Phytoremediation of metals: using plants to remove pollutants from the environment

Ilya Raskin*, Robert D Smith and David E Salt

Phytoremediation uses plants to remove pollutants from the environment. The use of metal-accumulating plants to clean soil and water contaminated with toxic metals is the most rapidly developing component of this environmentally friendly and cost-effective technology. The recent discovery that certain chelating agents greatly facilitate metal uptake by soil-grown plants can make this technology a commercial reality in the near future.

Addresses
AgBiotech Center, Cook College, Rutgers University, PO Box 231, New Brunswick, NJ 08903-0231, USA
*e-mail: raskin@aesop.rutgers.edu
Correspondence: Ilya Raskin

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Abbreviation
EDTA ethylenediaminetetraacetic acid

Introduction

The basic idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source; however, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising environmental technology called phytoremediation. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Phytoremediation is being developed as a potential remediation solution for thousands of contaminated sites in the US and abroad.

Soil and water contaminated with metals pose a major environmental and human health problem that is still in need of an effective and affordable technological solution. Nonradioactive As, Cd, Cu, Hg, Pb and Zn and radioactive Sr, Cs and U (referred to here as toxic metals) are the most environmentally important metallic pollutants. Microbial bioremediation has been somewhat successful for the degradation of certain organic contaminants, but is ineffective at addressing the challenge of toxic metal contamination, particularly in soil. Although organic molecules can be degraded, toxic metals can only be remediated by removal from soil. The current state-of-the-art technology for the clean-up of toxic metal-contaminated soils is the excavation and burial of the soil at a hazardous waste site at an average cost of $1 000 000 per acre. In the US alone, the cost of cleaning up sites contaminated with toxic and radioactive metals is estimated to be $300 billion. The problem is even more acute abroad, particularly when large areas are contaminated with radionuclides, for example, areas surrounding the Chernobyl nuclear reactor.

The phytoremediation of metals is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove toxic metals, including radionuclides, from soil and water. Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent reviews [1••,2,3••,4•].

Phytoremediation takes advantage of the fact that a living plant can be considered a solar-driven pump, which can extract and concentrate particular elements from the environment. Phytoremediation is becoming possible because of the productive interdisciplinary cooperation of plant biochemists, molecular biologists, soil chemists, agronomists, environmental engineers, and federal and state regulators. The metals targeted for phytoremediation include Pb, Cd, Cr, As and various radionuclides. The harvested plant tissue, rich in accumulated contaminant, is easily and safely processed by drying, ashing or composting. The volume of toxic waste produced as a result is generally a fraction of that of many current, more invasive remediation technologies, and the associated costs are much less. Some metals can be reclaimed from the ash, which further reduces the generation of hazardous waste and generates recycling revenues.

Discussed in this review are several specific subsets of metal phytoremediation being developed: phytoextraction, in which high biomass metal-accumulating plants and appropriate soil amendments are used to transport and concentrate metals from the soil into the above-ground shoots, which are harvested with conventional agricultural methods [5•]; phytofiltration, in which plant roots (rhizofiltration) [6•] or seedlings (blastofiltration) grown in aerated water precipitate and concentrate toxic metals from polluted effluents; phytovolatilization, in which plants extract volatile metals (e.g. Hg and Se) from soil and volatilize them from the foliage; and phytostabilization, in which plants stabilize pollutants in soils, thus rendering them harmless. Phytostabilization, which is related to soil reclamation, is a less-developed area of phytoremediation research; therefore, it will not be discussed in this review.

Phytoextraction

The phytoextraction of heavy metals and radionuclides represents one of the largest economic opportunities for phytoremediation because of the size and scope of environmental problems associated with metal-contaminated soils, and the competitive advantage offered by a

plant-based remediation technology. The total cost of the clean-up of all US sites contaminated with heavy metal and radionuclides using conventional technologies is estimated at $300 billion. The largest segment in this market is the clean-up of federal facilities contaminated with radionuclides as a legacy of nuclear weapon development during the Cold War.

The inspiration for the development of phytoextraction came from the discovery of a variety of wild plants, often endemic to naturally mineralized soils, that concentrate high amounts of essential and nonessential heavy metals in their foliage [7,8]. The degree of accumulation of metals such as Ni, Zn and possibly Cu in these plants, called hyperaccumulators, often reaches 1–5% of the dry weight. This is an order of magnitude greater than concentration of these metals in nonaccumulating plants growing nearby. The prevention of herbivory and disease is thought to be the main function of this unique phenomenon [9–11]. The suggestion that hyperaccumulating plants can be used for metal remediation was first published in the 1980s [12,13]; however, the very low biomass of known metal-accumulating plants, the lack of technology for their large-scale cultivation and a deficiency in understanding biological and environmental factors involved in metal hyperaccumulation prevented the development of phytoextraction for a long time. Thus, the research emphasis shifted to evaluating the metal accumulation capacity of high biomass plants that can be easily cultivated using established agronomic practices. Particular emphasis has been placed on the evaluation of shoot metal-accumulation capacity of the cultivated *Brassica* (mustard) species because of their relation to wild metal-accumulating mustards [5•]. As a result of this work, certain varieties of *Brassica juncea* (Indian mustard) were selected for their enhanced ability to accumulate metals from hydroponic solution into their above-ground (harvestable) parts. These plants concentrated toxic heavy metals (Pb, Cu and Ni) to a level up to several percent of their dried shoot biomass. Corn (*Zea mays*) and, to a lesser extent, ragweed (*Ambrosia artemisiifilia*) [14•] were also identified as good accumulators of Pb.

A major hurdle to the development of phytoextraction technology was that the shoot metal accumulation in the hydroponically cultivated plants greatly exceeded the metal accumulation measured in soil-grown plants. This phenomenon is explained by the low bioavailability of heavy metals in soils. For example, Pb, one of the most important environmental pollutants, is extremely insoluble and not generally available for plant uptake in the normal range of soil pH. Thus, vegetation growing in heavily contaminated areas often has less than 50 mg g^{-1} Pb in shoots [15]. Even plants that have a genetic capacity to accumulate Pb (e.g. *B. juncea*) will not contain much Pb in roots or shoots if cultivated in Pb-contaminated soil. The solution to the metal availability problem came with the discovery that certain soil-applied chelating agents greatly increase the translocation of heavy metals, including Pb, from soil into the shoots [16•]. EDTA (ethylenediaminetetraacetic acid) was particularly effective in facilitating the phytoextraction of Cd, Cu, Ni, Pb and Zn. For example, the application of 10 mmol kg^{-1} of EDTA to soil containing 1200 mg kg^{-1} Pb resulted in a 1.6% Pb accumulation in the shoots of *B. juncea* [16•]. EDTA was particularly effective when applied to established plants several days before harvest. EDTA acts by complexing soluble metals present in the soil solution. As the free-metal activity decreases, the dissolution of bound metal ions begins to compensate for the shift in equilibrium. The process continues until the supply of EDTA-extractable metal is exhausted. Interestingly, plants seem to take up and translocate Pb as an EDTA complex, accumulating large amounts of EDTA as well as Pb in their foliage (DE Salt, RD Smith, unpublished data).

Biological mechanisms of phytoextraction

The best long-term strategy for improving phytoextraction is to understand and exploit the biological processes involved in metal acquisition, transport and shoot accumulation. In combination with the continuous search for novel phytoextracting plants, this understanding will enable improvements in phytoextraction efficiency. Recent advances in plant biotechnology should provide the means to rapidly capitalize on the mechanistic understanding of phytoextraction. Unfortunately, we know very little about the biological mechanisms involved in phytoremediation.

Roots, which account for 20–50% of plant biomass, extract from the soil and deliver to the shoots most of the elements composing plant tissues, with the exception of carbon. Most of the work on the mechanisms of root and plant cell uptake has focused on the study of N, P, S, Fe, Ca, K and possibly Cl [17]. These studies produced some understanding of the processes involved in the acquisition of these essential elements. However, little is known about the mechanisms of mobilization, uptake and transport of most environmentally hazardous heavy metals, such as Pb, Cd, Cu, Zn, U, Sr, and Cs. It is clear that a large proportion of these metals remains sorbed to solid soil constituents. To acquire these 'soil-bound' metals, phytoextracting plants have to mobilize them into the soil solution. This so-called mobilization of 'soil-bound' metal can be accomplished in a number of ways:

1. Metal-chelating molecules can be secreted into the rhizosphere to chelate and solubilize 'soil-bound' metal. Until now, the major successes in phytoextraction were achieved by applying synthetic chelates to the soil (see above); however, there is a distinct advantage in using natural root-exuded compounds for this purpose. Only iron-chelating compounds, termed phytosiderophores, have been studied well in plants (see below). These phytosiderophores are released in response to iron deficiency and can, in principle, mobilize Cu, Zn and Mn from soil. Mugineic and deoxymugeneic acids from barley and corn and

avenic acid from oats are probably the best studied plant phytosiderophores [18]. Metal-chelating proteins, perhaps related to metallothioneins [19] or phytochelatins [20], may also function as siderophores in plants, although this has never been demonstrated; however, the contribution of phytosiderophores in toxic metal acquisition by the roots of phytoextracting plants remains largely unexplored. It has been recently reported that an Ni hyperaccumulator, *Alyssum lesbiacum*, may use histidine, an excellent Ni chelator, to acquire and transport Ni [21••].

2. Roots can reduce 'soil-bound' metal ions by specific plasma membrane bound metal reductases, which may increase metal availability. Pea plants deficient in Fe or Cu have an increased ability to reduce Fe^{3+} and Cu^{2+}, which is coupled with an increased uptake of Cu, Mn, Fe and Mg [22].

3. Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots. A similar mechanism has been observed for Fe mobilization in some Fe-deficient dicotyledonous plants [23].

4. Roots can employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to increase the bioavailability of metals. However, the significance of microorganisms in the phytoremediation of metals remains largely unknown. It is believed that plant uptake of certain mineral nutrients such as Fe [23], Mn [24], Cd [1••] and possibly Zn (Y Kapulnik, personal communication) may be facilitated by rhizospheric microorganisms.

Mobilized metals are taken up by plant roots from the soil solution and exported to the shoots. Very little is known about toxic metal transport into roots and their subsequent movement within the plant; however, some information is available on the transporter systems involved in the uptake of free and chelated Fe (for a review, see [17,25,26]). A putative iron transporter has recently been cloned from *Arabidopsis* [27]. Ca^{2+} and Mg^{2+} ions, which are present at high concentrations in soil solution and may not require mobilization, may enter the root via either extracellular (apoplastic) or intracellular (symplastic) pathways. These metal ions enter plant cells by an energy-dependent, saturable process via specific or generic metal ion carriers or channels [28]. Theoretically, toxic metals may compete for the same transmembrane carriers as those used by Ca and Mg; however, the high concentrations of these ions in soil solution makes this unlikely.

Most environmentally hazardous metals are too insoluble to move freely in the vascular system of the plant. Many form sulfate, carbonate or phosphate precipitates immobilizing these metals in apoplastic and symplastic compartments. Apoplastic transport of these metals is further limited by the high cation-exchange capacity of cell walls, unless the metal ion is transported as a noncationic metal chelate. Earlier studies showed that in hyperaccumulating and nonhyperaccumulating plant species, some toxic metals may be transported to the shoot complexed to organic acids, mainly citrate [8,29]. Recent studies of Cd movement in *B. juncea*, a good Cd accumulator, showed that, in roots, Cd was present as a CdS_4 complex, which may contain phytochelatins [30•]. In the xylem sap, Cd was coordinated predominantly with oxygen or nitrogen ligands, consistent with the involvement of organic acids [30•]. In the leaves, Cd preferentially accumulated in trichomes.

Phytofiltration

Aquatic and semiaquatic plants, as well as dried plant materials, have often been evaluated in various water purification systems [6•]. Commercial applications of these methods are hampered by the relatively slow growth rate and/or metal-binding capacities of tested plant material. Hydroponically cultivated roots of terrestrial plants were recently found to be more effective in removing heavy metals from water than earlier developed plant-based systems. An ideal plant for rhizofiltration should have rapidly growing roots with the ability to remove toxic metals from solution over extended periods of time.

Screening roots of hydroponically cultivated plants for their ability to remove and concentrate heavy metals from the solution resulted in the identification of certain varieties of sunflower as the most efficient plants for rhizofiltration [6•]. Sunflowers grown in a specially constructed rhizofiltration system can produce as much as 1.5 kg dry weight m^{-2} $month^{-1}$. Roots of *B. juncea* were also very effective in rhizofiltration [6•]. The biologically active, high surface area biofilter formed by plant roots can be extremely active in sorbing pollutants from water. Rhizofiltration technology has received a particular boost from the development of a 'feeder layer' fertilization system, which consists of a layer several centimeters deep of artificial soil which anchors the plant above the stream of contaminated water. The regular application of concentrated fertilizer to this feeder layer stimulates the development of an extensive root network inside the feeder layer. These roots supply nutrients to the whole plant. A much larger portion of the root system, responsible for the actual metal removal, grows through the screen on the bottom of the feeder layer and into the water below. Thus, no nutrients are added to the contaminated stream and the process of fertilization is separated from the process of remediation.

Different metals have different bioaccumulation coefficients (the ratio of metal concentration in dried tissues to that in the surrounding substrate) in the phytofiltration system. The coefficients range from several hundred for cationic species such as As to close to 10 000 for cationic species such as Pb and Cu. These coefficients are much higher if the polluting metal is present in relatively pure water where the activity of competing ions is low [1••,31].

In the process of attempting to improve rhizofiltration, it was discovered that young plant seedlings grown in aerated water (aquacultured) are often more effective than roots in removing heavy metals from water [31]. The technology of using plant seedlings to remove toxic metals from water was termed blastofiltration (blasto is 'seedling' in Greek). Blastofiltration may represent the second generation of plant-based water treatment technology. It takes advantage of the dramatic increase in surface to volume ratio that occurs after germination and the fact that some germinating seedlings also ab/adsorb large quantities of toxic metal ions. This property makes seedlings uniquely suitable for water remediation. Seedling cultures used for blastofiltration can be produced in light or in darkness, and seeds, water and air are the only components required.

Screening has identified Indian mustard seedlings as particularly effective in sorbing divalent cations of toxic metals [31]. This observation is particularly interesting because we previously identified *B. juncea* as one of the best plants for removing heavy metals from contaminated soils (see above). In addition, seedlings of *B. juncea* grew very rapidly in aerated water with very little microbial contamination. As a result, they developed an extremely large biomass with a very large surface area in 4–5 days. Data indicate that, for some metals, blastofiltration is more efficient and economical than rhizofiltration.

Biological mechanisms of phytofiltration

The mechanisms of toxic metal removal by plant roots or seedlings are not necessarily similar for different metals. In the case of Pb, two major components are involved: precipitation and exchangeable sorption. Analysis of *B. juncea* roots exposed to Pb showed the formation of precipitates in the cell walls that contained substantial amounts of Pb carbonates [5•]. The microscopic analysis of corn roots exposed to soluble Pb showed the presence of similar electron-dense deposits inside and outside cells [32]. Pb can also bind to the exchangeable cell wall anionic binding sites [33]. Cell wall fractionation studies showed that the largest amount of Pb reversibly binds to the pectic acid fraction [34]. Extended X-ray absorbance fine structure (EXAFS) analysis of *B. juncea* roots exposed to $Pb(NO_3)_2$ solution revealed that Pb was primarily bound to carboxyl groups, suggesting the involvement of polygalacturonic acid (DE Salt, unpublished data). Biological processes are responsible for the slower components of metal removal from solution. These processes are not very significant for Pb but are much more important for Cd [31]. These biological processes include intracellular uptake, vacuolar deposition and translocation to the shoots.

Phytovolatilization

Toxic metals such as Se, As and Hg can be biomethylated to form volatile molecules that can be lost to the atmosphere. Although it was known for a long time that microorganisms play an important role in the volatilization of Se from soils [35], a plant's ability to perform the same function was only recently discovered. Again, *B. juncea* was identified as a valuable plant for removing Se from soils [36,37]. Se volatilization in the form of methyl selenate was proposed as a major mechanism of Se removal by plants [38,39]. Some plants can also remove Se from soil by accumulating nonvolatile methyl selenate derivatives in the foliage. An enzyme responsible for the formation of methyl selenocystine in the Se accumulator *Astragalus bisculatus* was recently purified and characterized [40].

The unique property of elemental mercury is that it is a liquid at room temperature and thus is easily volatilized; however, because of its high reactivity, mercury in the environment exists mainly as a divalent cation Hg^{2+}. Bacteria can catalyze the reduction of the mercuric ion to elemental mercury using mercury reductase, a soluble NADPH-dependent FAD-containing disulfide oxidoreductase (NADPH, reduced nicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide) [41]. A modified bacterial gene encoding a functional mercuric ion reductase was recently introduced into *Arabidopsis thaliana* [42•]. Transformants showed greater resistance to $HgCl_2$ and produced large amounts of Hg vapor compared to control plants. Although the practicality of using mercury-volatilizing plants for environmental remediation is questionable, this elegant work points to a new environmental use of plant molecular biology.

From the laboratory to the field

Phytoremediation technology received a major boost after Phytotech Inc (Monmouth Junction, NJ, USA) was formed in 1993. During the past two years, Phytotech Inc undertook an extensive field demonstration program that focused primarily on lead-contaminated soil in the US and on radionuclide-contaminated soil in the Chernobyl region of the Ukraine. The results generated from two years of field trials are very promising and showed measurable decreases in soil pollutants (B Ensley, personal communication). Phytotech Inc also successfully tested rhizofiltration in the summer of 1995 at two locations: a DOE site contaminated with 100–400 parts per billion U in ground and surface water in Ashtabula, Ohio; and in a small pond within 1 km of the Chernobyl nuclear power plant in the Ukraine. The field results demonstrated that rhizofiltration is a practical way to treat radionuclide-contaminated water (B Ensley, personal communication). The successful transfer of phytoremediation from the laboratory to the field is a crucial step in the development of this technology.

Conclusions

At present, phytoextraction and phytofiltration are the best-developed subsets of toxic metal phytoremediation nearing commercialization. Although major opportunities for the phytostabilization of toxic metals also exist, this technology is relatively less developed than those described above. This situation should improve as more

researchers become interested in this area. Short-term advances in phytoextraction are likely to come from the development of effective chemical soil amendments and efficient ways of applying them. In addition, the ability of plants to accumulate toxic metals in their shoots may be enhanced through the use of specific chemicals (mainly metal-chelating agents) that facilitate the acquisition and transport of metals.

One of the major challenges for phytofiltration is to differentiate itself from the established water-treatment technologies. Phytofiltration is particularly effective and economically compelling when low concentrations of contaminants and large volumes of water are involved; therefore, phytofiltration may be particularly applicable to radionuclide-contaminated water and to the last polishing steps of water treatment. Major long-term improvements in phytoremediation should come when scientists isolate genes from various plant, bacterial and animal sources that can enhance the metal-accumulating potential of the plants in which these genes are inserted. Parallel developments in environmental and agricultural engineering should have a major impact on the efficiency of plant cultivation and disposal of metal-enriched biomass.

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