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Phytoremediation Technology: Hyper-accumulation Metals in Plants

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Abstract This paper reviews key aspects of phytoremediation technology and the biological mechanisms underlying phytoremediation. Current knowledge regarding the application of phytoremediation in alleviating heavy metal toxicity is summarized highlighting the relative merits of different options. The results reveal a cutting edge application of emerging strategies and technologies to problems of heavy metals in soil. Progress in phytoremediation is hindered by a lack of understanding of complex interactions in the rhizosphere and plant based interactions which allow metal translocation and accumulation in plants. The evolution of physiological and molecular mechanisms of phytoremediation, together with recently-developed biological and engineering strategies, has helped to improve the performance of both heavy metal phytoextraction and phytostabilization. The results reveal that phytoremediation includes a variety of remediation techniques which include many treatment strategies leading to contaminant degradation, removal (through accumulation or dissipation), or immobilization. For each of these processes, we review what is known for metal pollutants, gaps in knowledge, and the practical implications for phytoremediation strategies.

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1 Introduction

Heavy metals are ubiquitous environmental contaminants in industrialized societies. Soil pollution by metals differs from air or water pollution, because heavy metals persist in soil much longer than in other compartments of the biosphere (Lasat 2002). Over recent decades, the annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (Singh et al. 2003). Sources of heavy metal contaminants in soils include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway 1995). For example, mine tailings rich in sulphide minerals may form acid mine drainage (AMD) through reaction with atmospheric oxygen and water, and AMD contains elevated levels of metals that could be harmful to animals and plants (Stoltz 2004).

Ground-transportation also causes metal contamination. Highway traffic, maintenance, and de-icing operations generate continuous surface and ground-

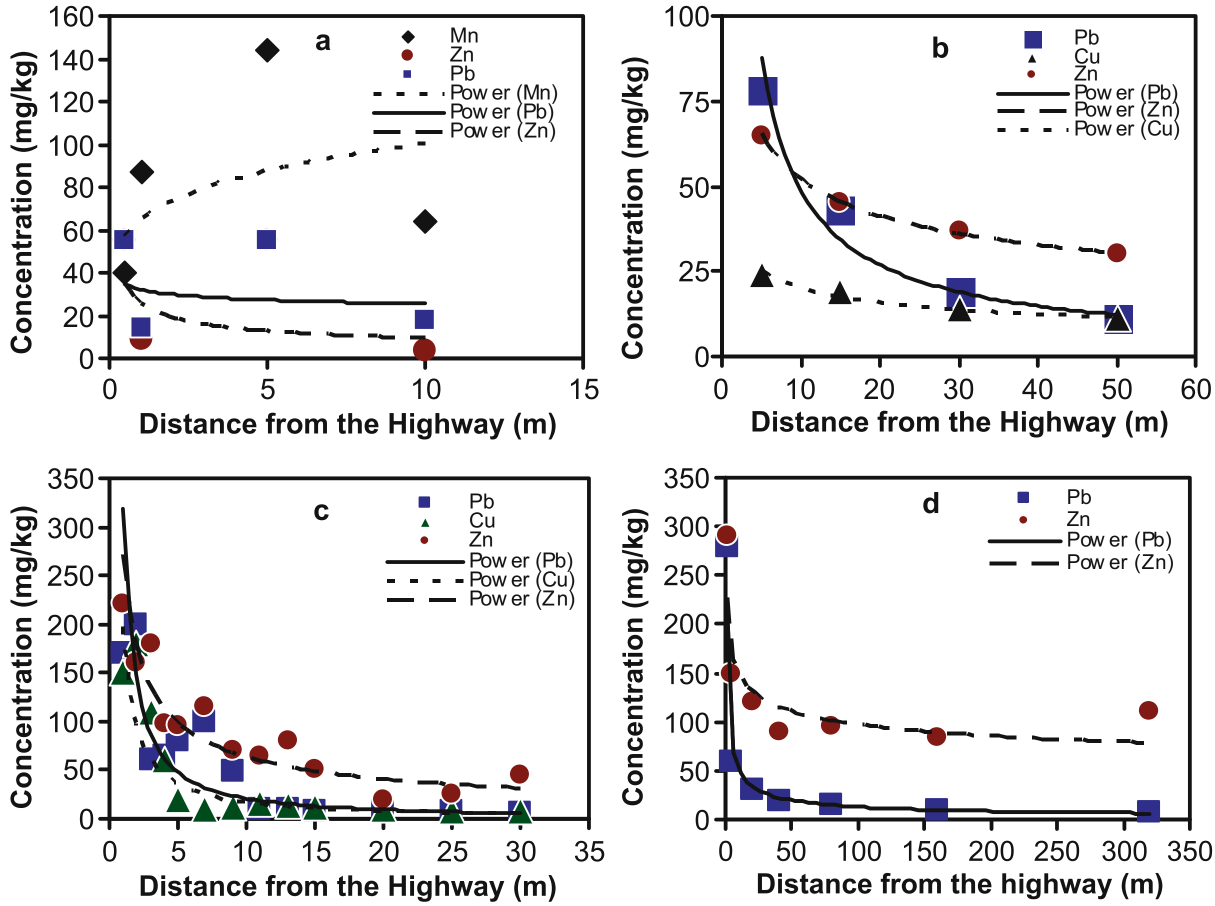

Figure	Metal	Regression Equation, y	Correlation, R^2
a	Mn	$64.487x^{0.1919}$	0.2453
	Zn	$25.616x^{-0.427}$	0.2013
	Pb	$31.996x^{-0.0989}$	0.0359
b	Pb	$347.5x^{-0.8549}$	0.966
	Cu	$43.347x^{-0.3368}$	0.9625
	Zn	$110.66x^{-0.3295}$	0.9971
c	Pb	$319.69x^{-1.1831}$	0.8269
	Cu	$197.25x^{-1.0689}$	0.8548
	Zn	$271.6x^{-0.6321}$	0.804
d	Pb	$206.93x^{-0.6}$	0.9731
	Zn	$227.69x^{-0.1842}$	0.7593

Fig. 1 Heavy metal content of road-side soils from **a** Brussels-Ortend, Belgium (Albasel and Cottenie 1985); **b** Osogobo, Nigeria (Fakayode and Olu-Owolabi 2003); **c** West bank, Palestine (Swaileh et al. 2004); **d** A31 between Nancy and France (Viard et al. 2004)

water contaminant sources. Tread ware, brake abrasion, and corrosion are well documented heavy metal sources associated with highway traffic (Ho and Tai 1988; Fatoki 1996; García and Millán 1998; Sánchez Martín et al. 2000). Heavy metal contaminants in roadside soils originate from engine and brake pad wear (e.g. Cd, Cu, and Ni) (Viklander 1998); lubricants (e.g. Cd, Cu and Zn) (Birch and Scollen

2003; Turer et al. 2001); exhaust emissions, (e.g. Pb) (Gulson et al. 1981; Al-Chalabi and Hawker 2000; Sutherland et al. 2003); and tire abrasion (e.g. Zn) (Smolders and Degryse 2002). The concentration ranges of metals of greatest importance in roadside soils are given in Fig. 1.

Toxic heavy metals cause DNA damage, and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller et al. 1998; Baudouin et al. 2002). 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 (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).

Metal-contaminated soil can be remediated by chemical, physical or biological techniques (McEldowney et al. 1993). Chemical and physical treatments irreversibly affect soil properties, destroy biodiversity and may render the soil useless as a medium for plant growth. These remediation methods can be costly. Table 1 summarizes the cost of different remediation technologies. Among the listed remediation technologies, phytoextraction is one of the lowest cost techniques for contaminated soil remediation. There is a need to develop suitable cost-effective biological soil remediation techniques to remove contaminants without affecting soil fertility. Phytoremediation could provide sustainable techniques for metal remediation. This paper summarizes the development of phytoremediation for metals in the past two decades.

Phytoremediation involves the use of plants to remove, transfer, stabilize and/or degrade contaminants in soil, sediment and water (Hughes et al. 1997). The idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source. The concentration of metal uptake in plants is shown in Fig. 2. A series of fascinating scientific discoveries, combined with interdisciplinary research, has allowed phytoremediation to develop into a promising, cost-effective, and environmentally friendly technology.

The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) refers to a diverse collection of plant-based technologies that use either naturally occurring, or genetically engineered, plants to clean contaminated environments (Cunningham et al. 1997; Flathman and Lanza 1998). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of indigenous metals in their tissues without symptoms of toxicity (Reeves and Brooks 1983; Baker and Brooks 1989; Baker et al. 1991; Entry et al. 1999). The idea of using plants to extract metals from contaminated soil was re-introduced and developed by Utsunamyia (1980) and Chaney (1983). The first field trial on Zn and Cd phytoextraction was conducted by Baker et al. (1991).

Several comprehensive reviews have been written, summarizing many important aspects of this novel plant-based technology (Salt et al. 1995, 1998; Chaney et al. 1997; Raskin et al. 1997; Chaudhry et al. 1998; Wenzel et al. 1999; Meagher 2000; Navari-Izzo and Quartacci 2001; Lasat 2002; McGrath et al. 2002; McGrath and Zhao 2003; McIntyre 2003; Singh et al. 2003; Garbisu and Alkorta 2001; Prasad and Freitas 2003; Alkorta et al. 2004; Ghosh and Singh 2005; Pilon-Smits 2005). These reviews give general guidance and recommendations for applying phytoremediation, highlighting the processes associated with applications and underlying biological mechanisms. The present review is intended to give an updated, more concise version of information so far available with respect to different subsets of phyoremediation. It provides a critical overview of the present state of the art, with particular emphasis on phytoextraction and phytostabilization of soil heavy metal contaminants.

Table 1 Cost of different remediation technologies (Glass 1999)

Process	Cost (US$/ton)	Other factors
Vitrification	75–425	Long-term monitoring
Land filling	100–500	Transport/excavation/ monitoring
Chemical treatment	100–500	Recycling of contaminants
Electrokinetics	20–200	Monitoring
Phytoextraction	5–40	Disposal of phytomass

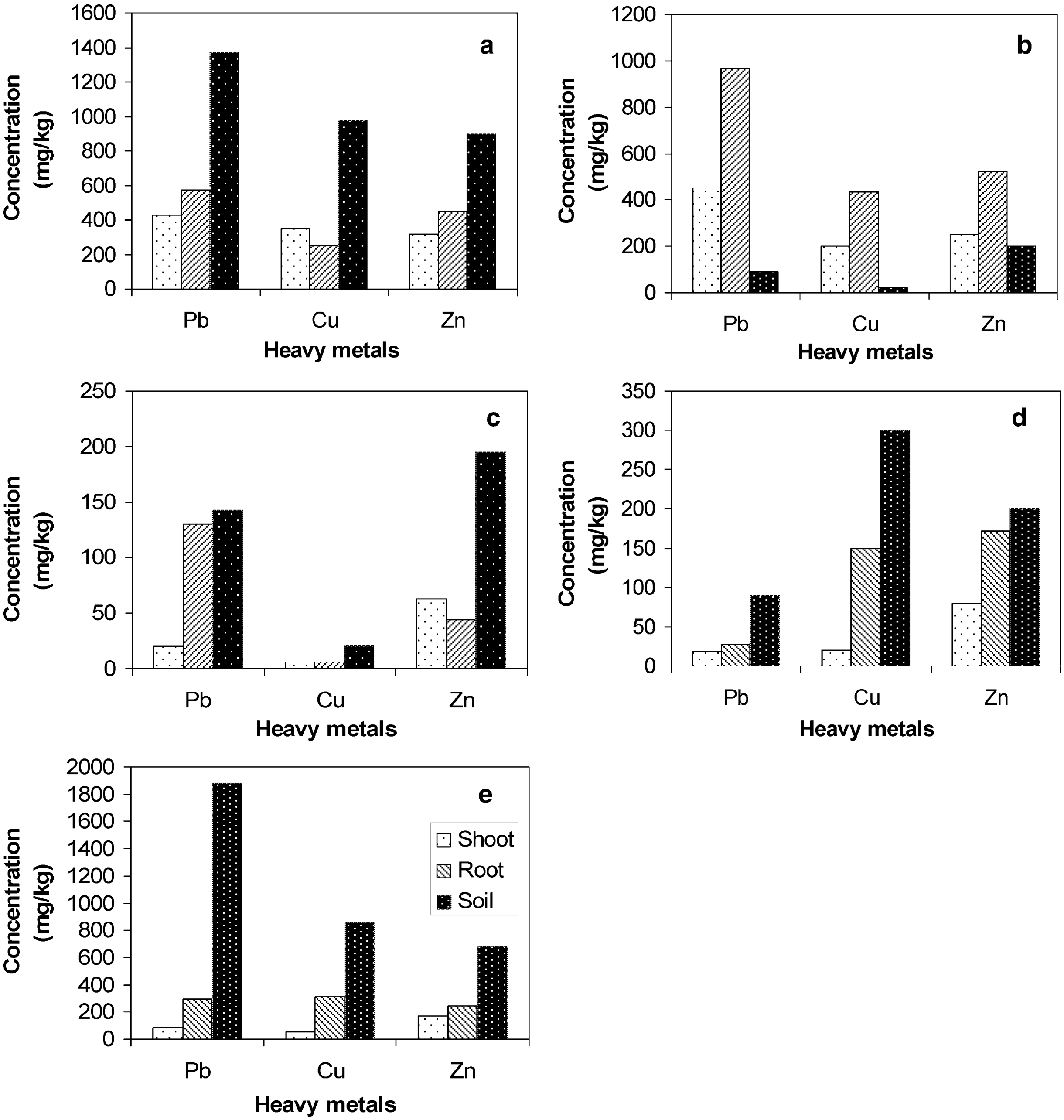

Fig. 2 Heavy metal content in plants growing on contaminated sites (Yoon et al. 2006). **a** Bahia grass (*Paspalum notatum*); **b** Wire grass (*Gentiana pennelliana*); **c** Ticktrefoil (*Desmodium paniculatum*); **d** Flats edge (*Cyperus esculentus*); **e** Bermuda grass (*Cynodon dactylon*)

2 Categories of Phytoremediation

Depending on the contaminants, the site conditions, the level of clean-up required, and the types of plants, phytoremediation technology can be used for containment (phytoimmobilization and phytostabilization) or removal (phytoextraction and phytovolatilization) purposes (Thangavel and Subhuram 2004). The four different plant-based technologies of phytoremediation, each having a different mechanism of action for remediating metal-polluted soil, sediment, or water: (1) phytostabilization, where plants stabilize, rather than remove contaminants by plant roots metal retention; (2) phytofiltration, involving plants to clean

Table 2 Different mechanisms of phytoremediation (Ghosh and Singh 2005)

Process	Mechanisms	Contaminant
Phytofiltration	Rhizosphere accumulation	Organics, Inorganic
Phytostabilisation	Complexation	Inorganic
Phytoextraction	Hyper accumulation	Inorganic
Phytovolatilization	Volatilisation by leaves	Organics, Inorganic

various aquatic environments; (3) phytovolatilization, utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization; and (4) phytoextraction, in which plants absorb metals from soil and translocate them to harvestable shoots where they accumulate. The different mechanisms of phytoremediation are summarized in Table 2.

Ecological issues also need to be evaluated when developing a phytoremediation strategy for a polluted site. In particular, one has to consider how the phytoremediation efforts might affect local ecological relationships, especially those involving other crops. Since the phytoremediation plants will be grown under contaminated soil/ water conditions, where other crops may not thrive because of contaminant toxicities, the competition problem is unlikely to arise.

2.1 Phytostabilization

Phytostabilization uses certain plant species to immobilize contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone and physical stabilization of soils. The schematic mechanism of phytostabilization is illustrated in Fig. 3. This process reduces the mobility of contaminants and prevents migration to groundwater or air. This can re-establish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations (Tordoff et al. 2000). Thorough planning is essential for successful revegetation, including physical and chemical analyses, bioassays and field trials. The main approaches to revegetation are summarized in Table 3.

Metal-tolerant species may be used to restore vegetation to such sites, thereby decreasing the potential migration of contaminants through wind, transport of exposed surface soils, leaching of soil and contamination of groundwater (Stoltz and Greger 2002). Unlike other phytoremediative techniques, phytostabilization is not intended to remove metal contaminants from a site, but rather to stabilize them by accumulation in roots or precipitation within root zones, reducing the risk to human health and the environment. It is applied in situations where there are potential human health impacts, and exposure to substances of concern can be reduced to acceptable levels by containment. The disruption to site activities may be less than with more intrusive soil remediation technologies.

Phytostabilization is most effective for fine-textured soils with high organic-matter content, but it is suitable for treating a wide range of sites where large areas are subject to surface contamination (Cunningham et al. 1997; Berti and Cunningham 2000). However, some highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is impossible (Berti and Cunningham 2000). Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, easier to implement, and preferable aesthetically. (Berti and Cunningham 2000; Schnoor 2000). When decontamination strategies are impractical because of the extent of the contaminated area or the lack of adequate funding, phytostabilization is advantageous (Berti and Cunningham 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique.

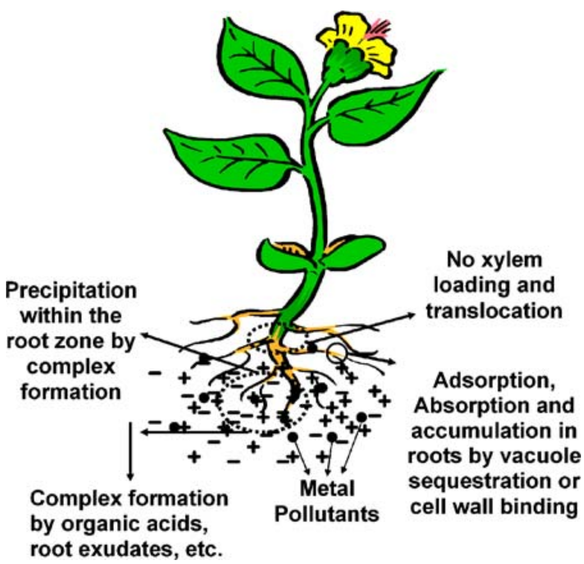

Fig. 3 Schematic mechanism of phytostabilization

Table 3 Approaches to revegetation (adapted from Williamson and Johnson 1981)

Soil characteristics	Reclamation technique	Problems encountered
Low toxicity – Total metal content <0.1%	Amelioration and direct seeding with grasses and legumes. Seed or transplant ecologically adapted native species. Apply lime, organic matter and fertilizers as necessary	Medium or long-term maintenance program. Expertise required on the characteristics of native flora. Grazing must be strictly monitored and excluded in some situations
High toxicity – Total metal content >0.1%	Amelioration and direct seeding with metal tolerant and salt tolerant (saline) ecotypes. Apply lime, organic matter and fertilizers as necessary. Amelioration with 10–50 cm of innocuous mineral waste and organic material and seeding with grasses and legumes. Apply lime and fertilizer if necessary	Commitment to regular management. Expertise required for the selection of tolerant ecotypes. Grazing management not possible. Regression will occur if depths of amendment are shallow or if upward movement of metals occurs. Availability and transport costs limiting.
Extreme toxicity	Isolation; surface treatment with 30–100 cm of innocuous barrier material and surface banding with 10–30 cm of rooting medium. Apply lime and fertilizer if necessary.	High cost and potential limitation of material availability.

Characteristics of plants appropriate for phytostabilization at a particular site include: tolerance to high levels of the contaminant(s) of concern; high production of root biomass able to immobilize these contaminants through uptake, precipitation, or reduction; and retention of applicable contaminants in roots, as opposed to transfer to shoots, to avoid special handling and disposal of shoots.

Yoon et al. (2006) evaluated the potential of 36 plants (17 species) growing on a contaminated site and found that plants with a high bio-concentration factor (BCF, metal concentration ratio of plant roots to soil) and low translocation factor (TF, metal concentration ratio of plant shoots to roots) have the potential for phytostabilization (Fig. 2a–e). The lack of appreciable metals in shoot tissue also eliminates the necessity to treat harvested shoot residue as a hazardous waste (Flathman and Lanza 1998). In a field study, mine wastes containing copper, lead, and zinc were stabilized by grasses (Agrostis tenuis cv. Goginan for acid lead and zinc mine wastes, Agrostis tenuis cv. Parys for copper mine wastes, and Festuca rubra cv. Merlin for calcareous lead and zinc mine wastes) (Smith and Bradshaw 1992). The research of Smith and Bradshaw (1992) led to the development of two cultivars of *Agrostis tenuis* Sibth and one of *Festuca rubra* L which are now commercially available for phytostabilizing Pb-, Zn-, and Cu-contaminated soils.

Stabilization also involves soil amendments to promote the formation of insoluble metal complexes that reduce biological availability and plant uptake, thus preventing metals from entering the food chain (Adriano et al. 2004; Berti and Cunningham 2000; Cunningham et al. 1997). One way to facilitate such immobilisation is by altering the physicochemical properties of the metal-soil complex by introducing a multipurpose anion, such as phosphate, that enhances metal adsorption via. anion-induced negative charge and metal precipitation (Bolan et al. 2003). Addition of humified organic matter (O.M.) such as compost, together with lime to raise soil pH (Kuo et al. 1985), is a common practice for immobilizing heavy metals and improving soil conditions, to facilitate re-vegetation of contaminated soils (Williamson and Johnson 1981). Soil acidification, due to the oxidation of metallic sulphides in the soil, increases heavy metal bioavailability; but liming can control soil acidification; also, organic materials generally promoted fixation of heavy metals in non-available soil fractions, with Cu bioavailability being particularly affected by organic treatments (Clemente et al. 2003). The production of sulphate by sulphide oxidation increased solubility of Zn and Mn, and therefore their concentrations in plant-available (DTPA-extractable) fractions. However, the bioavailability of Cu did not decrease with either soil pH increase or with lime, indicating that the organic treatments might have had a significant effect. Revegetation of mine tailings usually requires amendments of phosphorus, even though phosphate addition can mobilize arsenic (As) from the tailings. Leachates and uptakes of As were found to be higher with an organic fertilizer amendment than superphosphate, particularly in combination with barley (Mains et al. 2006b). Active phytoremediation followed by natural attenuation, was effective for remediation of the pyrite-polluted soil (Clemente et al. 2006).

The Met PAD [IM] bio test was used to assess the extent of metal accumulation by plants in mining areas. Plants were identified as hyper tolerant which can be used for phytostabilization (Boularbah et al. 2006). Two plant species, *Hyparrhenia hirta* and *Zygophyllum fabago*, that have naturally colonized some parts of mine tailings in South-East Spain, have been reported to tolerate high metal concentrations in their rhizospheres. These plant species do not take up high concentrations of metals, providing a good tool to achieve surface stabilization of tailings with low risk of affecting the food chain (Conesa et al. 2006). Phytostabilization efforts in the Mediterranean region have been found to be improved by using mixtures including local metallicolous legume and grass species (Frérot et al. 2006). It is better to identify the plants spontaneously colonizing the contaminated site, since they are more ecologically adapted than introduced species. Recent research results on phytostabilization are summarized in Table 4.

2.2 Phytofiltration

Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from water and aqueous-waste streams (Prasad and Freitas 2003). Plant roots or seedlings grown in aerated water absorb, precipitate and concentrate toxic metals from polluted effluents (Dushenkov and Kapulnik 2000; Elless et al. 2005). Mechanisms involved in biosorption include chemisorption, complexation, ion exchange, micro precipitation, hydroxide condensation onto the biosurface, and surface adsorption (Gardea-Torresdey et al. 2004).

Rhizofiltration uses terrestrial plants instead of aquatic plants because the former feature much larger fibrous root systems covered with root hairs with extremely large surface areas. Metal pollutants in industrial-process water and in groundwater are most commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge (Ensley 2000). The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov et al. 1995; Salt et al. 1995; Flathman and Lanza 1998; Zhu et al. 1999). Root exudates and changes in rhizosphere pH may also cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza 1998; Zhu et al. 1999).

Dushenkov et al. (1995), Salt et al. (1995), and Flathman and Lanza (1998) contend that plants for phytoremediation should accumulate metals only in the roots. Dushenkov et al. (1995) explain that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. However, Zhu et al. (1999) suggest that the efficiency of the process can be increased by using plants with a heightened ability to absorb and translocate metals.

Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes*, Kay et al. 1984; Zhu et al. 1999), pennywort (*Hydrocotyle umbellata* L., Dierberg et al. 1987), and duckweed (*Lemna minor* L., Mo et al. 1989). However, these plants have limited potential for rhizofiltration because they are not efficient in removing metals as a result of their small, slow-growing roots (Dushenkov et al. 1995). The high water content of aquatic plants complicates their drying, composting, or incineration. In spite of limitations, Zhu et al. (1999) indicated that water hyacinth is effective in removing trace elements in waste streams. Sunflower (*Helianthus annus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for removing metals from water. The roots of Indian mustard are effective in capturing Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al. 1995), whereas sunflower removes Pb (Dushenkov et al. 1995), U (Dushenkov et al. 1997a), ^{137}Cs, and ^{90}Sr (Dushenkov et al. 1997b) from hydroponic solutions. A novel phytofiltration technology has been proposed by Sekhar et al. (2004) for removal and recovery of lead (Pb) from wastewaters. This technology uses plant-based biomaterial from the bark of the plant commonly called Indian sarsaparilla (*Hemidesmus indicus*). The target of their research was polluted surface water and groundwater at industrially contaminated sites. Cassava waste biomass was also effective in removing two divalent metal ions, Cd (II) and Zn (II), from aqueous solutions (Horsfall and Abia 2003). Modification of the

Table 4 Summary of research results – Phytostabilisation

Plant species	Metal	Treatments	Results	Limitations	Reference
Horedeum vulgare, Lupinus angustifolius, Secale cereale	As	Different P amendment products (organic and inorganic)	P amendment of <3 gm^{-2} caused As leaching of 0.5 mg l^{-1} from unplanted lysimeters and up to 0.9 mg l^{-1} on average in planted lysimeters. Arsenic accumulated in plant biomass to 126 mg/kg in shoots and 469 mg/kg in roots.	Variable species – amendment combinations produced differences in the amount of As leached and uptake.	Mains et al. 2006a,b
Lolium italicum and *Festuca arundinaceae*	Pb and Zn	Compost at two rates (10%, and 30% v/v)	The concentration of Pb and Zn in aerial parts and in roots of *L. italicum* and *F. arundinacea* decreased more than five times in presence of compost. Pb content decreased from 218 to 32 mg/kg in shoot and 7,232 to 1,196 mg/kg in root. Zn decreased from 4,190 to 624 mg/kg in shoot and 7,120 to 1,993 mg/kg in root.	The level of contaminants in aerial parts of plants was still too high to be grazed by herbivores.	Rizzi et al. 2004
B. juncea	Cd	Soil amendments – liming materials, phosphate compounds and biosolids	Phosphate immobilized Cd, thereby reducing the phytotoxicity of Cd. The tissue metal concentration of Cd, Cu and Cr(VI) with biosolids application was 253, 157 and 12.4 mg/kg. (i.e. a decrease over nil amendment.)		Bolan et al. 2003
B. juncea	Zn, Cu, Mn, Fe, Pb and Cd	organic amendments (cow manure and compost) and lime	Active phytoremediation followed by natural attenuation, was effective for remediation of pyrite-polluted soil. Soil concentration decreased from: 363 to 166 mg/kg for Zn, 36 to 31 mg/kg for Cu, 1.94 to 1.48 mg/kg for Pb, 1.6 to 0.86 mg/kg for Cd, 679 to 303 mg/kg for Fe and 245 to 120 mg/kg for Mn. Available As concentration in soil decreased from 2.5–13.5 mg/kg after the first crop to 0.5–2.6 mg/kg after the second.	Bioavailability of Cu did not decrease with either soil pH increase or with lime.	Clemente et al. 2003; Clemente et al. 2006
Anthyllis vulneraria, Festuca arvernensis, Koeleria vallesiana, Armeria arenaria.	Zn, Cd and Pb	Local metallicolous legume and grass species.	Festuca and Koeleria in co-culture with Anthyllis showed a decreased concentration of heavy metals (Zn Pb Cd) in their leaves compared with monocultures. For Festuca, decreases of 2885 to	Armeria, one of the plants used in the study reduced the recruitment of Anthyllis seedlings.	Frérot et al. 2006

Table 4 (continued)

Plant species	Metal	Treatments	Results	Limitations	Reference
			1469 mg/kg for Zn, 1002 to 376 mg/kg for Pb and 19 to 8 mg/kg for Cd were reported. For Koeleria, a decrease of 3,514 to 2,786 mg/kg for Zn, 1,960 to 1,477 mg/kg for Pb and 34 to 26 mg/kg for Cd were reported.		
H. hirta and *Z. fabago*	Pb, Zn and Cu	Characterization of soil and plant samples from a mine tailing located in South-East Spain for further phytostabilisation research	*H. hirta* accumulated around 150 mg kg^{-1} Pb in both shoots and roots. Zn concentration was 750 mg kg^{-1} in *Z. fabago* shoots.	The plant species, *H. hirta* and *Z. fabago*, colonize only parts of the tailings with low electrical conductivity	Conesa et al. 2006

cassava waste biomass by treating it with thioglycollic acid resulted in increased adsorption rates for Cd, Cu, and Zn (Abia et al. 2003). Several species of *Sargassum* biomass (non living brown algae) were effective biosorbents for heavy metals such as Cd and Cu (Davis et al. 2000).

Plants used for phytofiltration should be able to accumulate and tolerate significant amounts of the target metals, in conjunction with easy handling, low maintenance costs, and a minimum of secondary waste requiring disposal. It is also desirable for plants to produce significant amounts of root biomass or root surface area (Dushenkov and Kapulnik 2000). Reports on phytofiltration are summarized in Table 5.

2.3 Phytovolatilization

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in the environment. In recent years, researchers have sought naturally-occurring or genetically-modified plants capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization. The mechanism of phytovolatilization is shown schematically in Fig. 4. Volatilization of Se from plant tissues may provide a mechanism of selenium detoxification. As early as 1894, Hofmeister proposed that selenium in animals is detoxified by releasing volatile dimethyl selenide from the lungs, based on the fact that the odour of dimethyl telluride was detected in the breath of dogs injected with sodium tellurite. Using the same logic, it was suggested that the garlicky odour of plants that accumulate selenium may indicate release of volatile selenium compounds. This is the most controversial of phytoremediation technologies. Hg and Se are toxic (Suszcynsky and Shann 1995), and there is doubt about whether the volatilization of these elements into the atmosphere is desirable or safe (Watanabe 1997).

The volatile selenium compound released from the selenium accumulator *Astragalus racemosus* was identified as dimethyl diselenide (Evans et al. 1968). Selenium released from alfalfa, a selenium non-accumulator, was different from the accumulator species and was identified as dimethyl selenide. Lewis et al. (1966) showed that both selenium nonaccumulator and accumulator species volatilize selenium. Selenium phytovolatilization has received the most attention to date (Lewis et al. 1966; Terry et al. 1992; Banuelos et al. 1993; McGrath 1998) because this element is a serious problem in many parts of the world where there are Se-rich soil (Brooks 1998). According to Brooks (1998), the release of volatile Se compounds from higher plants was first reported by Lewis et al. (1966). Terry et al. (1992) report that members of the Brassicaceae are capable of releasing up to 40 g Se ha^{-1} day^{-1} as various gaseous compounds. Some aquatic plants, such as cattail (*Typha latifolia* L.), have potential for Se phytoremediation (Pilon-Smits et al. 1999).

Volatile Se compounds such as dimethylselenide are 1/600 to 1/500 as toxic as inorganic forms of Se

Table 5 Summary of research results – Phytofiltration

Plant species	Metal	Treatments	Results	Reference
B. juncea, H. annuus	Cu, Cd, Cr, Ni, Pb, and Zn	Roots of hydroponically grown terrestrial plants used to remove toxic elements from aqueous solutions	Roots of B, juncea concentrated these metals 131–563-fold (on a DW basis) above initial solution concentrations. The recoveries of heavy metals were 45 % for Cd, 55% for Zn, 50% for Cr, 45% for Ni, 97% for Cu and 100 % for Pb.	Dushenkov et al. 1995
Sunflower plants	U	Rhizofiltration of U in water by roots of sunflower plants	U concentration in water reduced from 21–874 ug/l to <20 ug/l by rhizofiltration	Dushenkov et al. 1997a,b
Water Hyacinth	As, Cd Cr, Cu, Ni, and Se	The abilities of water hyacinth to take up and translocate six trace elements – As, Cd, Cr, Cu, Ni, and Se were studied under controlled conditions	The highest levels of Cd in shoots and roots were 371 and 6,103 mg/kg dry wt., and those of Cr were 119 and 3,951 mg/kg dry wt., Cadmium, Cr, Cu, Ni, and As were more highly accumulated in roots, whereas Se accumulated more in shoots.	Zhu et al. 1999
Duckweed	Hg	Effects of pH, copper and humic acid	Duckweed strongly absorbed Hg from water and after 3 days contained 2,000 ppm of Hg by weight	Mo et al. 1989
Duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata*).	Fe and Cu	Solutions enriched with 1·0, 2·0, 4·0, and 8·0 ppm of these 2 metal ions, renewed every 2 days over a 14-day test period.	When duckweed was kept in a solution containing Cu alone at 8·0 ppm level, the value of the metal concentration factor (i.e. the ratio of metals in the plant to the growth media) after 14 days was 51. However, in the presence of an equal concentration of Fe the value of this factor was 27, indicating the influence of Fe on the uptake rate of Cu.	Jain et al. 1989

found in soil (DeSouza et al. 2000). The volatilization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed, and gaseous species are not likely to redeposit at or near the site (Atkinson et al. 1990; Heaton et al. 1998). Furthermore, sites that utilize this technique may not require much management after the original planting. This remediation method has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al. 1998). Heaton et al. (1998) suggest that the transfer of Hg (O) to the atmosphere would not contribute significantly to the atmospheric pool. This technique appears to be a promising tool for remediating Se- and Hg- contaminated soils.

Volatilization of arsenic as dimethylarsenite has also been postulated as a resistance mechanism in marine algae. However, it is not known whether terrestrial plants also volatilize arsenic in significant quantities. Studies on arsenic uptake and distribution in higher plants indicate that arsenic predominantly accumulates in roots and that only small quantities are transported to shoots. However, plants may enhance the biotransformation of arsenic by rhizospheric bacteria, thus increasing the rates of volatilization (Salt et al. 1998).

Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Some authors suggest that the addition to atmospheric levels through phytovolatilization would not contribute significantly to the atmospheric pool, since the contaminants are likely to be subject to more effective or rapid natural degradation processes such as photodegradation (Azaizeh et al. 1997). However, phytovolatilization should be avoided for sites near population centres and at places with unique

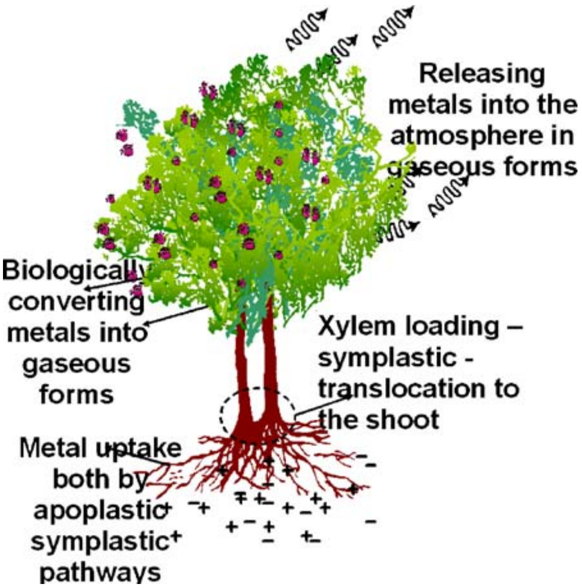

Fig. 4 Schematic mechanism of phytovolatilization

meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al. 1998). Hence the consequences of releasing the metals to the atmosphere need to be considered carefully before adopting this method as a remediation tool.

2.4 Phytoextraction

Phytoextraction, also called phytoaccumulation, refers to the uptake and translocation of metal contaminants in the soil by plant roots into above-ground components of the plants (Fig. 5). The typical levels of heavy metals concentration effects in plants are given in Table 6. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept, whereas phytoextraction is a specific clean-up technology (Prasad and Freitas 2003). Certain plants, called hyperaccumulators, absorb unusually large amounts of metals compared to other plants and the ambient metals concentration. Natural metal hyperaccumulators are plants that can accumulate and tolerate greater metal concentrations in shoots than those usually found in non-accumulators, without visible symptoms. Examples of commonly reported hyperaccumulators are given in Tables 7 and 8. According to Baker and Brooks (1989), hyperaccumulators should have a metal accumulation exceeding a threshold value of shoot metal concentration of 1% (Zn, Mn), 0.1% (Ni, Co, Cr, Cu, Pb and Al), 0.01% (Cd and Se) or 0.001% (Hg) of the dry weight shoot biomass.

Over 400 hyperaccumulator plants have been reported, including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae. Recently Environment Canada has released a database “Phytorem” which contains a worldwide inventory of more than 750 terrestrial and aquatic plants, both wild and cultivated species and varieties, of potential value for phytoremediation. These plants are selected and planted at a site based on the metals present and site conditions. After they have grown for several weeks or months, the plants are harvested. Planting and harvesting may be repeated to reduce contaminant levels to allowable limits (Kumar et al. 1995). The time required for remediation depends on the type and extent of metal contamination, the duration of the growing season, and the efficiency of metal removal by plants, but it normally ranges from 1 to 20 years (Kumar et al. 1995; Blaylock and Huang 2000). This technique is suitable for remediating large areas of land contaminated at shallow depths with low to moderate levels of metal-contaminants (Kumar et al. 1995; Blaylock and Huang 2000).

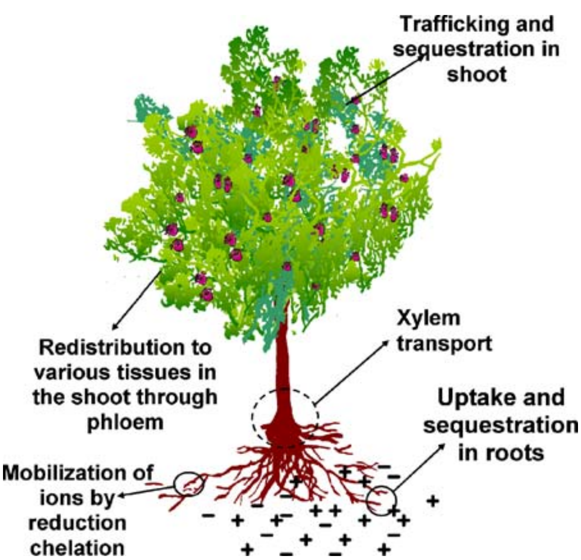

Fig. 5 Schematic mechanism of phytoextraction

Table 6 Effect of typical levels for heavy metals in plants

Status	Metal concentrations (mg kg^{-1})			
	Cd	Cu	Pb	Zn
Deficient	–	<1–5	–	<10
Normal	0.05–2	3–30	0.5–10	10–150
Phytotoxic	5–700	20–100	30–300	>100

Adapted from Pugh et al. (2002)

2.4.1 Types of Phytoextraction

Two basic strategies of phytoextraction are being developed: chelate-assisted phytoextraction, which we term induced phytoextraction; and long-term continuous phytoextraction. If metal availability is not adequate for sufficient plant uptake, chelates or acidifying agents may be added to the soil to liberate them (Cunningham and Ow 1996; Huang et al. 1997; Lasat et al. 1998). However, side effects of the addition of chelate to the soil microbial community are usually neglected. It has been reported (Wu et al. 1999) that many synthetic chelators capable of inducing phytoextraction might form chemically and microbiologically stable complexes with heavy metals, threatening soil quality and groundwater contamination. Several chelating agents, such as EDTA (ethylene diamine tetra acetic acid), EGTA (ethylene glycol-O,O′-bis-[2-amino-ethyl]-N,N, N′,N′,-tetra acetic acid), EDDHA (ethylenediamine di o-hyroxyphenylacetic acid), EDDS (ethylene diamine disuccinate) and citric acid, have been found to enhance phytoextraction by mobilizing metals and increasing metal accumulation (Tandy et al. 2006; Cooper et al. 1999). The increase in the phytoextraction of Pb by shoots of *Z. mays* L. was more pronounced than the increase of Pb in the soil solution with combined application of EDTA and EDDS (Luo et al. 2006). Although EDTA was, in general, more effective in soil metal solubilization, EDDS, less harmful to the environment, was more efficient in inducing metal accumulation in *B. decumbens* shoots (Santos et al. 2006). However, there is a potential risk of leaching of metals to groundwater, and a lack of reported detailed studies regarding the persistence of metal-chelating agent complexes in contaminated soils (Lombi et al. 2001a,b).

2.4.2 Successful Factors for Phytoextraction of Heavy Metals

As a plant-based technology, the success of phytoextraction is inherently dependent on several plant characteristics, the two most important being the ability to accumulate large quantities of biomass rapidly and the capacity to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar et al. 1995; Cunningham and Ow 1996; McGrath 1998; Pilon-Smits 2005). Effective phytoextraction requires both plant genetic ability and the development of optimal agronomic practices, including (1) soil management practices to improve the efficiency of phytoextraction, and (2) crop management practices to develop a commercial cropping system. Ebbs et al. (1997) reported that *B. juncea*, while having one-third the concentration of Zn in its tissue, is more effective at removing Zn from soil than *Thlaspi caerulescens*, a known hyperaccumulator of Zn. The advantage is due primarily to the fact that *B. juncea* produces ten-times more biomass than *T. caerulescens*. Plants for phytoextraction should be able to grow outside their area of collection, have profuse root systems and be able to transport metals to their shoots.

Table 7 Examples of hyperaccumulators and their bioaccumulation potential

Plant species	Metal	Content (mg kg^{-1})	Reference
T. caerulescens	Zn	39,600 (shoots)	Reeves and Brooks (1983)
T. caerulescens	Cd	1,800	Baker and Walker (1989)
Ipomea alpine	Cu	12,300	Baker and Walker (1989)
Sebertia acuminate	Ni	25% by wt. dried sap	Jaffre et al. (1976)
Haumaniastrum robertii	Co	10,200	Brooks (1998)
A. racemosus	Se	14,900	Beath et al. (1937)
P. vittata	As	27,000	Wang et al. 2002
Berkheya coddii	Ni	5,500	Robinson et al. 1997
Iberis intermedia	Ti	3,070	Leblanc et al. 1999

Table 8 Examples of hyperaccumulators and their accumulation characteristics

Plant species	Metal	Results	Reference
Pistia stratiotes	Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn	All elements accumulated mainly in the root system.	Odjegba and Fasidi 2004
Spartina plants	Hg	Organic Hg was absorbed and transformed into an inorganic form (Hg^{+}, Hg^{2+}) and accumulated in roots	Tian et al. 2004
H. annuus	Pb	Pb concentrated in the leaf and stem indicating the prerequisites of a hyperaccumulator plant	Boonyapookana et al. 2005
H. indicus	Pb	Heavy metal mainly accumulated in roots and shoots	Chandra Sekhar et al. 2005
Sesbania drummondii	Pb	Pb accumulated as lead acetate in roots and leaves, although lead sulfate and sulfide were also detected in leaves, whereas lead sulfide was detected in root samples. Lead nitrate in the nutrient solution biotransformed to lead acetate and sulfate in its tissues. Complexation with acetate and sulfate may be a lead detoxification strategy in this plant species	Sharma et al. 2004
Lemna gibba	As	A preliminary bioindicator for As transfer from substrate to plants. Used for As phytoremediation of mine tailing waters because of its high accumulation capacity	Mkandawire and Dudel 2005
P. vittata, P. cretica, P. longifolia and *P. umbrosa*	As	Suitable for phytoremediation in the moderately contaminated soils	Caille et al. 2004
Alyssum	Ni	Majority of Ni is stored either in the leaf epidermal cell vacuoles, or in the basal portions of the numerous stellate trichomes. The metal concentration in the trichome basal compartment was the highest ever reported for healthy vascular plant tissue, approximately 15–20% dry weight	Broadhurst et al. 2004
Solanum nigrum and *C. Canadensis*	Cd	High concentration of Cd accumulated. Tolerant to combined action of Cd, Pb, Cu and Zn	Wei et al. 2004
T. caerulescens	Cd	High Cd-accumulating capability, acquiring Cd from the same soil pools as non-accumulating species.	Schwartz et al. 2003
Arabis gemmifera	Cd and Zn	Hyperaccumulator of Cd and Zn, with phytoextraction capacities almost equal to *T. caerulescens*	Kubota and Takenaka 2003
Sedum alfredii Hance	Cd	Mined ecotype had a greater ability to tolerate, transport, and accumulate Cd, compared to non-mined ecotype	Xiong et al. 2004
Stanleya pinnata	Se	Adapted to semi-arid western U. S. soils and environments. Uptake, metabolism and volatilization of Se	Parker et al. 2003
Austromyrtus bidwilli. P. acinosa Roxb	Mn	Australian native hyperaccumulator of Mn, grows rapidly, has substantial biomass, wide distribution and a broad ecological amplitude	Bidwell et al. 2002; Xue et al. 2004

They should have high metal tolerance, be able to accumulate several metals in large amounts, exhibit high biomass production and fast growth, resist diseases and pests, and be unattractive to animals, minimizing the risk of transferring metals to higher trophic levels of the terrestrial food chain (Thangavel and Subhuram 2004). Phytoextraction is applicable only to sites containing low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow normal cultivation practices, utilizing agricultural equipment. Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth.

Basic et al. (2006a,b) investigated the parameters influencing the Cd concentration in plants, as well as the biological implications of Cd hyperaccumulation in nine natural populations of *T. caerulescens*. Cd concentrations in the plant were positively correlated with plant Zn, Fe and Cu concentrations. The physiological and/or molecular mechanisms for uptake, transport and/or accumulation of these four heavy metals interact with each other. They specified a measure of Cd hyperaccumulation capacity by populations and showed that *T. caerulescens* plants originating from populations with high Cd hyperaccumulation capacity had better growth, by developing more and bigger leaves, taller stems, and produced more fruits and heavier seeds. Liu et al. (2006) conducted a survey of Mn mine tailing soils and eight plants growing on Mn mine tailings. The concentrations of soil Mn, Pb, and Cd and the metal-enrichment traits of these eight plants were analyzed. It was found that *Poa pratensis, Gnaphalium affine, Pteris vittata, Conyza Canadensis* and *Phytolacca acinosa* possessed specially good metal-enrichment and metal-tolerant traits. In spite of the high concentration of Mn in *P. pratensis,* its lifecycle was too short, and its shoots were too difficult to collect for it to be suitable for soil remediation.

The effectiveness of phytoextraction of heavy metals in soils also depends on the availability of metals for plant uptake (Li et al. 2000). The rates of redistribution of metals and their binding intensity are affected by the metal species, loading levels, aging and soil properties (Han et al. 2003). Generally, the solubility of metal fractions is in the order: exchangeable > carbonate specifically adsorbed > Fe–Mn oxide > organic sulfide > residual (Li and Thornton 2001). Ammonium nutrition of higher plants results in rhizosphere acidification due to proton excretion by root cells. Ammonium-fed sunflowers induced a strong acidification of the solution and, compared to the nitrate-fed sunflowers, a small modification in mineral nutrition and different Cd partitioning between root and shoot. Moreover, ammonium nutrition was found to induce a great mobilisation of a sparingly soluble form of cadmium ($CdCO_3$) (Zaccheo et al. 2006). A lipid-transfer protein isolated from a domestic cultivar of brewer's barley grain, Hordeum vulgare has the affinity to bind Co (II) and Pb (II), but not Cd (II), Cu (II), Zn (II) or Cr (III). This suggests a new possible role of barley lipid-transfer protein for phytoextraction (Gorjanovic et al. 2006).

The slow desorption of heavy metals in soils has been a major impediment to the successful phytoextraction of metal contaminated sites. Except for Hg, metal uptake into roots occurs from the aqueous phase. In soil, easily mobile metals such as Zn and Cd occur primarily as soluble or exchangeable, readily bioavailable form. Cu and Mo predominate in inorganically bound and exchangeable fractions. Slightly mobile metals such as Ni and Cr are mainly bound in silicates (residual fraction). Soluble, exchangeable and chelated species of trace elements are the most mobile components in soils, facilitating their migration and phytoavailability (Williams et al. 2006). Other species such as Pb occur as insoluble precipitates (phosphates, carbonates and hydroxyl-oxides) which are largely unavailable for plant uptake (Pitchel et al. 1999).

Understanding the mechanisms of rhizosphere interaction, uptake, transport and sequestration of metals in hyperaccumulator plants will lead to designing novel transgenic plants with improved remediation traits (Eapen and D'Souza 2005). Moreover, the selection and testing of multiple hyperaccumulator plants could enhance the rate of phytoremediation, giving this process a promise one for bioremediation of environmental contamination (Suresh and Ravishankar 2004). Some of the recent reports on phytoextraction are summarized in Table 9. Phytoremediation has been combined with electrokinetic remediation, applying a constant voltage of 30 V across the soil. The combination of both techniques could represent a very promising approach to the decontamination of metal-polluted soils (O'Connor et al. 2003).

3 Handling of Hazardous Plant Biomass after Phytoremediation

Phytoextraction involves repeated cropping of plants in contaminated soil until the metal concentration drops to an acceptable level. Each crop is removed from the site. This leads to accumulation of huge quantities of hazardous biomass, which must be stored or disposed appropriately to minimize environmental risk. After harvesting, the methods of disposal of contaminated plants include approved secure landfills, surface impoundments, deep well injection, ocean dumping or incineration. The waste volume can be reduced by thermal, microbial, physical or chemical means.

In one study, the dry weight of *B. juncea* for induced phytoextraction of lead amounted to 6 tons/ha con-

Table 9 Recent reports on phytoextraction

Metal	Plant studied	Method of Phytoremediation	Results	Reference
Cd, Zn	*T. caerulescens*	PE-C	Physiological and molecular mechanisms for uptake, transport and accumulation of four heavy metals Cd, Fe, Cu and Zn interact with each other. T. caerulescens plants originating from populations with high Cd hyperaccumulation capacity had better growth. Revegetation of metal polluted soils with T. caerulescens could help activate their biochemical and microbial functionality. Different soils had various responses to acidification. A different optimum pH may exist for phytoextraction.	Basic et al. 2006a,b; Keller et al. 2006; Hammer et al. 2006; Hernandez-Allica et al. (2006); Wang et al. (2006)
Mn	*G. affine* D. Don *C. canadensis* (L.) Cronq	PE-C	*G. affine* and *C. canadensis* had excessive accumulation of Mn and could be useful in phytoremediation. The perennial herb *P. acinosa* Roxb. (Phytolaccaceae), which occurs in Southern China, was found to be a new manganese hyperaccumulator.	Liu et al. 2006; Xue et al. 2004
Cu	*Elsholtzia splendens*, and *Trifolium repens*	PE-CA	Application of glucose or citric acid significantly increased the extractable Cu concentration in planted and unplanted soils. Concentrations of Cu in the shoots of *E. splendens* were 2.6, 1.9 and 2.9 times of those of *T. repens* under no chelate, citric acid and glucose treatments, respectively.	Chen et al. 2006
Pb, As, Pb, Cu, Zn, Cd	Carrot, Lettuce and Tomato. Euphorbia, Verbascum. and Astragalus	PE-C	Except for carrot roots, concentration less than ICP-OES detectable limits. Plants with high metal intake abilities escalate mobility of metals and increase contaminations on surface and subsurface.	Pendergrass and Butcher (2006); Sagiroglu et al. (2006)
Cu, Zn, Pb	Sunflower	PE-CA	Synthetic Chelating agents did not increase the uptake of heavy metals for equal soluble concentrations in the presence and absence of chelates. Proper use of soil amendments increased the phytoextraction of Zn, Cu, Pb, Cd from contaminated soils	Tandy et al. 2006; Clemente et al. 2006; Chen et al. 2006
Cu and Fe	*Athyrium vokoscense*	PE and PM	1 g Cu and 0.1 g Fe recovered from 500 g soil. Removal rates of Cu and Fe in the contaminated soil were 82 and 95% respectively. Application of (NLMWOA (Natural Low Molecular Weight Organic Acids) increased the extraction of Cu, with no enhancement of lead phytoextraction.	Kobayashi et al. 2005; Evangelou et al. 2006
Se	*A. bisulcatus* and *B. juncea*	PE	There was a substantial improvement in Se accumulation (4 to 9 times increase) in transgenic plants.	LeDuc et al. 2006

Table 9 (continued)

Metal	Plant studied	Method of Phytoremediation	Results	Reference
Cd	*B. napus* and *B. juncea*	PE	Lipid changes in *B. juncea*, the well-known Cd-hyperaccumulator species, revealed greater stability of its cellular membranes to cadmium-stress compared to a Cd-sensitive specie, *B. napus*. An increase in cadmium content varying from 16 to 74%, compared to the non-inoculated control, was observed in rape plants cultivated in soil treated with 100 mg Cd kg^{-1} (as $CdCl_2$) and inoculated with the cadmium-resistance bacterial strains from heavy metal-polluted soils.	Quartacci et al. 2006; Belimov et al. 2005; Nouairi et al. 2006; Sheng and Xia 2006

PE PhytoExtraction, *CA* Chelate Assisted, *C* Continuous, *PM* Phytomining

taining 10,000–15,000 mg/kg metal on a dry weight basis (Blaylock et al. 1997). Composting and compaction can provide post-harvest treatment (Raskin et al. 1997 and Kumar et al. 1995). Even though composting can significantly reduce the volume of the harvested biomass, metal-contaminated biomass still requires treatment prior to disposal. In the case of compaction, care should be taken to collect and dispose of the leachate. A conventional and promising route to utilize biomass produced by phytoremediation is through thermo-chemical conversion processes such as combustion, gasification and pyrolysis.

If phytoextraction could be combined with biomass generation and its commercial utilization as an energy source, then it could be turned into a profitable operation, with the residual ash available to be used as an ore (Brooks 1998; Comis 1996; Cunningham and Ow 1996). Phytomining includes the generation of revenue by extracting soluble metals produced by the plant biomass ash, also known as bio-ore. With some metals like Ni, Zn, Cu, etc., the value of reclaimed metal may provide an additional incentive for phytoremediation (Chaney et al. 1997, Watanabe 1997, Thangavel and Subhuram 2004).

4 Conclusions

Phytoremediation is still in its research and development phase, with many technical issues needing to be addressed. The results, though encouraging, suggest that further development is needed. Phytoremediation is an interdisciplinary technology that can benefit from many different approaches. Results already obtained have indicated that some plants can be effective in toxic metal remediation. The processes that affect metal availability, metal uptake, translocation, chelation, degradation, and volatilization need to be investigated in detail. Better knowledge of these biochemical mechanisms may lead to: (1) Identification of novel genes and the subsequent development of transgenic plants with superior remediation capacities; (2) Better understanding of the ecological interactions involved (e.g. plant-microbe interactions); (3) Appreciation of the effect of the remediation process on ecological interactions; and (4) Knowledge of the entry and movement of the pollutant in the ecosystem. In addition to being desirable from a fundamental biological perspective, findings will help improve risk assessment during the design of remediation plans, as well as alleviation of risks associated with the remediation. It is important that public awareness of this technology be considered, with clear and precise information made available to the general public to enhance its acceptability as a global sustainable technology. So far, most phytoremediation experiments have taken place on a laboratory scale, with plants grown in hydroponic settings fed heavy metal diets. Both agronomic management practices and plant genetic abilities need to be optimized to develop commercially useful practice.

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