The Use of Plants for the Removal of Toxic Metals from Contaminated Soil

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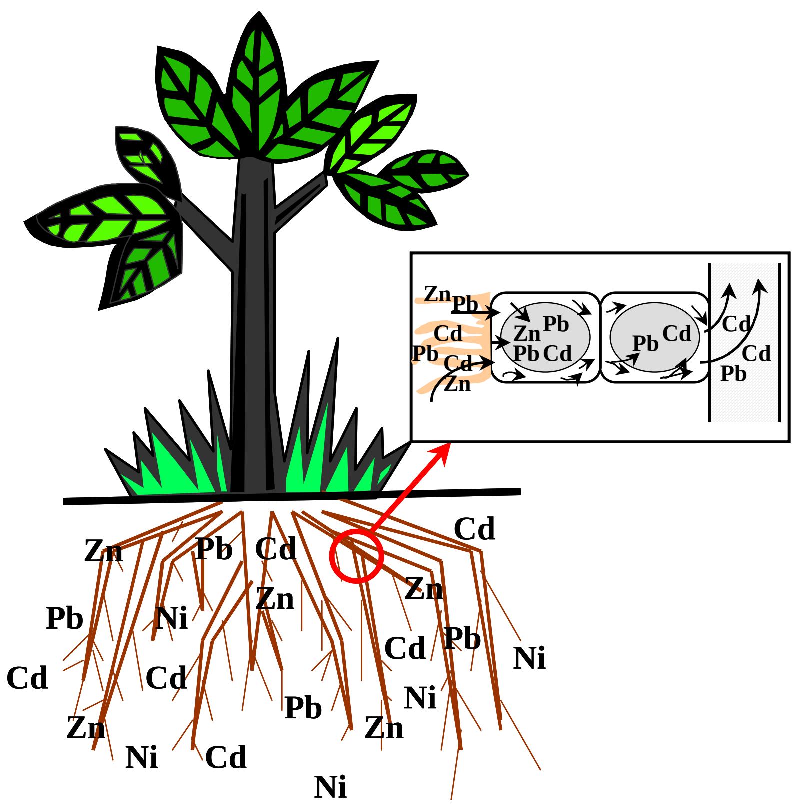

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Abstract

Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments. Fundamental and applied research have unequivocally demonstrated that selected plant species possess the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants. Despite this tremendous potential, phytoremediation is yet to become a commercial technology. Progress in the field is precluded by limited knowledge of basic plant remedial mechanisms. In addition, the effect of agronomic practices on these mechanisms is poorly understood. Another limitation lies within the very biological nature of this novel approach. For example, potential for phytoremediation depends upon the interaction among soil, contaminants, microbes, and plants. This complex interaction, affected by a variety of factors, such as climatic conditions, soil properties, and site hydro-geology, argues against generalization, and in favor of site-specific phytoremediating practices. Thus, an understanding of the basic plant mechanisms, and the effect of agronomic practices on plant/soil/contaminant interaction would allow practitioners to optimize phytoremediation by customizing the process to site specific conditions.

Remediation of metal contaminated soil faces a particular challenge. Unlike organic contaminants, metals cannot be degraded. Commonly, decontamination of metal-contaminated soils requires the removal of toxic metals. Recently, phytoextraction, the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly cleanup alternative. In this paper, we review the processes and mechanisms that allow plants to remove metals from contaminated soils and discuss the effects of agronomic practices on these processes.

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I. INTRODUCTION

Background

The concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of wastewater (Hartman,1975). At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminaria* were the first plant species documented to accumulate high levels of metals in leaves (Baumann,1885). In 1935, Byers reported that plants of the genus *Astragalus* were capable of accumulating up to 0.6 % selenium in dry shoot biomass. One decade later, Minguzzi and Vergnano (1948) identified plants able to accumulate up to 1% Ni in shoots. More recently, Rascio, (1977) reported tolerance and high Zn accumulation in shoots of *Thlaspi caerulescens*. Despite subsequent reports claiming identification of Co, Cu, and Mn hyperaccumulators, the existence of plants hyperaccumulating metals other than Cd, Ni, Se and Zn has been questioned and requires additional confirmation (Salt et al., 1995). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia (1980) and Chaney (1983), and the first field trial on Zn and Cd phytoextraction was conducted in 1991 (Baker et al.). In the last decade, extensive research has been conducted to investigate the biology of metal phytoextraction. Despite significant success, our understanding of the plant mechanisms that allow metal extraction is still emerging. In addition, relevant applied aspects, such as the effect of agronomic practices on metal removal by plants are largely unknown. It is conceivable that maturation of phytoextraction into a commercial technology will ultimately depend on the elucidation of plant mechanisms and application of adequate agronomic practices. Natural occurrence of plant species capable of accumulating extraordinarily high metal levels makes the investigation of this process particularly interesting.

Advantages and disadvantages of phytoremediation

Metal-contaminated soils are notoriously hard to remediate. Current technologies resort to soil excavation and either landfilling or soil washing followed by physical or chemical separation of the contaminants. The cost of soil remediation is highly variable and depends on the contaminants of concern, soil properties, and site conditions. Cost estimates associated with the use of several technologies for the cleanup of metal-contaminated soil are shown in Table 1.

Table 1. Cost of soil treatment (Glass, 1999a)

Treatment	Cost ($/ton)	Additional factors/expenses
Vitrification	75-425	Long-term monitoring
Landfilling	100-500	Transport/excavation/monitoring
Chemical treatment	100-500	Recycling of contaminants
Electrokinetics	20-200	Monitoring
Phytoextraction	5- 40	Monitoring

Cleaning of metal-contaminated soils via conventional engineering methods can be prohibitively expensive (Salt et al., 1995). The costs estimated for the remediation of sites contaminated with heavy metals, and heavy metals mixed with organic compounds are shown in Table 2.

Table 2. Projected five-year costs for remediation of sites contaminated with toxic metals only, and mixtures of toxic metals and organics (U.S. EPA, 1993).

Sector	Metals Only	Metals and Organics
	---------------------$ million------------------------	
Superfund[1]	2,400	10,400
RCRA[2]	3,000	12,800
DOD[3]	400	2,400
DOE[4]	900	6,500
State[5]	200	800
Private[6]	200	2,500
Total	7,100	35,400

[1] Sites ranked on the National Priorities List
[2] Sites requiring corrective action under the provisions of Resource Conservation and Recovery Act RCRA
[3] Department of Defense
[4] Department of Energy
[5] State-funded contaminated sites
[6] Private-funded contaminated sites

Because of the high cost, there is a need for less-expensive cleanup technologies. Phytoremediation is emerging as a cost-effective alternative. Several analyses have demonstrated that the cost of metal phytoextraction is only a fraction of that associated with conventional engineering technologies (Table 1). In addition, because it remediates the soil *in situ*, phytoremediation avoids dramatic landscape disruption, and preserves the ecosystem. Despite these advantages, several disadvantages and constraints restrict the applicability of phytoextraction (Table 3).

Table 3. Major factors limiting the success and applicability of phytoextraction

Plant-based biological limitation	Regulatory limitations	Other limitations
1) Low plant tolerance 2) Lack of contaminant translocation from root to shoot 3) Small size of remediating plants	1) Lack of cost and performance data 2) Regulators unfamiliarity with the technology 3) Disposal of contaminated plant waste 4) Risk of food chain contamination	1) Contaminant beneath root zone 2) Lengthy process 3) Contaminant in biologically unavailable form 4) Lack of remediating plant species

Markets for phytoremediation

A comprehensive analysis of phytoremediation markets was published by Glass (1999a; 1999b). The author indicated that the estimated 1999 phytoremediation markets was two fold greater than 1998 estimates. This growth has been attributed to an increased number of companies offering services, particularly companies in the consulting engineering sector, and to growing acceptance of the technology. An estimate of 1999 U.S. phytoremediation markets related to a variety of contaminated media and contaminants of concern is shown in Table 4.

Table 4. Estimated 1999 U.S. phytoremediation markets (Glass, 1999b)

Organics in groundwater	$ 7-12 million
Landfill leachate	$ 5-8 million
Organics in soil	$ 5-7 million
Metals in soil	$ 4.5-6 million
Inorganics in wastewater	$ 2-4 million
Inorganics in groundwater	$ 2-3 million
Organics in wastewater	$ 1-2 million
Metals in groundwater	$ 1-2 million
Radionuclides	$ 0.5-1 million
Metals in wastewater	$ 0.1-0.2 million
Other	$ 1.9-3.8 million
Total	$ 30 -49 million

Current estimates of 1999, and 2000 revenues were slightly lower than what had been previously projected, largely due to slower commercialization of the technology for the cleanup of metal- and radionuclide-contaminated sites (Glass, 1999b).

The second largest market for phytoremediation was identified in Europe, although European market was estimated to be 10-fold smaller than the U.S. market (Glass 1999b).

II. TOXIC METALS IN SOIL

Sources of contamination

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.) and atomic number >20. The most common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil. Contamination, however, has resulted from industrial activities, such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste (Kabata-Pendias and Pendias,1989). Soil concentration range and regulatory limits for several major metal contaminants are shown in Table 5.

Table 5. Soil concentration ranges and regulatory guidelines for some toxic metals

Metal	Soil concentration range[a] ($mg\ kg^{-1}$)	Regulatory limits[b] ($mg\ kg^{-1}$)
Pb	1.00-6,900	600
Cd	0.10-345	100
Cr	0.05-3,950	100
Hg	<0.01-1,800	270
Zn	150.00-5,000	1,500

a) Riley *et al.*, 1992

b) Nonresidential direct contact soil cleanup criteria (NJDEP, 1996)

High levels of metals in soil can be phytotoxic. Poor plant growth and soil cover caused by metal toxicity can lead to metal mobilization in runoff water and subsequent deposition into nearby bodies of water. Furthermore, bare soil is more susceptible to wind erosion and spreading of contamination by airborne dust. In such situations, the immediate goal of remediation is to reclaim the site by establishing a vegetative cover to minimize soil erosion and pollution spread.

Risk assessment

Soil remediation is needed to eliminate risk to humans or the environment from toxic metals. Human disease has resulted from Cd (Nogawa et al., 1987; Kobayashi 1978; Cai et al., 1990), Se (Yang et al., 1983), and Pb in soil (Chaney et al., 1999). Livestock and wildlife have suffered from Se poisoning (Rosenfeld and Beath, 1964; Ohlendorf et al., 1986). In addition, soil contamination with Zn, Ni and Cu caused by mine wastes and smelters is known to be phytotoxic to sensitive plants (Chaney et al., 1999). One of the greatest concerns for human health is caused by Pb contamination. Exposure to Pb can occur through multiple pathways, including inhalation of air and ingestion of Pb in food, water, soil or dust. Excessive Pb exposure can cause seizures, mental retardation and behavioral disorders. The danger of Pb is aggravated by low environmental mobility even under high precipitations.

Total and bioavailable soil fractions

In soil, metals are associated with several fractions: (1) in soil solution, as free metal ions and soluble metal complexes, (2) adsorbed to inorganic soil constituents at ion exchange sites, (3) bound to soil organic matter, (4) precipitated such as oxides, hydroxides, carbonates, and (5) embedded in structure of the silicate minerals. Soil sequential extractions are employed to isolate and quantify metals associated with different fractions (Tessier et al., 1979).

For phytoextraction to occur, contaminants must be bioavailable (ready to be absorbed by roots). Bioavailability depends on metal solubility in soil solution. Only metals associated with fractions 1 and 2 (above) are readily available for plant uptake. Some metals, such as Zn and Cd, occur primarily in exchangeable, readily bioavailable form. Others, such as Pb, occur as soil precipitate, a significantly less bioavailable form.

Effect of soil properties on metal bioavailability

The chemistry of metal interaction with soil matrix is central to the phytoremediation concept. In general, sorption to soil particles reduces the activity of metals in the system. Thus, the higher the cation exchange capacity (CEC) of the soil, the greater the sorption and immobilization of the metals. In acidic soils, metal desorption from soil binding sites into solution is stimulated due to H^+competition for binding sites. Soil pH affects not only metal bioavailabilty, but also the very process of metal uptake into roots. This effect appears to be metal specific. For example, in *T. caerulescens,* Zn uptake in roots showed a small pH dependence, whereas uptake of Mn and Cd was more dependent on soil acidity (Brown et al., 1995a).

III. PHYTOREMEDIATING PLANTS

Why do plants take up toxic metals?

To grow and complete the life cycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Plants have evolved highly specific mechanisms to take up, translocate, and store these nutrients. For example, metal movement across biological membranes is mediated by proteins with transport functions. In addition, sensitive mechanisms maintain intracellular concentration of metal ions within the physiological range. In general, the uptake mechanism is selective, plants preferentially acquiring some ions over others. Ion uptake selectivity depends upon the structure and properties of membrane transporters. These characteristics allow transporters to recognize, bind and mediate the trans-membrane transport of specific ions. For example, some transporters mediate the transport of divalent cations, but do not recognize mono- or trivalent ions.

Many metals such as Zn, Mn, Ni and Cu are essential micronutrients. In common nonaccumulator plants, accumulation of these micronutrients does not exceed their metabolic needs (<10ppm). In contrast, metal hyperaccumulator plants can accumulate exceptionally high amounts of metals (in the thousands of ppm). Since metal accumulation is ultimately an energy consuming process, one would wonder what evolutionary advantage does metal hyperaccumulation give to these species? Recent studies have shown that metal accumulation in the foliage may allow hyperaccumulator species to evade predators including caterpillars, fungi and bacteria (Boyd and Martens, 1994; Pollard and Baker, 1997).

Hyperaccumulator plants do not only accumulate high levels of essential micronutrients, but can also absorb significant amounts of nonessential metals, such as Cd. The mechanism of Cd accumulation has not been elucidated. It is possible that the uptake of this metal in roots is via a system involved in the transport of another essential divalent micronutrient, possibly Zn^{2+}. Cadmium is a chemical analogue of the latter, and plants may not be able to differentiate between the two ions (Chaney et al., 1994).

What is a hyperaccumulator species?

Interest in phytoremediation has grown significantly following the identification of metal hyperaccumulator plant species. Hyperaccumulators are conventionally defined as species capable of accumulating metals at levels 100-fold greater than those typically measured in common nonaccumulator plants. Thus, a hyperaccumulator will concentrate more than: 10 ppm Hg; 100 ppm Cd; 1,000 ppm Co, Cr, Cu, and Pb; 10,000 ppm Ni and Zn. To date, approximately 400 plant species from at least 45 plant families have been reported to hyperaccumulate metals. Most hyperaccumulators bioconcentrate Ni, about 30 absorb either Co, Cu, and/or Zn, even fewer species accumulate Mn and Cd, and there are no known natural Pb-hyperaccumulators (Reeves and Baker, 1999). Several hyperaccumulators and their bioaccumulation potential are listed in Table 6.

Table 6. Several metal hyperaccumulator species and their bioaccumulation potential

Plant species	Metal	Leaf content (ppm)	Reference
Thlaspi caerulescens	Zn:Cd	39,600:1,800	Reeves&Brooks(1983);Baker&Walker(1990)
Ipomea alpina	Cu	12,300	Baker&Walker (1990)
Haumaniastrum robertii	Co	10,200	Brooks (1977)
Astragalus racemosus	Se	14,900	Beath et al. (1937)
Sebertia acuminata	Ni	25% by wt dried sap	Jaffre et al. (1976)

Possibly, the best-known metal hyperaccumulator is *Thlaspi caerulescens* (alpine pennycress). While most plants show toxicity symptoms at Zn accumulation of about 100 ppm, *T. caerulescens* was shown to accumulate up to 26,000 ppm without showing any injury (Brown et al., 1995b). Possibly, hyperaccumulator plants may have a higher requirement for metals such as Zn than non-accumulator species (Hajar, 1997). In support of this, many hyperaccumulators, including *T. caerulescens*, have been shown to colonize metal-rich soils such as calamine soil (soil enriched in Pb, Zn, and Cd). Because of this ability, considerable efforts have been directed to identify hyperaccumulator plants endemic to metal rich soils (Baker and Proctor, 1990).

How do plants tolerate high metal concentration in soil?

Ecological studies have revealed the existence of specific plant communities, endemic floras, which have adapted on soils contaminated with elevated levels of Zn Cu, and Ni. Different ecotypes of the same species may occur in areas uncontaminated by metals. To plants endemic to metal-contaminated soils, metal tolerance is an indispensable property. In comparison, in related populations inhabiting uncontaminated areas, a continuous gradation between ecotypes with high and low tolerance usually occurs. Plants evolved several effective mechanisms for tolerating high concentrations of metals in soil. In some species, tolerance is achieved by preventing toxic metals uptake into root cells. These plants, coined excluders, have little potential for metal extraction. Such an excluder is “Merlin,” a commercial variety of red fescue (*Festuca rubra*), used to stabilize erosion-susceptible metal-contaminated soils. A second group of plants, accumulators, does not prevent metals from entering the root. Accumulator species have evolved specific mechanisms for detoxifying high metal levels accumulated in the cells. These mechanisms allow bioaccumulation of extremely high concentration of metals. In addition, a third group of plants, termed indicators, shows poor control over metal uptake and transport processes. In these plants, the extent of metal accumulation reflects metal concentration in the rhizospheric soil. Indicator species have been used for mine prospecting to find new ore bodies (Raskin et al, 1994).

Mechanisms of metals uptake into roots and translocation to shoots

Because of their charge, metal ions cannot move freely across the cellular membranes, which are lipophilic structures. Therefore, ion transport into cells must be mediated by membrane proteins with transport functions, generically known as transporters. Transmembrane transporters possess an extracellular binding domain to which the ions attach just before the transport, and a transmembrane structure which connects extracelluar and intracellular media. The binding domain is receptive only to specific ions and is responsible for transporter specificity. The transmembrane structure facilitates the transfer of bound ions from extracellular space through the hydrophobic environment of the membrane into the cell. These transporters are characterized by certain kinetic parameters, such as transport capacity (V_{max}) and affinity for ion (K_m). V_{max} measures the maximum rate of ion transport across the cellular membranes. K_m measures transporter affinity for a specific ion and represents the ion concentration in the external solution at which the transport rate equals $V_{max}/2$. A low K_m value, high affinity, indicates that high levels of ions are transported into the cells even at low external ion concentration. By studying kinetic parameters, K_m and V_{max}, plant biologists gain insights to specificity and selectivity of the transport system.

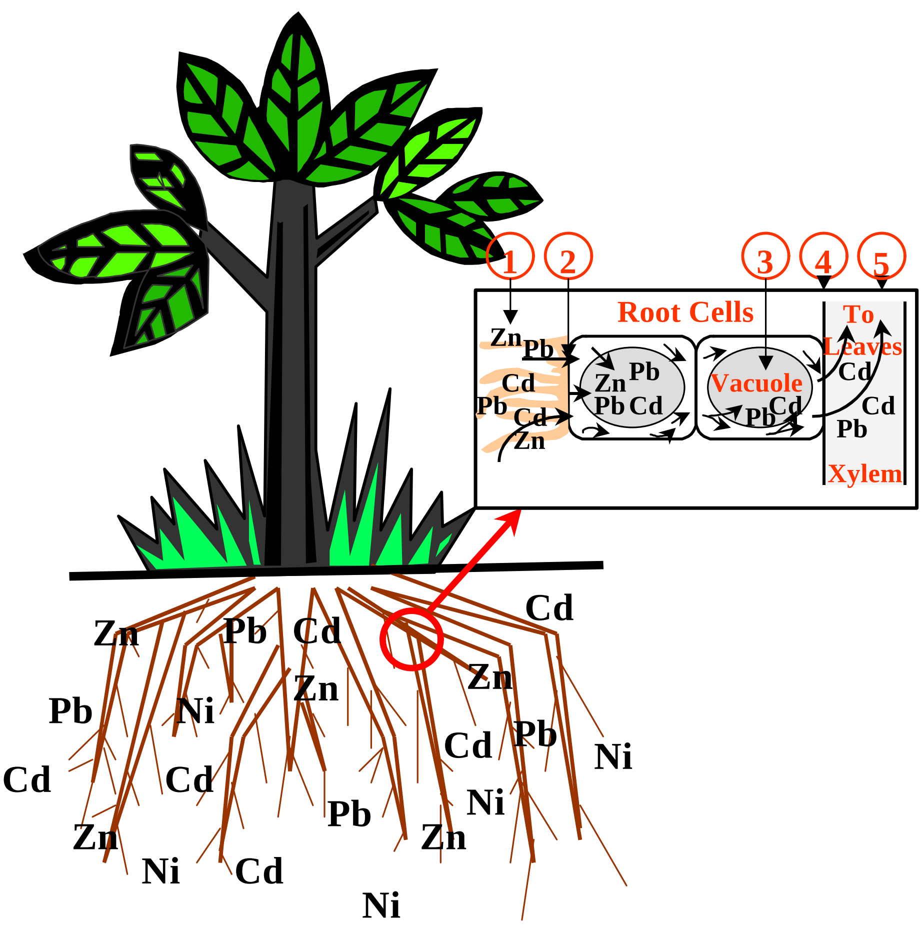
It is important to note that of the total amount of ions associated with the root, only a part is absorbed into cells. A significant ion fraction is physically adsorbed at the extracellular negatively charged sites (COO^-) of the root cell walls. The cell wall-bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass (phytoextraction). Thus, it is possible that a plant exhibiting significant metal accumulation into the root, to express a limited capacity for phytoextraction. For example, many plants accumulate Pb in roots, but Pb translocation to shoot is very low. In support of this, Blaylock and Huang (1999) concluded that the limiting step for Pb phytoextraction is the long distance translocation from roots to shoots.

Binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoot. Metals can also be complexed and sequestered in cellular structures (e.g., vacuole) becoming unavailable for translocation to the shoot (Lasat et al., 1998). In addition, some plants, coined excluders, possess specialized mechanisms to restrict metal uptake into roots. However, the concept of metal exclusion is not well understood (Peterson, 1983).

Uptake of metals into root cells, the point of entry into living tissues, is a step of major importance for the process of phytoextraction. However, for phytoextraction to occur metals must also be transported from the root to the shoot. Movement of metal-containing sap from the root to the shoot, termed translocation, is primarily controlled by two processes: root pressure and leaf transpiration. Following translocation to leaves, metals can be reabsorbed from the sap into leaf cells. A schematic representation of metal transport processes that take place in roots and shoots is shown in Figure 1.

Figure 1. Metal uptake and accumulation in plants

1. **A metal fraction is sorbed at root surface**
2. **Bioavailable metal moves across cellular membrane into root cells**
3. **A fraction of the metal absorbed into roots is immobilized in the vacuole**
4. **Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem)**
5. **Metal is translocated from the root to aerial tissues (stems and leaves)**



Plant mechanisms for metal detoxification

Although micronutrients such as Zn, Mn, Ni and Cu are essential for plant growth and development, high intracellular concentrations of these ions can be toxic. To deal with this potential stress, common nonaccumulator plants have evolved several mechanisms to control the homeostasis of intracellular ions. Such mechanisms include regulation of ion influx (stimulation of transporter activity at low intracellular ion supply, and inhibition at high concentrations), and extrusion of intracellular ions back into the external solution. Metal hyperaccumulator species, capable of taking up metals in the thousands of ppm, possess additional detoxification mechanisms. For example, research has shown that in *T. goesingense*, a Ni hyperacccumulator, high tolerance was due to Ni complexation by histidine which rendered the metal inactive (Krämer et al., 1997; Krämer et al., 1996). Sequestration in the vacuole has been suggested to be responsible for Zn tolerance in the shoots of the Zn-hyperaccumulator *T. caerulescens* (Lasat et al., 1996; Lasat et al., 1998). Several mechanisms have been proposed to account for Zn inactivation in the vacuole including precipitation as Zn-phytate (Van Steveninck et al., 1990), and binding to low molecular weight organic acids (Mathys, 1977; Tolrà et al., 1996; Salt et al., 1999). Complexation to low molecular weight organic compounds (<10 kD) was also shown to play a role in tolerance to Ni (Lee et al., 1977). Cadmium, a potentially toxic metal, has been shown to accumulate in plants, where it is detoxified by binding to phytochelatins (Wagner 1984; Steffens, 1990; Cobbett and Goldsbrough, 1999), a family of thiol (SH)- rich peptides (Rauser,1990). Metallothioneins (MT), identified in numerous animals and more recently, in plants and bacteria (Kägi, 1991), are also compounds (proteins) with heavy metal-binding properties (Tomsett et al., 1992).

Plant limitations

When the concept of phytoextraction was reintroduced (approximately two decades ago), engineering calculations suggested that a successful plant-based decontamination of even moderately contaminated soils would require crops able to concentrate metals in excess of 1-2%. Accumulation of such high levels of heavy metals is highly toxic and would certainly kill the common nonaccumulator plant. However, in hyperaccumulator species, such concentrations are attainable. Nevertheless, the extent of metal removal is ultimately limited by plant ability to extract and tolerate only a finite amount of metals. On a dry weight basis, this threshold is around 3% for Zn and Ni, and considerably less for more toxic metals, such as Cd and Pb. The other biological parameter which limits the potential for metal phytoextraction is biomass production. With highly productive species, the potential for biomass production is about 100 tons fresh weight/hectare. The values of these parameters limit the annual removal potential to a maximum of 400 kg metal/ha/yr. It should be mentioned, however, that most metal hyperaccumulators are slow growing and produce little biomass. These characteristics severely limit the use of hyperaccumulator plants for environment cleanup.

Improving phytoremediating plants

It has been suggested that phytoremediation would rapidly become commercially available if metal removal properties of hyperaccumulator plants, such as *T. caerulescens,* could be transferred to high-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) (Brown et al., 1995b). Biotechnology has already been successfully employed to manipulate metal uptake and tolerance properties in several species. For example, in tobacco (*Nicotiana tabacum*) increased metal tolerance has been obtained by expressing the mammalian metallothionein, metal binding proteins, genes (Lefebvre et al., 1987; Maiti et al., 1991).

Possibly, the most spectacular application of biotechnology for environmental restoration has been the bioengineering of plants capable of volatilizing mercury from soil contaminated with methyl-mercury. Methyl-mercury, a strong neurotoxic agents, is biosynthesized in Hg-contaminated soils. To detoxify this toxin, transgenic plants (*Arabidopsis* and tobacco) were engineered to express bacterial genes *merB* and *merA*. In these modified plants, *merB* catalyzes the protonolysis of the carbon-mercury bond with the generation of Hg^{2+}, a less mobile mercury species. Subsequently, *MerA* converts Hg(II) to Hg (0) a less toxic, volatile element which is released into the atmosphere (Rugh et al., 1996; Heaton et al., 1988). Although regulatory concerns restrict the use of plants modified with *merA* and *merB* , this research illustrates the tremendous potential of biotechnology for environment restoration. In an effort to address regulatory concerns related to phytovolatilization of mercury, Bizili et al. (1999) demonstrated that plants engineered to express *MerBpe* (an organomercurial lyase under the control of a plant promoter) may be used to degrade methyl-mercury and subsequently remove ionic mercury via extraction. Despite recent advances in biotechnology, little is known about the genetics of metal hyperaccumulation in plants. Particularly, the heredity of relevant plant mechanisms, such as metal transport and storage (Lasat et al., 2000) and metal tolerance (Ortiz et al., 1992; Ortiz et al., 1995) must be better understood. Recently, Chaney et al. (1999) proposed the use of traditional breeding approaches for improving metal hyperaccumulator species and possibly incorporating significant traits such as metal tolerance and uptake characteristics into high-biomass-producing plants. Partial success has been reported in the literature. For example, in an effort to correct for small size of hyperaccumulator plants, Brewer et al. (1997) generated somatic hybrids between *T. caerulescens* (a Zn hyperaccumulator) and *Brassica napus* (canola) followed by hybrid selection for Zn tolerance. High biomass hybrids with superior Zn tolerance were recovered. These authors have also advocated a coordinated effort to collect and preserve germplasm of accumulator species.

IV. PLANT-METAL INTERACTION IN THE RHIZOSPHERE

Metal bioavailability for uptake into roots

A major factor limiting metal uptake into roots is slow transport from soil particles to root surfaces (Nye and Tinker, 1977; Barber, 1984). With the possible exception of volatile mercury, for all other metals, this transport takes place in soil solution. In soil, metal solubility is restricted due to adsorption to soil particles. Some of the soil binding sites are not particularly selective. For example, they bind Cd as strong as Ca. Nonspecific binding occurs at clay cation exchange sites and carboxylic groups associated with soil organic matter. Other sites are more selective and bind Cd stronger than Ca. For example, most clay particles are covered with a thin layer of hydrous Fe, Mn, and Al oxides. These selective sites maintain Cd activity in the soil solution at low levels (Chaney, 1988). Lead, a major contaminant, is notorious for the lack of soil mobility, primarily due to metal precipitation as insoluble phosphates, carbonates and (hydr)oxides (Blaylock and Huang, 1999). Thus, increasing metal solubility in the soil is an important prerequisite to enhance the potential for Pb phytoextraction. This subject is detailed in the next section.

Two mechanisms are responsible for metal transport from the bulk soil to plant roots: 1) convection or mass flow, and 2) diffusion (Corey et al., 1981; Barber, 1984). Due to convection, soluble metal ions move from soil solids to root surface. From the rhizosphere, water is absorbed by roots to replace water transpired by leaves. Water uptake from rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. Some ions are absorbed by roots faster than the rate of supply via mass flow. Thus, a depleted zone is created in soil immediately adjacent to the root. This generates a concentration gradient directed from the bulk soil solution and soil particles holding the adsorbed elements, to the solution in contact with the root surface. This concentration gradient drives the diffusion of ions toward the depleted layer surrounding the roots.

Plants have evolved specialized mechanisms to increase the concentration of metal ions in soil solution. For example, at low ion supply, plants may alter the chemical environment of the rhizosphere to stimulate the desorption of ions from soil solids into solution. Such a mechanism is rhizosphere acidification due to H^+ extrusion from roots (Crowley et al., 1991). Protons compete and replace metal ions from binding sites, stimulating their desorption from soil solids into solution. In addition, some plants can regulate metal solubility in the rhizosphere by exuding a variety of organic compounds from roots. Root exudates complex metal ions keeping them in solution available for uptake into roots (Romheld and Marschner, 1986).

Effect of soil microorganisms on metal uptake

Root growth affects the properties of the rhizospheric soil and stimulates the growth of the microbial consortium. To illustrate this, research has shown that the population of microorganisms in the rhizosphere is several orders of magnitude greater than in the surrounding soil (Anderson, 1997). In turn, rhizospheric microorganisms may interact symbiotically with roots to enhance the potential for metal uptake. In addition, some microorganisms may excrete organic compounds which increase bioavailability, and facilitate root absorption of essential metals, such as Fe (Crowley et al., 1991) and Mn (Barber and Lee, 1974) as well as nonessential metals, such as Cd (Salt et al., 1995). Soil microorganisms can also directly influence metal solubility by altering their chemical properties. For example, a strain of *Pseudomonas maltophilia* was shown to reduce the mobile and toxic Cr^{6+} to nontoxic and immobile Cr^{3+}, and also to minimize environmental mobility of other toxic ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+} (Blake et al., 1993; Park et al., 1999). In addition, it has been estimated that microbial reduction of Hg^{2+} generates a significant fraction of global atmospheric Hg^0 emissions (Keating et al., 1977)

Effect of root exudates on metal uptake

Root exudates have an important role in the acquisition of several essential metals. For example, some grass species can exude from roots a class of organic acids called siderophores (mugineic and avenic acids), which were shown to significantly enhance the bioavailability of soil-bound iron (Kanazawa et al., 1995), and possibly zinc (Cakmak 1996a; 1996b). In addition, root exudates have been shown to be involved in plant tolerance. In support of this, it has been demonstrated that some plant species tolerate Al in the rhizosphere, by a mechanism involving exudation of citric and malic acids (Pellet et al., 1995; Larsen et al., 1998). These organic acids chelate rhizospheric Al^{3+} which is highly phytotoxic to form a significantly less toxic complex.

V. OPTIMIZATION OF METAL PHYTOEXTRACTION WITH AGRONOMIC PRACTICES

Plant selection

The selection of phytoremediating species is possibly the single most important factor affecting the extent of metal removal. Although, the potential for metal extraction is of primary importance, other criteria, such as ecosystem protection must be also considered when selecting remediating plants. As a general rule, native species are preferred to exotic plants which can be invasive and endanger the harmony of the ecosystem. To avoid propagation of weedy species, crops are in general preferred although some crops may be too palatable and pose a risk to grazing animals.

The rate of metal removal depends upon the biomass harvested and metal concentration in harvested biomass. Possibly, one of the most debated controversies in the field refers to the choice of remediative species; metal hyperaccumulators vs. common nonaccumulator species. Hyperaccumulator plants have the potential to bioconcentrate high metal levels. However, their use may be limited by small size and slow growth. In common nonaccumulator species, low potential for metal bioconcentration is often compensated by the production of significant biomass (Ebbs et al., 1997). Chaney et al. (1999), analyzed the rate of Zn and Cd removal, and reached the conclusion that non-accumulator crops will not remove enough metal to support phytoextraction. Furthermore, these authors argued that at many sites metal contamination is high enough to cause toxicity to crop species and significant biomass reduction. In support of this, several maize (one of the most productive crops) inbred lines have been identified which can accumulate high levels of Cd (Hinesly et al., 1978). However, these lines were susceptible to Zn toxicity and, therefore, could not be used to cleanup soils at the normal Zn:Cd ratio of 100:1 (Chaney et al., 1999). In addition, when appropriate disposal is an important regulatory concern, the use of lower biomass producing hyperaccumulator species would be an advantage because less contaminated biomass will have to be handled.

For Pb, a major soil contaminant, no hyperaccumulator species has been identified. However, several species, such as hemp dogbane (*Apocynum cannabinum*), common ragweed (*Ambrosia artemisiifolia*), nodding thistle (*Carduus nutans*), and Asiatic dayflower (*Commelina communis*), were shown to have superior Pb accumulating properties (Berti and Cunningham, 1993). Practices have been developed to increase the potential of common nonaccumulator plants for Pb phytoextraction. Particularly, the uptake-inducing properties of synthetic chelates open the possibility of using high biomass producing crops for Pb phytoextraction. Under chelate-induced conditions, maize (Huang and Cunningham, 1996) and Indian mustard (Blaylock et al., 1997) have been successfully used to remove Pb from solution culture and contaminated soil, respectively.

Physical characteristics of soil contamination are also important for the selection of remediating plants. For example, for the remediation of surface-contaminated soils, shallow-rooted species would be appropriate to use, whereas deep-rooted plants would be the choice for more profound contamination.

Soil fertilization, and conditioning

Phytoremediation is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. The importance of employing effective agronomic practices has been discussed by Chaney et al. (1999). These authors investigated the effect of soil acidification on Zn and Cd phytoextraction and proposed the use of $(NH_4)_2SO_4$ as a soil additive to provide nutrients (N and S) needed for high yield, and to acidify the soil for greater metal bioavailability. It should be noted that there may be some negative side effects associated with soil acidification. For example, due to increased solubility some toxic metals may leach into the groundwater creating an additional environmental risk. Chaney et al. (1999) indicated that following metal phytoextraction, soil can be limed to elevate the pH near a neutral value, so that normal farm uses or ecosystem development could resume. However, premature liming may increase soil capacity for metal binding and restrict the potential for phytoextraction. A similar effect can be expected following the addition of organic fertilizers. In addition, the raising of pH may stimulate the formation of metal hydroxy ions, such as $ZnOH^+$ which is more strongly sorbed to soil solids than the uncomplexed ions.

Phosphorus is a major nutrient, and plants respond favorably to the application of P fertilizer by increasing biomass production. The addition of P fertilizer, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloro-pyromorphite (Chaney et al., 2000). This underlines the importance of finding new approaches for P application. Such an alternative may be foliage application. This method may lead to improvement of plant P status without inhibiting Pb mobility in soil.

Enhancing metal bioavailability with synthetic chelators

For some toxic metals such as Pb, a major factor limiting the potential for phytoextraction is limited solubility and bioavailability for uptake into roots. One way to induce Pb solubility is to decrease soil pH (McBride, 1994). Following soil acidification, however, mobilized Pb can leach rapidly below the root zone. In addition, soluble ionic lead has little propensity for uptake into roots. The use of specific chemicals, synthetic chelates, has been shown to dramatically stimulate the potential for Pb accumulation in plants. These compounds prevent Pb precipitation and keep the metal as soluble chelate-Pb complexes available for uptake into roots and transport within plant. For example, addition of EDTA (ethylene-diamine-tetraacetic acid), at a rate of 10 mmol/kg soil, stimulated Pb accumulation in shoots of maize up to 1.6 % (Blaylock et al., 1997). In a subsequent study, Indian mustard exposed to Pb and EDTA in hydroponic solution was able to accumulate more than 1%Pb in dry shoots (Vassil et al., 1998). Another synthetic chelator, HEDTA (hydroxyethyl- ethylenediamine-triacetic acid) applied at 2.0 g/kg soil contaminated with 2,500 ppm Pb, increased Pb accumulation in shoots of Indian mustard from 40 ppm to

10,600 ppm (Huang and Cunningham, 1996). Accumulation of elevated Pb levels is highly toxic and can cause plant death. Because of the toxic effects, it is recommended that chelates be applied only after a maximum amount of plant biomass was produced. Prompt harvesting (within one week of treatment) is required to minimize the loss of Pb-laden shoots.

Blaylock et al. (1997), indicated that, in addition to Pb, chelate-assisted phytoextraction is applicable to other metals. These authors indicated that application of EDTA also stimulated Cd, Cu, Ni, and Zn phytoaccumulation. Chelate ability to facilitate phytoextraction was shown to be directly related to its affinity for metals. For example, EGTA (ethylenebis (oxyethylenetrinitrilo) tetraacetic acid) has a high affinity for Cd^{2+}, but does not bind Zn^{2+}. EDTA, HEDTA, and DTPA (diethylene-triamine-pentaacetic acid) are selective for Zn. In fact, zinc binding by DTPA is so strong that plants cannot use Zn from this complex and potentially suffer from Zn deficiency.

Sowing

The extent of metal extraction depends on the amount of plant biomass produced. An important factor that controls biomass production is plant density (number of plants/m^2). Density affects both yield/plant and yield/ha. In general, higher density tends to minimize yield per plant and maximize yield per hectare. Density is also likely to affect the pattern of plant growth and development. For example, at higher stand density, plants will compete more strongly for light. Thus, more resources (nutrients and energy) may be allocated for plant growth as opposed to developmental processes (flowering). An extended growth period may be beneficial if plant metal absorption and accumulation depend upon growth processes. Furthermore, the distance between plants is likely to affect the architecture of the root system with possible further implications on metal uptake. However, the effect of this interaction is unknown and awaits investigation.

Crop rotation

Another agronomic principle, which has been neglected in phytoremediation research, is crop rotation. Because of the proliferation of weeds, predators, and diseases, which can cause significant yield reduction, crops, including those used for soil remediation, must be rotated. In general, crops are rotated less frequently today than 30 years ago. From crop science, it can be extrapolated that short-term (two to three years) monoculture (the use of the same species in consecutive seasons), may be acceptable for metal phytoremediation. However, for longer-term applications, as most metal phytoextraction projects are anticipated, it is unlikely that successful metal cleanup can be achieved with only one remediative species used exclusively in monoculture. Plant rotation is even more important when multiple crops per year are projected.

Crop maintenance: pest control and irrigation

Weed control and irrigation are major crop maintenance practices. Weeds can be controlled by mechanical or chemical methods. Herbicides can be applied before or after the emergence of phytoremediating species. Application of pre-emergent herbicides ensures good weed control, quick emergence, and establishment of selected plants. Post-emergent herbicides control weeds that occur later in the growing season. Because metal uptake into roots depends on the movement of soil solution from the bulk soil to root surface, maintaining an adequate soil moisture is important. Depending on the local climate, irrigation may be required to achieve adequate soil moisture. The volume of water delivered must be carefully considered. This volume should compensate for losses due to evaporation and transpiration. Excessive water delivery will not only inflate operational cost, but may also restrict root growth and depress metal extraction rates. The method of irrigation must also be carefully considered. For example, when delivered under low pressure directly to the soil, as dripping, losses due to evaporation are kept to a minimum. In addition, this method will have little effect on air humidity. In contrast, water delivered under pressure from a nozzle, will elevate air humidity and possibly inhibit leaf transpiration. Since the movement of metal-containing sap from the root to the shoot depends on transpiration, transport and rate of metal accumulation in shoots may be affected. Furthermore, when applied under pressure, water losses due to evaporation are significant and add to the operational cost.

Handling and disposal of contaminated waste

One concern associated with the application of phytotechnology is handling and disposal of contaminated plant waste. The need to harvest contaminated biomass and possibly dispose of it as hazardous waste subject to RCRA standards creates an added cost and represents a potential drawback to the technology. One option is disposal of contaminated biomass to a regulated landfill. To decrease handling, processing, and potential landfilling costs, waste volume can be reduced by thermal, microbial, physical or chemical means. With some metals (Ni, Zn, and Cu) the value of the reclaimed metal may provide an additional incentive for phytoextraction. Chaney et al. (1999) proposed incineration of plant biomass to further concentrate the bio-ore. These authors showed that the value of the metal recovered in the biomass was shown to offset the cost of the technology. Furthermore, Watanabe (1997) showed that Zn and Cd recovered from a typically contaminated site could have a resale value of $1,060/ha.

Cost and time projections

Cost analysis of metal phytoextraction is hampered by a lack of information. In support of this, to date no metal-contaminated site has been completely remediated with plants. Therefore, available cost data are limited to short-term (two- to three-year-old) field studies. It is doubtful that these results can be used to accurately estimate the cost of a full-scale project that can last as long as 15 years. In addition, complexity argues against generic, and in favor of site-specific cost analysis. Despite these limitations, several authors, have investigated the time-frame and cost of metal phytoextraction. For example, Brown et al. (1995a) considered a soil contaminated with 400 mg kg^{-1} Zn, and a desired cleanup level of 40 mg kg^{-1}. These authors used *T. caerulescens* in their analysis and assumed a constant rate of uptake of 4,000 mg kg^{-1}, and an annual yield of 10 t/ha. They estimated that it would take 18 growing seasons to remove excess Zn from the soil. In a subsequent study, the cost of remediating a metal-contaminated soil by conventional engineering techniques was estimated between $50 and $500 per ton (Cunningham and Ow, 1996). Thus, the price tag of remediating an acre of soil (3-foot-deep contamination), weighing some 4,500 tons, would be in excess of $ 250,000. These authors estimated that growing a crop on an acre of land can be accomplished at cost ranging from two to four orders of magnitude less than current cost for soil excavation and burial. Salt et al. (1995) estimated that using phytoextraction to cleanup one acre of soil to a depth of 50 cm will cost $60,000-100,000 compared to at least $400,000 for soil excavation and storage alone.

Research needs

There is a need to optimize the agronomic practices to maximize the cleanup potential of remediative plants. Since in many instances metal absorption in roots is limited by low solubility in soil solution, it is important to further investigate the use of chemical amendments to induce metal bioavailability. Significant results have been obtained in this area. However, there is a need to find cheaper, environmentally benign chemical compounds with metal chelating properties. Research is also needed to identify phytoremediating species capable of being rotated to sustain the rate of metal extraction. More information is also needed to optimize the time of harvest. Plants should be harvested when the rate of metal accumulation in plants declines. This will minimize the duration of each growth cycle and allow more crops to be harvested in a growing season. The current status of agronomic practices as they apply to metal phytoextraction and further research needs are assessed in Table 7 .

Table 7. Assessment of the current status of agronomic practices as they apply to metal phytoextraction and further research needs.

Agronomic issue	Readiness[1]	Purpose	Current status	Research needs
Soil mobilization	1	To control crop pests and condition surface soil for seed germination.	Achieved via rototilling or plowing. Information available on crop species can be applied to remediating plants.	Research needed to determine whether plowing may displace soil contaminants to root inaccessible depths.
Fertilization/ Conditioning	3	Fertilizers are used to improve soil nutrient supply and nutrient availability for uptake into roots. Conditioners are used to improve soil aeration and water holding capacity.	Soil fertilization has been researched extensively and a wealth of information is available in the literature. However, with the exception of Pb/P interaction little is known about the effect of fertilizers/ conditioners on the mobility of toxic metals and their bioavailability in soil.	The effect of fertilizers/conditioners on the chemistry of soil metals and plants' ability to absorb metals must be better understood. Of particular concern is the application of synthetic chelates (themselves potentially toxic). Research should clarify the fate of these compounds in soil and groundwater, as well as the effect of chelate application on metal mobility and uptake into plants.
Crop rotation	4	To control crop pests such as weeds, insects and diseases, and to manage nutrient soil supply.	Today crops are rotated less frequently than 30 years ago. For conventional crops, economics favor the repeated use (rotation) of one or two profitable species. However, in phytoremediation, where two or more crops per year are expected, species rotation may prove a valuable crop management tool. Very little is known about the rotation of phytoremediating plants.	Research must identify metal accumulator species that can be rotated within a specific application.

Sowing	2	The proper amount of seed must be incorporated in soil to obtain a plant stand of desired density.	A wealth of information exists on sowing of crop species. This knowledge can be applied to related phytoremediating species. For example, similar techniques would be employed to sow canola and Indian mustard.	It is not clear how plant density will affect the rate of metal phytoextraction and potential for biomass production. These effects should be investigated.
Irrigation	2	To compensate for water loss due to evaporation from soil and plant transpiration.	A great deal of information exists on the irrigation of crop species. This information can be directly applied to plants grown for environmental remediation.	The effect of various irrigation methods on root growth and expansion, and metal phytoextraction rate must be better understood.
Weed control	1	To suppress the growth of weeds to the point that its effect on the rate of metal extraction is minimal.	Mechanical and chemical methods are the most common approaches. Biological control (the use of natural enemies to reduce weed population) is emerging as an alternative.	RPMs must identify the appropriate weed control strategy (e.g., herbicides rotation) to prevent the buildup of weed populations and maximize the rate of metal phytoextraction.
Harvesting	3	Remove plant biomass loaded with metal contaminants.	In general, techniques and equipment are readily available.	Research must be conducted to determine when to harvest. It is important to harvest when the rate of metal extraction starts declining. This would allow more crops to be obtained in a season and increase the amount of metal removed. Appropriate timing would also maximize the amount of biomass removed at harvest.

[1)] Readiness for field deployment: 1) very little research is needed in this area; 2) practice is ready for field deployment although research may improve its effectiveness; 3) significant research is needed to efficiently apply the practice; 4) practice has not been investigated for metal extraction.

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