 summarizes selected initial studies of the removal by biosorption or phytoextraction of heavy metal contaminants from natural or synthetic water. The use of the water hyacinth *Eichhornia crassipes* for the phytoremediation of nutrients and heavy metal contaminants in various aquatic systems has become widespread in Thailand. Reoxygenation of water is an additional benefit of using *Eichhornia sp.* in phytoremediation applications. The potential utility of the water hyacinth for nutrient removal and biomass production, based on the continuous harvest at the maximum sustainable yield (MSY), was modeled using the Tha Chin River, a tributary of the Chao Phraya River (Mahujchariyawong and Ikeda 2001). Results indicated that nitrogen and phosphorus were reduced by maintaining maximum removal rates of 0.42 and 0.09 for nitrogen and phosphorus, respectively. Other phytoremediation research in Thailand focused on the sorption of metal contaminants from water using native species of bacteria and plants. Research indicated that most standard biological treatment processes for removing heavy metals from wastewater were maximized when contaminant concentrations were relatively high, i.e., above 100 mg/l. Applying live or dead cells of the cyanobacterium *Spirulina platensis* to phytoremediate Cd-contaminated water using the process of biosorption can play an important role in the treatment of wastewater with Cd concentrations below 100 mg/l (Rangsayatorn et al. 2002; Rangsayatorn et al. 2004). *S. platensis* removed up to 98 mg/l Cd from water in laboratory microcosms and between 36 and 71 mg Cd/g/cells immobilized in either silicate or alginate gels. Phytoextraction of Cd by 13 species of aquatic plants was studied in synthetic water lab microcosms amended with 0.1, 1.0, and 10 mg Cd/l (Bunluesin et al. 2004). Depending on species, total Cd was removed in a range of 23–7,942 mg/l. Lead removal from water with concentrations of 0.1, 1.0, and 10 mg/l in lab microcosms using the aquatic macrophytes *Ceratophyllum demersum*, *Hygrophila difformis*, *Cabomba caroliniana*, and *Ludwigia hyssopifolia* has been reported (Yaowakhan et al. 2005). They noted that 80–100 % Pb was removed.

The interaction of metal/metalloid contaminants can influence uptake and sorption in aquatic systems. Information about the interaction of Cd and Zn as they affect phytoextraction by *C. demersum* in the presence of humic substances was studied (Bunluesin et al. 2007). In general, Cd decreased Zn accumulation in *C. demersum* except at the lowest concentration of Zn in which the Zn accumulation was similar to that without Cd. *C. demersum* could accumulate high concentrations of both Cd and Zn. Humic acid had a significant effect on total Zn accumulation in plants, and 2 mg/l of humic acid reduced total Zn accumulation at 1 mg/l levels in water from 2,167 to 803 mg/kg. Cd uptake by plant tissue and toxicity symptoms and accumulation at 0.25 and 0.5 mg/1 were reduced from 515 to 154 mg kg/1 and from 816 to 305 mg kg/1, respectively, by the addition of 2 mg/1 of humic acid.

The hydroponic removal of Pb, Cd, and Zn using phytoextraction by *Chromolaena odorata* was studied in lab microcosms using synthetic water (Tanhan et al. 2007). Plants were collected from a field site at the Bo Ngam (Pb) lead mine site in Kanchanaburi Province in Thailand and exposed to Pb concentrations of 0.25 and 0.50 mg/l and Zn concentrations of 10 and 20 mg/l. Results indicated that bioconcentration factors (BCF) in the range of 2,131–6,362 Pb and 2,673–3,705 Cd were achieved. Another hydroponic study using *Sonchus arvensis* from the Bo Ngam lead mine site examined Pb extraction from water with concentrations

Table 24.1 Summary of selected studies of the phytoremediation of inorganic contaminants from water

Plant species	Total contaminants removed[a]
Eichhornia crassipes	N and P reduced by maintaining maximum removal rates of 0.42.N and 0.09 P in a river water model Mahujchariyawong and Ikeda (2001)
Spirulina platensis	98 mg Cd/g cells
	36–71 mg Cd/g/cells[b]
	Rangsayatorn et al. (2002; 2004)
Pistia stratiotes	23–7,942 mg Cd/1 from 0.1 to 10 mg/1 water by different species
Colocasia esculenta	Bunluesin et al. (2004)
Lemna minor	
Azolla pinnata	
Ceratophyllum demersum	
Neptunia oleracea	
Typha angustifolia	
Hydrophila verticillata	
Hygrophila difformis	
Ipomoea aquatic	
Cyperus strigosus	
Trapa bispinosa	
Pandanus amaryllifolius	
Ceratophyllum demersum	80–100 % Pb from water with Pb at 0.1–10 mg/1 water
Hygrophila difformis	Yaowakhan et al. (2005)
Cabomba caroliniana	
Ludwigia hyssopifolia	
Ceratophyllum demersum	92-587mg/kg dw Cd from water with
	0.5–5.0 mg/l Zn and 0.05–0.25 Cd
	464–765 mg/kg dw Zn from water
	with 0.05–0.25 mg/l Cd and 0.5–5.0 Zn
	Bunluesin et al. (2007)
Chromolaena odoratum	BCF[c] of 2,131–6,362 Pb, 2,673–3,705 Cd,
	341–839 Zn from water
	Tanhan et al. (2007)
Sonchus arvensis	849 mg/kg Pb from water with 5 mg/kg Pb
	TF[d] Pb 0.24 BC[e] Pb 170.8
	Surat et al. (2008)
Buddleja asiatica	TF Pb 0.04 BC 1,308
	TF Cd 0.02 BC 1,420
	TF Zn 0.06 BC 193
Buddleja paniculata	TF Pb 0.01 BC 1,221
	TF Cd 0.02 BC 1,253
	TF Zn 0.05 BC 271
	All plants in water for 15 days with Pb at 10–20
	mg/l; Cd at 0.25–0.50; mg/1; Zn at 10–20 mg/1
	Waranusantigul et al. (2008)
Pteris vittata	TF 0.03 BCF 478–773
Pityrogramma calomelanos	TF 0.03–0.04 BCF 728–773
Nephrolepsis exaltata	TF 0.03–0.04 BCF 308–405
cv. Gracillimum	
N. exaltata	TF 0.04 BCF 205–243
cv. Smirha	All ferns in water for 15 days with 10–20 mg/1 Pb
	Soongsombat et al. (2009)

[a] Synthetic water

[b] Immobilized cells

[c] Bioconcentration Factor (BCF)

[d] *Translocation Factor (TF)*

[e] Bioaccumulation Coefficient (BC)

in the range of 5–20 mg/l (Surat et al. 2008). Results indicated that *S. arvensis* could remove up to 849 mg/kg Pb from water with 5 mg/kg and had a transformation factor (TF) of 0.24 and a bioaccumulation coefficient (BC) of 170.8. The phytoextraction potential of *Buddleja asiatica* and *B. paniculata* to remove Pb, Zn, and Cd from synthetic water was also investigated in laboratory hydroponic studies (Waranusantigul et al. 2008). Both species of *Buddleja* were collected from the Bo Ngam Pb mine site, and results indicated that *B. asiatica* had maximum TF values of 0.04, 0.02, and 0.06 for Pb, Cd, and Zn, respectively. Maximum BC values for *B. asiatica* were 1,308, 1,420, and 193 for Pb, Cd, and Zn, respectively. Maximum TF values noted for *B. paniculata* were 0.01, 0.02, and 0.05, respectively, and maximum BC values were 1,221, 1,253, and 271 for Pb, Cd, and Zn, respectively.

Lead tolerance and hydroponic accumulation in the ferns *Pteris vittata*, *Pityrogramma calomelanos*, and *Nephrolepis exaltata* cv. *gracillimum* collected from the Bo Ngam lead mine site were studied with a view toward potential Pb phytoremediation applications. The ferns tested had TF values in the range of 0.3–0.4, and both *P. vittata* and *P. calomelanos* had bioconcentration factors (BCF) of 773 (Soongsombat et al. 2009).

24.2.2 Phytoextraction/Phytostabilization of Inorganics from Soils/Sediments

Table 24.2 summarizes selected studies of the phytoextraction/phytostabilization of heavy metal/metalloid contaminants from soils and sediments. The data represent a diverse array of soil or sediment types with different chemical and physical characteristics studied in laboratories, greenhouses, and field sites including standardized potting soils, soils from field sites, and mining waste soils and sediments. In Thailand, vetiver grass is found widely distributed naturally in all parts of the country and has been used for erosion control and slope stabilization. The phytoremediation potential of *Vetiveria zizanioides* and *V. nemoralis* growing in field plots treated with Pb at concentrations of 5–11 g/l has been studied (Chantachon et al. 2003). Results indicated that phytoextraction coefficients (PC) fell in the desirable range of 0.5–10.0 with reported values of 2.7 and 1.3 in *V. zizanioides* and *V. nemoralis*, respectively. The phytoremediation potential of *Chrysopogon nemoralis* and *Chrysopogon zizanioides* to treat wastewaters with Mn, Fe, Cu, Zn, and Pb contaminants from dairy, battery, ink, and electric bulb facilities was reported by Roongtanakiat (2009). They reported average TF values of 0.67 (Mn), 0.07 (Fe), 0.41 (Cu), 0.19 (Zn), and 0.07 (Pb). Studies using electron microscopic surveys (Panich-Pat et al. 2005) revealed that most of the Pb phytoextracted from soil by *Typha angustifolia* was in the root biomass with most of the contaminant in the rhizome near the cell wall. Most Pb transported to the above ground biomass was deposited in the leaf chloroplasts. The interaction of Pb and Cd in greenhouse lab soil-water microcosms at contaminant concentrations of 1,666 mg/l Pb and 38.5 mg/l Cd was also examined (Panich-Pat et al. 2010). They reported total plant biomass accumulations of Pb and Cd as 14,675 and 390 mg/l, respectively. Several soil and/or sediment studies focused on the heavily contaminated Bo Ngam lead mine site in Kanchanaburi Province in Thailand. A survey of the uptake and accumulation of Pb contaminants by the plant community growing at the Bo Ngam Pb mine site identified 48 plant species in 14 families (Rotkittikhun et al. 2006). *Microstegium ciliatum*, *Polygala umbonata*, and *Spermacoce mauritiana* were noted as the most efficient phytoextractors in soil with Pb concentrations up to 164,333 mg/kg dw Pb. Other studies examined the extraction of Pb, Cd, and Zn by *Chromolaena odorata* from soils collected from different areas of the Bo Ngam Pb mine site (Tanhan et al. 2007). Values reported indicate that TFs were 1.69, 2.25, and 1.00 for Pb, Cd, and Zn, respectively, and BC values were 6,243, 3,705, and 787 for Pb, Cd, and Zn, respectively. The phytoextraction potential of *Sonchus arvensis* collected at the Bo Ngam lead mine site growing in pots with mine site soils amended with organic fertilizer and the chelator EDTA was also examined (Surat et al. 2008). They reported Pb TF and BC values of 2.19 and 2.38, respectively. The phytoremediation potential of *Buddleja asiatica* and *B. paniculata* using hydroponic and pot studies in laboratory and field studies was also estimated using materials from the Bo Ngam lead mine site. Plants were exposed to Pb concentrations up to 20 mg/l in water and 101,405 mg/kg dw in soils (Waranusantigul et al. 2008). Results indicated that Pb TF values of 1.1 and 0.9 were achieved in pot studies with *B. asiatica* and *B. paniculata*, respectively, and TF values up to 5.0 and 2.2 for *B. asiatica* and *B. paniculata*, respectively.

Studies of Cr removal from tannery industry soils by six plant species considered to be weeds was examined to determine their potential applications in phytoremediation (Sampanpanish et al. 2006). *Pluchea indica* and *Cynodon dactylon* phytoextracted 152 mg/kg Cr from the soils used in pot studies.

The effects of phosphorus fertilizer and rhizosphere microbe amendments on arsenic accumulation by the silverback fern, *Pityrogramma calomelanos*, were investigated in both greenhouse and field experiments (Jankong et al. 2007). Field experiments were conducted on site and in greenhouse experiments using contaminated soils from Ron Phibun District of Nakhon Si Thammarat Province in Thailand with As concentrations of 136–269 mg/kg. The results showed that phosphorus significantly increased plant biomass, rhizosphere microbes, and As accumulation in *P. calomelanos*. Phosphorus and rhizosphere bacteria enhanced As phytoextraction, while rhizofungi

Table 24.2 Summary of selected studies of the phytoextraction/phytostablization of inorganic contaminants from soils/sediments

Plant species	Contaminant removed
Vetiveria zizanioides	PC[a] 2.7
Vetiveria nemoralis	PC 1.3
	In soil plots treated with PB at 5-11g/l for 3 months Chantachon et al. (2003)
Chrysopogon nemoralis	TF[b] Mn 0.67
Chrysopogon zizanioides	Fe 0.07
	Cu 0.41
	Zn 0.19
	Pb 0.07
	Average values Roongtanakiat (2009)
Typha angustifolia	Up to 14,675 mg/1Pb In soil-water lab microcosms with Pb at 1,666 mg/1 and Cd at 38.5 mg/1 Panich-pat et al. (2005; 2010)
Microstegium cilatum *Polygala umbonata* *Spermacoce mauritiana*	12,200–28,370 mg/kg Pb in shoots of 3 species of 48 species examined from Bo Ngam Pb mine site Rotkittikhun et al. (2006)
Chromolaena odorata	TF 1.69 Pb
	TF 2.25 Cd
	TF 1.00 Zn
	BC[c] 6,243 Pb
	BC 3.705 Cd
	BC 787 Zn
	In soils with 117,728 mg/kg dw Pb; 1.6 mg/kg 261 Mg/kg dw Zn Tanhan et al. (2007)
Sonchus avensis	TF Pb 2.19
	BF[d] Pb 2.38
	Studies in mine site soils amended with EDTA and organic fertilizer Surat et al. (2008)
Buddleja asiatica	TF Pb 1.1
Buddleja paniculata	TF Pb 0.9
	In pot studies with mine soils at Pb concentrations up to 89,084 mg/kw dw
B. asiatica	TF Pb 0–5.0
B. paniculata	TF Pb 0–5.0 101,405 mg/kg dw
	In mine site soils with Pb concentrations up to 101,405 mg/kg dw Waranusantigul et al (2008)
Cynodon dactylon	Total Cr accumulation of 152 mg/kg dw in
Pluchea indica	Cynodon dactylon and *Pluchea reticulatus*
Phyllanthus reticulatus	Sampanpanish et al. (2006)
Vetiveria nemoralis	
Amaranthus viridis	
Pityrogramma calomelanos	In pot studies with tannery site soils with 100 mg
	Cr (VI)/mg
	TF 7.6 in greenhouse
	TF 86.6 in field 8 weeks
	With P fertilizer inoculated with rhizosphere
	microbes
	TF 17.8 bacteria in greenhouse
	TF 14.9 fungi in greenhouse
	TF 59.0 bacteria in field
	Tf 70.4 fungi in field
	Jankong et al. (2007)
Pteris vittata	TF 0.29–0.86 BCF[e] 0.17–2.83
Pityrogramma calomelanos	TF 0.09–0.26 BCF 0.12–1.40
Nephrolepsis exaltat cv. Gracillium	TF 0.10–0.72 BCF 0.12–0.44
	In pot studies with soil with various Pb
	Concentrations in the range of 52–103,065 mg/kg
	Soongsombat et al. (2009)

(continued)

Table 24.2 (continued)

Plant species	Contaminant removed
Pteris vittata	TF 0.12–0.52 BCF 0.20–0.88
Pityrogramma calomelanos	TF 0.06–0.28 BCF 0.07–0.31
	Studies at the Bo Ngam mine site with soil
	concentrations in the range of 269–97,995 mg/kg
	Soongsombat et al. (2009)
Chromolaena odoratum	TF 1.5 BAF^{c} 1.33 EC^{f} 0.13
Gynura pseudochina	TF 6.00 BAF 20.48 EC 2.48
Justicia procumbens	TF 1.04 BAF 3.15 EC 0.71
Impatien violaeflora	TF 1.15 BAF 1.29 EC 0.61
	Cd removal from studies at the Padaeng Zn mine site with soil and sediment concentrations of Cd in the range of 596–1,458 and Zn in the range of 2,733–57,012 mg/k Phaenark et al. (2009)
Leucaena leucocephala	TF ranged between 0.00 and 1.09
Acacia mangium	ECR^{g} ranged between 0.00 and 0.44
Peltophorum pterocarpum	*Pb uptake in field studies ranged between 0.00 and 812 ug/plant.*
Pterocarpus macrocarpus	*A. mangium* plus organic fertilizer was the best option for phytostabilization of Pb-contaminated mine tailing. Meeinkuirt et al. (2012)
Lagerstroemia floribunda	
Eucalyptus camaldulensis	
Thysanolaena maxima	TF 0.79 BCFR^{h} 8.30
Vetiveriia zizanioides	TR 0.45 BCFR 6.46
	Pot studies for 3 months with Pb mine tailings up
	To 15,597 mg/kg and various fertilizer
	amendments
	Meeinkuirt et al. (2013)
T. maxima	TF 0.88 BCRF 23.80
V. zizanioides	TF 23.40 BCFR 11/15
	In field studies for 12 months with Pb mine tailings
	Up to 19,234 mg/kg and various fertilizers
	amendments
	Meeinkuirt et al. (2013)

[a] Phytoextration Coefficient (PC^{1})
[b] Translocation Factor (TF)
[c] Bioaccumulation Coefficient (BC)
[d] Bioaccumulatation Factor (BF or BAF)
[e] Bioconcentration Factor (BCF)
[f] Extraction Coefficient (EC)
[g] Enrichment Coefficient of Roots (ECR)
[h] Bioconcentrations Factor For Root (BCFR)

significantly reduced total As concentration in plants but increased plant biomass providing soil phytostabilization.

Lead tolerance and accumulation in the ferns *Pteris vittata* and *Pityrogramma calomelanos* were studied to estimate the potential for the phytoremediation of Pb-contaminated soils (Soongsombat et al. 2009). Plants were exposed to Pb concentrations in the range of 50–103,065 mg/kg in soil at the Bo Ngam lead mine site and in laboratory pot studies. In the pot studies, *P. vittata* achieved a range of Pb TF values of 0.29–0.86 and BCF values of 0.17–2.83, *P. calomelanos* achieved a range of TF values of 0.09–0.26 and BCF values in the range of 0.12–0.44, and *N. exaltata* cv. *gracillimum* achieved values of 0.10–0.72 and BCF values of 0.12–0.44. Additional studies done at the Bo Ngam lead mine site with *P. vittata* and *P. calomelanos* produced TF and BCF values in the range of 0.12–0.52 and 0.20–0.88, respectively, for *P. vittata* and 0.06–0.28 and 0.07–0.31 for *P. calomelanos*, respectively.

Significant Cd contamination in soil and rice was reported in 2003 in Mae Sot, Tak Province, Thailand, where rice-based agricultural systems were established in the vicinity of the Padaeng Zn mine. The prolonged consumption of Cd-contaminated rice poses risks to public health, and the health impacts of Cd-exposed populations in Mae Sot have been demonstrated. The Thai government has prohibited rice cultivation in the area as an effort to prevent further exposure. To identify potential plants for phytoremediation, sampling sites at the Zn mine were selected to collect plant and soil samples. Total Cd and Zn concentrations in sediments or soils were approximately 596 and 20,673 mg/kg, respectively, in tailing pond area, 543 and 20,272 mg/kg in an open pit area, and 894 and 31,319 mg kg/kg in a stockpile area. Cadmium and Zn levels of 1,458 and 57,012 mg/kg, respectively, were noted in a forest area and 64 and 2,733 mg kg/l, respectively, in a Cd- and Zn-contaminated rice field. Samples of 36 plant species representing 16 families were collected at the study site along with soils and were analyzed in the laboratory (Phaenark et al. 2009). Four species (*Chromolaena odorata*, *Gynura pseudochina*, *Impatiens violaeflora*, and *Justicia procumbens*) could be considered as Cd hyperaccumulators since their shoot Cd concentrations exceeded 100 mg Cd kg/1 dry mass and they showed a translocation factor >1. Only *Justicia procumbens* could be considered as a Zn hyperaccumulator with a Zn concentration in its shoot of more than 10,000 mg Zn kg/1 dry mass with a translocation factor >1.

The Pb phytostabilization potential of six tree species (*Leucaena leucocephala*, *Acacia mangium*, *Peltophorum pterocarpum*, *Pterocarpus macrocarpus*, *Lagerstroemia floribunda*, *Eucalyptus camaldulensis*) was examined at the KEMCO Pb mine in Kanchanaburi Province, Thailand (Meeinkuirt et al. 2012). The studies included a pot experiment for 3 months and a field trial experiment for 12 months using soil with a Pb concentration greater than 9,850 mg/kg. In the pot study, *E. camaldulensis* treated with Osmocote fertilizer attained the highest total biomass (15.3 g plant/l) followed by *P. pterocarpum* (12.6 g plant/1) and *A. mangium* (10.8 g plant/1), both treated with cow manure. Cow manure application resulted in the highest root Pb accumulation (>10,000 mg kg/1) in *L. floribunda* and *P. macrocarpus*. These two species also exhibited the highest Pb uptake (85–88 mg plant−1). Results from field trials also showed that Osmocote promoted the best growth performance in *E. camaldulensis* (biomass 385.7 g plant/1, height 141.7 cm) followed by *A. mangium* (biomass 215.9 g plant/1, height 102.7 cm). Total Pb accumulation in plants was 600 and 812 ug/plant in *E. camaldulensis* and *A. mangium*, respectively. *A. mangium* with the addition of organic fertilizer was the best option for the phytostabilization of Pb-contaminated mine soils and sediments. Additional research (Meeinkuirt et al. 2013) using pot studies of *Thysanolaena maxima* and *Vetiveria zizanioides* growing in Pb mine tailings as high as 15,597 mg/kg and various fertilizer amendments showed TF and BCFR values up to 0.79 and 8.30, respectively, in *T. maxima* and TF and BCFR values up to 0.45 and 6.46, respectively, in *V. zizanioides*. Field studies lasting up to 12 months in Pb mine tailings up to concentrations of 19,234 mg/kg with various fertilizer amendments from the same site showed TF and BCFR values of 0.88 and 23.80, respectively, for *T. maxima* and TF and BCFR values up to 23.40 and 6.46, respectively, for *V. zizanioides*.

24.2.3 Selected Studies of the Phytoextraction/Conversion of Organic Contaminants

Table 24.3 summarizes selected studies of the phytoremediation of organic contaminants from soils, sediments, and water. Organic chemicals as soil and sediment contaminants have received increased interest by researchers in Thailand in recent years. Phytoremediation approaches to remediate or restore industrial and agricultural sites contaminated with xenobiotic compounds have been studied by universities, private companies, and research institutes. Early research on the phytoremediation of organic contaminants in Thailand noted that vetiver hedges can play an important role in the processes of captivity and decontamination of pesticides, preventing them from contaminating and accumulating in crops (Pinthong et al. 1998). Another study described the potential to use either individual agricultural plants (*Zea sp.*, *Cucumis sp.*, and *Psophocarpus sp.*) or mixtures of the plants to remove polyaromatic hydrocarbons (PAHs) in soil (Somtrakoon et al. 2014). Results indicated that the most effective plant community to remove anthracene and fluorine contaminants at concentrations of 138.9 and 95.9 mg/kg was a mixture of *Zea sp.* and *Psophocarpus sp.*

The use of nanotechnology coupled with phytotechnology to treat soils contaminated with 100 mg/kg trinitrotoluene (TNT) was the subject of a recent research project (Jiamjitrpanich et al. 2012). The investigators used pot studies to examine the potential of combining phytoremediation and nanoscale zero-valent iron (nZVI) for the removal of TNT from contaminated soil. *Panicum maximum* was chosen and used as a hyperaccumulator plant for this study. The results indicated that nano-phytoremediation was more effective than either nano-remediation or phytoremediation alone as a method for the degradation and removal of TNT-contaminated soil. Overall, the highest removal efficiency of nano-phytoremediation (100 %) was found in soil with the TNT/nZVI ratio of 1/10 (100 mg/kg initial TNT concentration).

Pot studies were also used to estimate the removal potential of trichloroethylene (TCE) from contaminated soils by several ecotypes of *Vetiveria zizanioides* (Janngam et al. 2010). Two ecotypes (Sri Lanka and Songkhla) were seen to remove more than 98 % TCE in 1 month, and the Sri Lanka

Table 24.3 Summary of selected studies of the phytoremediation of organic contaminants from soils/sediments/water

Plant species	Contaminant removed
Zea sp.	86–97 % reduction
Cucumis sp.	Pot studies with mixtures of 138.9 and 95.9 mg/kg anthracene and fluorine respectively for 1 month
Psophocarpus sp.	Somtrakoon et al. (2014)
Panicum maximum	100 % reduction
	Pot studies with 100 mg/kg Trinitrotoluene (TNT) and nanoscale zero valence Fe for 2 months Jiamjitrpanich et al. (2012)
Vetiveria zizanioides	98.39 % reduction
Ecotype Sri Lanka	TF[a] 2.32
Ecotype Songkhla	TF 1.36
	Pot studies with 549 mg/kg Trichloroethylene (TCE) for 1 month
	Janngam et al. (2010)
Vetiveria zizanioides	94 % degradation efficiency
Vetiveria zizanioides	91 % degradation efficiency
Vetiveria zizanioides with fertilizer and	Pot studies with 10,000 mg/kg diesel and 10,000 mg/kg lube oil mixture for 4 months
Pseudomonas putida	Subba et al. (2012)
Bruguiera gymnorrhiza	[b]99 % reduction COD (from 15,408 mg/1) and 100 %
	reduction Bisphenol A at several concentrations
	Saiyood et al. (2013)

[a] Translocation Factor TF
[b] Wastewater

and Songkhla ecotypes had TF values of 2.32 and 1.36, respectively.

The degradation efficiency of total petroleum hydrocarbons (TPH) in a two percent diesel and lubrication oil mixture using potential phytoremediation approaches was studied in a series of pot and landfarming experiments (Subba et al. 2012). Treatments included the use of *Vetiveria zizanioides* with and without amendments of fertilizer, aeration, and *Pseudomonas putida* inoculations. The highest degradation efficiencies of TPH (91–94 %) were noted in *V. zizanioides* alone and *V. zizanioides* with fertilizer and *P. putida* amendments. Other research demonstrated that *Bruguiera gymnorrhiza* reduced concentrations of COD (at 15,408 mg/l) and various concentrations of bisphenol A by 99 and 100 %, respectively, in contaminated wastewater (Saiyood et al. 2013).

24.2.3.1 Summary

The collaborative studies completed in Thailand, Canada, and the USA generally indicate that practical applications of phytoextraction may be limited due to wide variation in contaminated sites and the requirement that multiple seasons and harvest sequences will be necessary for success. Currently phytoextraction is far from being considered a mature technology by others as well (Luca et al. 2007). Most of data published have been extrapolated from experiments performed under conditions that are not adequate to give results applicable for the future remediation of contaminated areas (Baker and Whiting 2002; McGrath et al. 2006; Luca et al. 2007). However, marginally contaminated agricultural soils provide some opportunities where phytoextraction can be used as a polishing technology (Dickinson et al. 2009).

There has been an increased interest in phytostabilization as a viable approach to phytoremediate contaminated soils and sediments in Thailand in recent years. *Acacia mangium* with the addition of organic fertilizer was reported as one option for the phytostabilization of Pb-contaminated mine tailings because it retained higher Pb concentrations in the roots than other trees tested (Meeinkuirit et al. 2012; 2013). Organic chemicals as soil and sediment contaminants have received increased interest by researchers in Thailand in recent years, and efforts to learn more about the phytoremediation of organic contaminants in water, soils, and sediments are expected to continue at an increased rate. Phytoremediation approaches to remediate or restore industrial and agricultural sites contaminated with xenobiotic compounds offer special challenges and will continue to be studied by universities, private companies, and research institutes.

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