Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants

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Toxic metal pollution of waters and soils is a major environmental problem, and most conventional remediation approaches do not provide acceptable solutions. The use of specially selected and engineered metal-accumulating plants for environmental clean-up is an emerging technology called phytoremediation. Three subsets of this technology are applicable to toxic metal remediation: (1) Phytoextraction—the use of metal-accumulating plants to remove toxic metals from soil; (2) Rhizofiltration—the use of plant roots to remove toxic metals from polluted waters; and (3) Phytostabilization—the use of plants to eliminate the bioavailability of toxic metals in soils. Biological mechanisms of toxic metal uptake, translocation and resistance as well as strategies for improving phytoremediation are also discussed.

Pollution of the biosphere with toxic metals, has accelerated dramatically since the beginning of the industrial revolution[1,2]. The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and sewage[3]. Toxic metal contamination of soil, aqueous waste streams and ground water poses a major environmental and human health problem which is still in need of an effective and affordable technological solution. In this review we divide toxic metals into two major groups: heavy metals and radionuclides. These two groups may differ greatly in the mass of the metal which constitutes environmental hazard. The bioremediation paradigm, "using microorganisms to degrade pollutants *in situ*," has been recently attracting a lot of public attention and R&D spending[4]. Unfortunately, heavy metals and radionuclides can not be chemically degraded. Therfore, application of microbial bioremediation to the *in situ* removal of heavy metals from contaminated substrates is mainly limited to their immobilization by precipitation or reduction[5].

In many ways living plants can be compared to solar driven pumps which can extract and concentrate certain elements from their environment. All plants have the ability to accumulate, from soil and water, those heavy metals which are essential for their growth and development. These metals include Fe, Mn, Zn, Cu, Mg, Mo, and possibly Ni. Certain plants also have the ability to accumulate heavy metals which have no known biological function, these include Cd, Cr, Pb, Co, Ag, Se, and Hg[6,7]. However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals, and to accumulate them to unusually high concentrations has evolved both independently and together in a number of different plant species[8,6]. Accumulators of Ni[9], Co and Cu[10], Mn[11], Pb and Zn[12], and Se[13], have been reported.

The basic idea that plants can be used for environmental remediation is certainly very old and cannot be traced to any particular reference. For example, extensive research on using semi-aquatic plants and ecosystems for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era[13a]. The knowledge that aquatic or semiaquatic vascular plants such as water hyacinth (*Eichhornia crassipes*)[14], pennyworth (*Hydrocotyle umbellata*)[15], duckweed (*Lemna minor*)[16,17], and water velvet (*Azolla pinnata*)[16], can take up Pb, Cu, Cd, Fe, and Hg from contaminated solutions has been around for a long time. This ability is currently utilized in many constructed wetlands, which may be effective in removing some heavy metals as well as organics from water[18]. Unfortunately, constructed wetlands simply move heavy metal contaminants to a different location, without removing them from the environment.

It is fair to say, that only recently has the value of metal-accumulating terrestrial plants for environmental remediation been fully realized[19–23]. Phytoremediation defines the use of plants for environmental cleanup. This review concentrates on phytoremediation of heavy metals, which can be divided into (1) *phytoextraction*, in which metal-accumulating plants are used to transport and concentrate metals from the soil into the harvestable parts of roots and above-ground shoots[24]; (2) *rhizofiltration*, in which plant roots absorb, precipitate and concentrate toxic metals from polluted effluents[25]; (3) *phytostabilization*, in which heavy metal tolerant plants are used to reduce the mobility of heavy metals, thereby reducing the risk of further environmental degradation by leaching into the ground water or by airborne spread. We do not discuss here *plant assisted bioremediation*, in which plant roots in conjunction with their rhizospheric microorganisms are used to remediate soils contaminated with organics[26,27] and the air purifying uses of some plants.

Need

Cleanup of hazardous wastes by conventional technologies is projected to cost at least $400 billion in the U.S. alone, based on estimates obtained from a variety of federal and private sources. Cleanup of the U.S. sites contaminated with heavy metals alone can cost $7.1 billion while mixtures of heavy metals and organics bear an additional $35.4 billion price tag. Radionuclide contamination represent another major opportunity for phytoremediation. The extent of radionuclide contamination problems in soil and water at U.S. DOE and DOD sites

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FIGURE 1. Michael Blaylock (Rutgers University) tending field trials of a metal-accumulating *B. juncea* cultivar near Liberty Park, NJ. The site is contaminated with chromium. (Photographed by Viatcheslav Dushenkov).

TABLE 1. Shoot and root bioaccumulation coefficients[1] of *Brassica juncea* and *Thlaspi caerulescens*. Hydroponically grown plants were exposed to metal solutions for 8 days.

	Bioaccumulation coefficient ± SE			
	Shoot		Root	
Metal	*Brassica*	*Thlaspi*	*Brassic*	*Thlaspi*
Cd (5)[2]	175±16	59±12	20574±4295	4258±168
Cu (1)	159±32	623±265	55809±9221	60716±21510
Cr (0.4)	80±8	89±15	5486±393	8545±2677
Ni (1)	587±115	2739±383	11475±125	8425±4220
Pb (5)	3±1	29±23	1432±1409	7011±3616
Zn (3)	49±31	770±320	1816±1739	2990±1424

[1]Bioaccumulation coefficient is the ratio of metal concentration in plant tissue (μg/g DW) to initial metal concentration in solution (mg/L).
[2]Initial concentration of metal in the solution (mg/L).

are still being evaluated. However, the cleanup of contaminated sites that have been identified and characterized to date will cost over $10 billion using current treatment technologies.

This overpowering cost burden has opened a path to the marketplace for innovative technologies. For example, bioremediation, an innovative technology employing bacteria to break down hazardous chemical compounds, will have an estimated $1 billion or more market in North America and Europe by the year 2000. Heavy metal contamination in soils, a segment of hazardous waste market amenable for phytoremediation could constitute a $400 million per year opportunity. Customers for phytoremediation services will include all industrial producers generating water and solid waste contaminated with toxic metals, as well as private companies and municipal, state and federal agencies responsible for the reclamation of contaminated sites.

Several approaches are currently used for treating soils contaminated with toxic metals: (1) Landfilling: the excavation, transport and deposition of contaminated soil in a permitted hazardous waste landfill; (2) Fixation: the chemical processing of soils to immobilize the metals, usually followed by treatment of the soil surface to eliminate penetration by water; (3) Leaching: using acid solutions or proprietary leachants to desorb and leach metals from soil followed by the return of clean soil residue to the site.

Phytoextraction may be a cost-effective alternative to these approaches. Using phytoextraction to clean up one acre of sandy loam soil to a depth of 50 cm will cost $60,000–100,000 compared to at least $400,000 for excavation and storage alone using traditional soil removal methods. Growing several sequential crops of metal-accumulating plants will still be up to an order of magnitude less expensive than soil removal methods. Furthermore, this method is ecologically preferable, since it reclaims soil at the site, recycling it in a biologically safe state rather than permanently disposing of it by removal to a storage site.

Current approaches to treating heavy metal contamination in water include: (1) Precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge; (2) ion exchange; (3) reverse osmosis; and (4) microfiltration.

Rhizofiltration may offer a cost advantage in water treatment because of the ability of plants to remove up to 60% of their dry weight as toxic metals, thus markedly reducing the generation and disposal cost of the hazardous or radioactive residue. Rhizofiltration will also be a particularly cost-competitive technology in the treatment of surface or ground water containing relatively low concentrations of toxic metals.

Phytoextraction

The optimum plant for the phytoextraction process should not only be able to tolerate and accumulate high levels of heavy metals in its harvestable parts but also have a rapid growth rate and the potential to produce a high biomass in the field. Because most of the metal-accumulating wild plants are relatively small in size and have slow growth rates, their potential for phytoextration may be limited. Nevertheless, the first reported field trials of wild metal accumulators of Ni and Zn, growing on soils contaminated by long term application of heavy metal containing sludges demonstrated the feasibility of phytoextraction[28]. However, even the best metal accumulator identified in this trial, *Thlaspi caerulescens*, belonging to the Brassicaceae (mustard family) would take 13 to 14 years of continuous cultivation to clean the site.

Recently in our laboratory we have found that several high biomass crop species, related to wild metal accumulating mustards, can accumulate heavy metals in their shoots. Of all the species screened, certain cultivars of *Brassica juncea* (Indian mustard) had the highest shoot Pb accumulation as well as an ability to accumulate and tolerate Cd, Cr(VI), Ni, Zn, and Cu[24]. In general our screen demonstrated that the ability to accumulate heavy metals varied greatly between species and between cultivars within a species. Using a hydroponic culture system and relatively low metal concentrations, we compared the abilities of *B. juncea* and a wild metal accumulator, *T. caerulescens* (see above) to accumulate heavy metals in their shoots (Table 1, first two columns). For all metals except Cd, the ratio of metal concentration in plant tissue (μg/g DW) to initial metal concentration in solution (mg/L), were greater for *T. caerulescens* than for *B. juncea*. This direct comparison demonstrated the ability of *T. caerulescens* to accumulate higher levels of heavy metals, particularly Zn and Ni, in its shoots when compared to *B. juncea*. Recent work[29,30] also demonstrated that *T. caerulescens* has higher resistance to the toxic effects of both Cd and Zn. However *B. juncea* produces at least 20 times more biomass than *T. caerulescens* under field conditions giving it the potential to remove more metal in a single cropping.

Our group recently performed field trials of a metal accumulating cultivar of *B. juncea* at sites in New Jersey (Fig. 1), in the Mariupol and Chernobyl regions of the Ukraine (in collaboration with Dr. B.V. Sorochinsky), and in the Pennine region of England (in collaboration with A.J.M. Baker). *B. juncea* was able to grow and accumulate Pb, Cr, Cd, and Ni from soils at these sites. *B. juncea* also demonstrated a strong accumulation of ^{90}Sr, a radionuclide found in the soils in the Chernobyl region of the Ukraine. Accumulation of ^{90}Sr in *B. juncea* shoots was 3-fold higher than that seen in *Zea mays*, and the final concentration of ^{90}Sr in shoots of *B. juncea* was 12-fold higher than in the soil. Another group also demonstrated that *B. juncea* was also able to reduce the total Se and B content of soil in a single cropping[23].

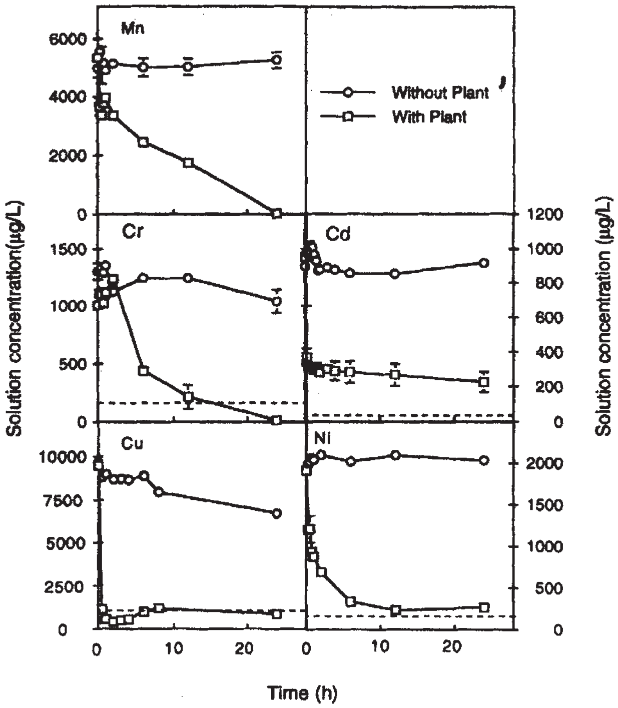

FIGURE 2. Concentration of metals in aqueous solutions in the presence (□) and absence (○) of sunflower (*Helianthus Annuus* L.) roots. Metal ions are indicated on the top of each panel. Metal concentrations in 10 mL aliquots removed from the solution were determined by graphite furnace atomic absorption spectroscopy. Roots immersed in the 800 mL of solution had dry weights of 0.7 to 1.7g. Vertical bars denote S.E. (n=4). Dashed line represents the New Jersey ground water standard (μg/L).

Rhizofiltration

An ideal plant for rhizofiltration should have rapidly growing roots with the ability to remove toxic metals from solution over extended periods of time. Using a 3000 L pilot scale rhizofiltration systems, we have demonstrated the feasibility of growing large amounts of densely packed roots hydroponically, and we estimate that the production of up to 1.5 Kg DW/m^2/month of roots is possible for a number of plants including *B. juncea*, rye, corn and sunflower. Our recent screening of "large root" species demonstrated that many of these plants have an intrinsic ability to absorb and precipitate heavy metals from solution[25]. We have found that within 24 hours roots of sunflower (*Helianthus annuus* L.) were able to dramatically reduce the levels of Cr(VI), Mn, Cd, Ni, and Cu in water (Fig. 2), bringing metal concentration close to or below the available regulated discharge limits. Similar results were obtained with U(VI), Pb, Zn and Sr.

Mechanisms of toxic metal removal by plant roots are not necessarily similar for different metals. In the case of Pb, sorption by the root is probably the fastest component of metal removal. Surface sorption is a combination of such physical and chemical processes as chelation, ion exchange and specific adsorption. This component does not require biological activity and will take place in dead roots. Biological processes are responsible for the slower components of metal removal from the solution. These biological processes include intracellular uptake, vacuolar deposition and translocation to the shoots[24,31]. Because metal transport to the shoot makes rhizofiltration less efficient by producing more contaminated plant residue, plants used for rhizofiltration should not be efficient translocators of metal. Fortunately, the ability of plants to translocate heavy metals to shoots varies much more than their ability to accumulate metals in roots[24]. The third and slowest component of metal removal, reported for high Pb concentrations[25], involves root-mediated precipitation from the solution in the form of insoluble Pb phosphate. This precipitation probably involves a release of root exudates. In addition, cell walls of roots exposed to Pb accumulate large amounts of insoluble $PbCO_3$, formed from the respiratory CO_2. The combination of these processes produces the characteristic non-linear kinetics of metal disappearance from solution.

Metal bioaccumulation coefficients of roots of some plants are dramatic and can be as high as 60,000. The last two columns in Table 1 compare the bioaccumulation coefficients of different metals in roots of *B. juncea* and *T. caerulescens*. Results show that the relative differences in root metal uptake between two species is less dramatic than differences in shoot uptake.

Rhizofiltration is particularly effective and economically compelling when low concentrations of contaminants and large volumes of water are involved. Therefore, rhizofiltration may be particularly applicable to radionuclide contaminated water. Uptake of radionuclides by plants is not well studied, but promising results obtained in our laboratory suggest that many cationic and anionic radionuclide contaminants can be substantially or completely removed from water with selected metal accumulating plants, cultivated in a specially developed and optimized rhizofiltration system. Rhizofiltration of radionuclides may be particularly effective when used in combination with a microorganism-based bioremediation strategy[32].

Phytostabilization

Heavy metal polluted soils usually lack established vegetation cover due to the toxic effects of pollutants or recent physical disturbance. Barren soils are more prone to erosion and leaching which spread pollutants in the environment. A simple solution to the stabilization of these wastes is re-vegetation with metal-tolerant plant species. For the stabilization of metallifer-

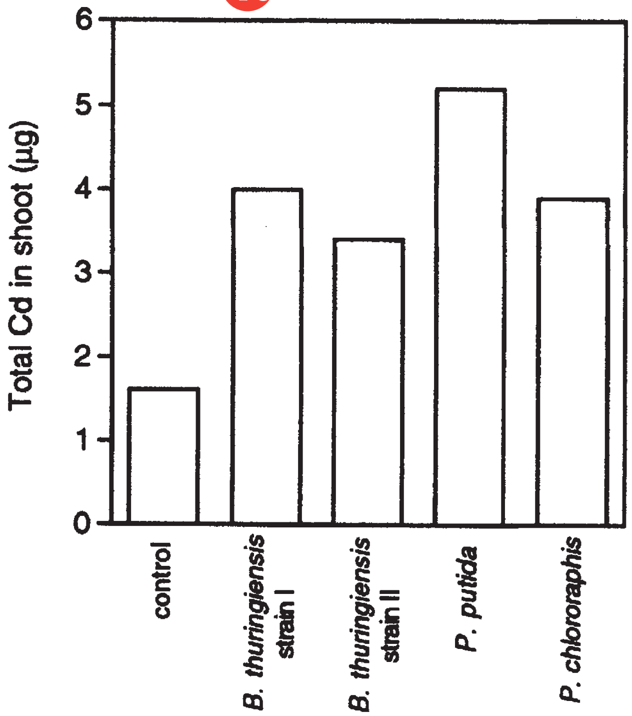

FIGURE 3. Total Cd accumulation in the shoots of 2 week old *B. juncea* seedlings grown hydroponically in the presence of selected rhizospheric microorganisms, including *Bacillus thuringiensis* strain I and II, *Pseudomonas putida* and *Pseudomonas chlororaphis*. Seedlings were then exposed to 0.1 μg/mL Cd, containing ^{109}Cd, for 24 h. Shoots were then harvested and their Cd content estimated as accumulation of ^{109}Cd.

ous mine wastes in the UK, a group in Liverpool[33], utilized local metal-tolerant plant species. By extensive fertilization and planting of the endemic metal-tolerant varieties, they were able to stabilize the site and establish excellent vegetative cover. Based on these results three cultivars of different grasses were made commercially available: *Agrostis tenuis*, cv Goginan for acid lead/zinc wastes; *Festuca rubra*, cv Merlin for calcareous lead/zinc wastes; and *Agrostis tenuis*, cv Parys for copper wastes. An extensive effort to stabilize Cd and Zn contaminated soils with metal tolerant grasses is currently taking place in Palmerton, PA (Chaney, personal communications).

Our group has investigated the potential of metal-accumulating plants to reduce leaching of metals from soils into the ground water. Three week old *B. juncea* seedlings grown in a sand-Perlite mixture containing 625 μg/g Pb were able to reduce the Pb level in the leachate from 740 μg/mL, in the absence of a plant, to 22 μg/ml in the presence of a plant (Kumar and Raskin, unpublished). Plants may also reduce metal leaching by converting metals from a soluble oxidation state to an insoluble oxidation state. Recently it has been suggested that reduction of Cr may be useful in remediating some Cr(VI) contaminated sites[34]. Evidence obtained with X-ray absorbance spectroscopy (XAS) suggest that roots of *B. juncea* are able to reduce available and toxic Cr(VI) to unavailable and less toxic Cr(III) (Salt, Prince, and Pickering, unpublished). A good phytostabilizing plant should tolerate high levels of heavy metals and immobilize these metals in the soil via root uptake, precipitation or reduction. In addition, these plants should have low shoot accumulation of heavy metals to eliminate the necessity to treat harvested shoot residues as hazardous waste.

Bioavailability of Metals in Soils

The effectiveness of phytoextraction for remediation of heavy metal contaminated soils is highly dependent on the availability of metals for plant uptake. Metals in the soil environment exist as components of several different fractions: (1) free metal ions and soluble metal complexes in the soil solution; (2) metal ions occupying ion exchangeable sites and specifically adsorbed on inorganic soil constituents; (3) organically bound metals; (4) precipitated or insoluble compounds, particularly of oxides, carbonates and hydroxides; and (5) metals in the structure of silicate minerals. Anthropogenic metal contamination of soils generally results in metals occurring in fractions (1)–(4) while metal in fraction (5) is indicative of background or indigenous soil concentrations[35]. A major hurdle for phytoextraction is that only fraction (1), and, possibly some components of fraction (2) are readily available to plants.

Manipulation of the soil environment to enhance the availability of metals is critical for effective phytoremediation. Although significant amounts of research regarding the soil chemistry and bioavailability of heavy metals in soils has been conducted, most of it has been directed towards inhibiting or reducing metal availability. There is very little data available on practices designed to enhance metal uptake, except in the case of micronutrient deficiencies, usually overcome through addition of chelated micronutrient compounds.

Increasing Metal Bioavailability with Soil Amendments

Chelating agents have been used as soil extractants, a source for micronutrient fertilizers, and to maintain solubility of micronutrients in hydroponic solutions. The formation of metal-chelate complexes prevents precipitation and sorption of the metals thereby maintaining their availability for plant uptake. The addition of chelates to the soil can also bring metals into solution through desorption of sorbed species, dissolution of Fe and Mn oxides, and dissolution of precipitated compounds[36]. We have shown, for example, that the shoots of *B. juncea* seedlings grown for 4 weeks in soil containing 0.9 mmol/Kg Cd (100 mg/Kg) and 1 mmol/Kg chelating agent contained 875 μg/g dry weight Cd, this compared to only 164 μg/g dry weight Cd in the absence of a chelator (Blaylock, Zakharova, and Raskin, unpublished). Therefore, amending soils with metal chelates is an effective way to increase metal solubility and plant uptake.

Soil pH is another important factor controlling the solubility of metals in soils. Numerous studies have shown that lowering the pH of a soil will decrease the adsorption of heavy metals and thus increase their concentration in soil solution[37]. Therefore, metal toxicities are often observed in plants growing in extremely acid soils, due to the increased soluble concentrations of Fe, Mn, and Al. By maintaining a moderately acid pH in the soil through the use of ammonium containing fertilizers or soil acidifiers it may be possible to increase metal bioavailability and hence plant uptake.

Because many of the heavy metals in soils are bound to or sorbed on oxides there is a potential for enhancing solubility through dissolution of oxide materials. Many plants are known to release reductants from their roots to obtain insoluble metals from the soil. Plant roots have an ability to reduce insoluble oxidized forms of Fe and Mn through the release of organic acids and reductants[38,39]. The addition of ascorbic acid to soils high in Mn oxides amended with selenite increased the solubility of Se by enhancement of the Mn mediated oxidation of selenite to selenate[40]. Manganese oxides have also been shown to oxidize insoluble Cr(III) to soluble Cr(VI)[41]. While it is not always possible to alter the redox status of a soil in the field, reducing organic acids or other redox active amendments may contribute to the success of phytoextraction.

The competition of metal ions in solution for sorption sites may also be a useful tool to control metal availability. For example, phosphate might be used to extract the anions of Cr, Se and As from the soil. It has been suggested[42] that Se uptake in wheat can be increased by changing from a sulfate based NPK fertilizer to a chloride based form to avoid the competition between sulfate and selenate for plant uptake. Application of Ca increases soil mobility and plant uptake of its analog Sr (Sorochinski, personal communication).

Increasing Metal Bioavailability with Soil Microorganisms

Plant assisted bioremediation, the use of plants in combination with microorganisms to degrade organic pollutants within the environment has been reviewed in several recent publications[43,27,26]. However, the use of microorganisms to improve the plant uptake of heavy metals from soils and water has not been investigated. It is known that plant uptake of certain mineral nutrients such as Fe[44] and Mn[45] may be facilitated by rhizospheric microorganisms. Data from our laboratory suggest that plant uptake of nonessential heavy metals is also effected by rhizospheric microorganisms. A number of microorganisms selected in nutrient cultures from heavy metal contaminated soils stimulated metal uptake by plants. For example, several strains of *Pseudomonas* and *Bacillus* were capable of increasing the total amount of Cd accumulated from hydroponic solution by 2 week old *B. juncea* seedlings (Fig. 3). By populating the rhizosphere with selected microorganisms during the process of phytoextration and rhizofiltration it should be possible to enhance uptake of heavy metals from soils or aqueous streams. Specially selected microorganisms could be applied to the plant via a seed treatment or added in the irrigation water.

Plant Biology of Heavy Metal Accumulation

A long term strategy for the improvement of heavy metal accumulation by plants must contain a commitment to gaining a better understanding of the biological mechanisms involved in this process. Heavy metal accumulation within plants can be divided into three major areas. The biology of heavy metal uptake, translocation, and resistance.

Root uptake. Even in the presence of metal mobilizing soil amendments, a large proportion of metal remains sorbed to soil constituents. For plants to accumulate these 'soil-bound' metals they must first mobilize them into the soil solution. This mobilization of 'soil-bound' metal can be achieved in a number of different ways (Fig. 4A). First, metal-chelating molecules (phytosiderophores) can be secreted into the rhizosphere to chelate and solubilize 'soil-bound' metal. For example, mugineic acid and avenic acid serve as phytosiderophores of graminaceous species[46]. These phytosiderophores are released in response to Fe and Zn deficiency and can mobilize Cu, Zn and Mn from soil[47]. Metal-chelating proteins, perhaps related to metallothioneins[48] or phytochelatins[49] may also function as siderophores in plants. Secondly, roots can reduce 'soil-bound' metal ions by specific plasma membrane bound metal reductases. Pea plants deficient in Fe or Cu have an increased ability to reduce Fe(III) and Cu(II) which is coupled with an increased uptake of Cu, Mn, Fe and Mg[50]. Thirdly, plant roots can solubilize heavy metals by acidifying their soil environment with protons extruded from the roots. A lower pH releases 'soil-bound' metal ions into the soil solution. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants[44]. It should be noted that all three of the above processes could also be performed by mycorrhizal fungi or root-colonizing bacteria (see the earlier discussion on rhizospheric microorganisms).

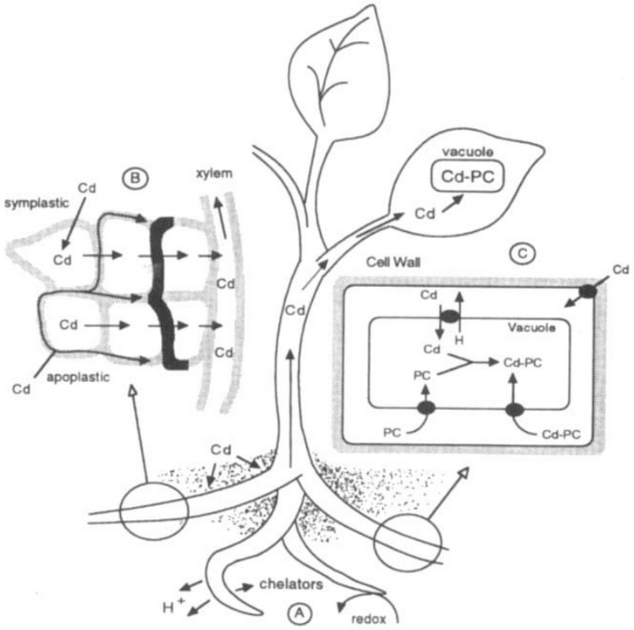

FIGURE 4. Model of the possible routes for Cd accumulation within plants.

Solubilized metal ions may enter the root either via the extracellular (apoplastic) or intracellular (symplastic) pathways (Fig. 4B). Most metal ions enter plant cells by an energy-dependent, saturable process via specific or generic metal ion carriers or channels[51]. Non-essential heavy metals may effectively compete for the same transmembrane carriers as used by essential heavy metals. This relative lack of selectivity in transmembrane ion transport may partially explain why non-essential heavy metals can enter cells even against a concentration gradient. For example, kinetic data demonstrate that essential Cu and Zn and non-essential Ni and Cd compete for the same transmembrane carrier[51]. Metal-chelate complexes may also be transported across the plasma membrane via specialized carriers, as is the case for Fe-phytosiderophore transport in graminaceous species[44].

Transport within plants. Once metal ions have entered the root they can either be stored or exported to the shoot. Metal transport to the shoot probably takes place in the xylem. However, metals may redistribute in the shoot via the phloem[52]. For metal ions to enter the xylem vessels they must first cross the casparian strip (Fig. 4B) which divides the endodermis and the epidermis. To cross this strip of water impermeable cell wall metal ions must move symplastically, as apoplastic transport is blocked. It is therefore feasible that symplastic transport of metals within the endodermis is a rate limiting step in metal translocation to the shoot. Xylem cell walls have a high cation exchange capacity which would be expected to severely retard the movement of metal cations. Therefore, metal-chelate complexes, such as Cd-citrate should facilitate metal movement in the transpiration stream[53]. Analysis of the xylem sap of certain metal accumulators has demonstrated the involvement of organic acids in metal transport[6]. Using X-ray spectroscopy (EXAF) we have recently demonstrated that Cd in the xylem sap of *B. juncea*, exposed to Cd for 7 days, is chelated by oxygen atoms, supporting the involvement of organic acids in Cd translocation (Salt, Prince and Pickering, unpublished). Recent work also suggests that phytochelatins may be involved in metal binding in xylem sap[54]. Nicotianamine, involved in phloem transport of Fe, will also bind Zn, Co, Ni and Cu, and therefore may serve as a general heavy metal transporter in the phloem[52]. In addition, metals may be transported in the phloem chelated to either organic acids, phytochelatins or metallothioneins.

Heavy metal resistance. This topic has recently been extensively reviewed[55–57]. For a plant to resist the toxic effects of heavy metals they must either limit their cellular uptake (avoidance)[58], detoxify the heavy metals once they enter the cells or develop heavy metal resistant metabolisms. The evidence for the avoidance of heavy metal toxicity, by reduced cellular uptake, is very limited. Nevertheless, avoidance may be a viable strategy for certain sensitive tissues like the root-tip meristem. Some plant ecotypes endemic to heavy metal polluted soils have been shown to contain heavy metal resistant enzymes, for example cell wall acid phosphatases[59]. However, it is unlikely that the development of heavy metal resistant biochemical processes could be a viable heavy metal resistance mechanism. Once heavy metals accumulate within cells they will need to be detoxified. This can occur in a number of ways depending on the metal, either through chelation, compartmentalization or precipitation. For example Zn may be chelated to organic acids and accumulated within the vacuole[60,61]. Intact vacuoles isolated from tobacco and barley exposed to Zn were shown to contain high levels of Zn[62,63]. This has also been confirmed in roots and shoots of the Zn accumulator *Thlaspi caerulescens* J & C Presl[64,65]. Zinc accumulation within the vacuole, as a Zn detoxification mechanism, is supported by the observation that the vacuolar volume fraction of meristematic cells within the root tip of *Festuca rubra* increases during Zn exposure[66]. Another mechanism for the detoxification of intracellular Zn is its precipitation as Zn-phytate[66–69].

Cadmium is also known to accumulate within the vacuole[69–71] (Fig. 4C) where it associates with the family of thiol rich peptides, called phytochelatins[49,72]. The recent discovery of mechanisms for the transport of both Cd and the Cd-phytochelatin complex across the tonoplast[73,74] supports this suggestion that Cd detoxification is achieved by the accumulation of Cd, associated with phytochelatins, within the vacuole. This is also supported by recent EXAF data obtained by our group in collaboration with others (Salt, Prince and Pickering, unpublished). These experiments demonstrated that in roots of *B. juncea* the majority of Cd is chelated with sulfur, the major chelating group in phytochelatins. The Cd-phytochelatin complex also contains inorganic sulfide[75–77] which is thought to stabilize and increase its Cd binding capacity. On exposure to Cu plants also appear to produce phytochelatins[78], however their response to Cu differs from Cd in that Cu-binding metallothionein-like molecules also appear to be produced[79,80]. Data from our laboratory also shows that phytochelatins are produced in roots of *B. juncea* exposed to Pb (Salt and Raskin unpublished), and the possibility exists that phytochelatins may also be involved in Pb detoxification.

Future

A better understanding of the biochemical processes involved in plant heavy metal uptake, transport, accumulation, and resistance will foster systematic improvements in phytoremediation using modern genetic approaches. Some successes have been already scored along this path. For example, genes encoding the Cd-binding protein, metallothionein, have been expressed in plants, in a seemingly successful attempt to increase Cd resistance[81–83]. Another strategy for improving the phytoremediation potential of high biomass plant species is the introduction of genes responsible for metal accumulation and resistance from the wild metal accumulators. In the absence of known "phytoremediation" genes this may be accomplished via somatic and sexual hybridization followed by extensive screening and backcrossing of progeny. However, a long term effort should be directed towards developing a 'molecular toolbox' composed of genes valuable for phytoremediation. Systematic screening of plant species and genotypes for metal accumulation and resistance will broaden the spectra of genetic material available for optimization and transfer. Mutagenesis of selected high biomass plant species may also produce improved phytoremediating cultivars.

On the other hand, optimizing agronomic practices employed during phytoremediation, such as irrigation, fertilization, planting and harvest time and the timing of amendment application, should increase the efficiency of both the phytoextraction and rhizofiltration processes. In addition, the problems of design and engineering of phytoextraction and, particularly, rhizofiltration systems have to be addressed.

Phytoremediation of heavy metals is designed to concentrate metals in plant tissues, thus minimizing the amount of solid or liquid hazardous waste which needs to be treated and deposited at hazardous waste sites, with the ultimate goal of developing an economical method of reclaiming metals from plant residue. This will completely eliminate the need for costly off-site disposal. At present, the following methods for the further concentration of metals in plant tissues are being investigated by our group: sun, heat and air drying; environmentally-safe ashing or incineration; composting; pressing and compacting; leaching.

Phytoremediation is clearly a very new field, and one which holds great potential. In order to realize this promise it will be necessary to build a greater understanding of the many and varied processes that are involved. This will require a multidisciplinary approach, spanning fields as diverse as plant biology, agricultural engineering, agronomy, soil science, microbiology and genetic engineering.

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