

Phytoremediation of soil metals

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The phytoremediation of metal-contaminated soils offers a low-cost method for soil remediation and some extracted metals may be recycled for value. Both the phytoextraction of metals and the phytovolatilization of Se or Hg by plants offer great promise for commercial development. Natural metal hyperaccumulator phenotype is much more important than high-yield ability when using plants to remove metals from contaminated soils. The hypertolerance of metals is the key plant characteristic required for hyperaccumulation; vacuolar compartmentalization appears to be the source of hypertolerance of natural hyperaccumulator plants. Alternatively, soil Pb and Cr⁶⁺ may be inactivated in the soil by plants and soil amendments (phytostabilization). Little molecular understanding of plant activities critical to phytoremediation has been achieved, but recent progress in characterizing Fe, Cd and Zn uptake by *Arabidopsis* and yeast mutants indicates strategies for developing transgenic improved phytoremediation cultivars for commercial use.

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Abbreviations

EDTA ethylenediaminetetraacetic acid
MT metallothionein
PC phytochelatin

Introduction

Because the costs of growing a crop are minimal compared to those of soil removal and replacement, the use of plants to remediate hazardous soils is seen as having great promise; several recent reviews on many aspects of soil metal phytoremediation are available [1,2^{••},3[•],4^{••},5[•],6,7^{••}]. Phytoremediation is the use of plants to make soil contaminants nontoxic, and is often also referred to as bioremediation, botanical bioremediation and, Green Remediation. The idea of using rare plants that hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced in 1983 [8], gained public exposure in 1990 [9], and has increasingly been examined as a potential practical and more cost-effective technology than the soil replacement, solidification and washing strategies

presently used [2^{••},3[•],7^{••}]. Categories of phytoremediation include phytoextraction (the use of plants to remove contaminants from soils), phytovolatilization (the use of plants to make volatile chemical species of soil elements), rhizofiltration (the use of plant roots to remove contaminants from flowing water) and phytostabilization (the use of plants to transform soil metals to less toxic forms, but not remove the metal from the soil). The use of plants and associated rhizosphere organisms or bioengineered plants to metabolize toxic organic compounds also appears promising (recently reviewed by Cunningham *et al.* [10^{••}]).

Phytostabilization appears to have strong promise for two toxic elements, chromium and lead. The reduction of Cr⁶⁺, which poses an environmental risk, to Cr³⁺, which is highly insoluble and not demonstrated to pose an environmental risk [11], by deep rooted plants can be very effective. Chemical species of Pb in soil are usually somewhat bioavailable if the soil is ingested by children, livestock or wildlife [12], whereas a Pb phosphate mineral, chloropyromorphite, is both extremely insoluble and non bioavailable [13,14^{••},15,16,17^{••}] but it is formed slowly, apparently because the reactants have low solubility. The roots of *Agrostis capillaris* growing in highly contaminated Pb/Zn mine wastes caused the formation of pyromorphite from soil Pb and phosphate, but the mechanism remains unknown [17^{••}]. Although it was believed that *Thlaspi rotundifolium* hyperaccumulated Pb, *Zea mays* accumulated higher Pb levels in controlled tests if soil pH and P were low [18[•]]. The addition of chelating agents (e.g. N-hydroxyethyl-ethylenediamine-N, N'-triacetate [HEDTA], ethylenediaminetetraacetic acid [EDTA]) to such soils increased Pb solubility and mobility within plants: shoot Pb reached 1%, allowing the removal of enough Pb to encourage further evaluation of this approach [18[•],19^{••}]. Methods to prevent the leaching of Pb chelates down the soil profile would be required to permit such additions in the field in regions where net infiltration occurs. Inactivating soil Pb using of soil amendments and revegetation to prevent erosion is increasingly seen as the promising soil Pb remediation technology [12,20].

Different views of the potential for use of phytoremediation to clean up contaminated soils have developed among researchers. Some have examined the naturally occurring metal hyperaccumulators, plants that can accumulate 10–500 times higher levels of elements than crops; Reeves [21] suggested a widely accepted definition of Ni hyperaccumulators: 'a plant in which a nickel concentration of at least 1000 µg g⁻¹ has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat'. This definition can be adapted to other elements. Most plant species suffer significant

yield reduction when shoots reach 50–100 mg Ni kg⁻¹ dry weight whereas Ni hyperaccumulators tolerate at least 10–20 times the normal maximum tolerable levels; and among the smaller group of plants that can tolerate at least 1% Ni in shoots, a few can reach 5% Ni, or 500 times the shoot Ni tolerated by crop plants. Species that accumulate over 1% Ni have been called ‘hypernickelophores’ by Jaffré [22]. This term seems appropriate for the plant species that accumulate over 1% of several elements (hypernickelophore, hyperzincophore, etc.) because this ability is qualitatively different than the hyperaccumulators as defined by Reeves [21]. Crop plants tolerate higher shoot Zn and Mn levels than Ni (about 300–500 mg Zn kg⁻¹), so ‘hyperaccumulators’ contain >1% shoot Zn or Mn [7••]. Shoot Cd levels are usually <1 mg kg⁻¹, so ‘hyperaccumulators’ must accumulate and tolerate ≥ 100 mg Cd kg⁻¹; some tolerate >1% Cd [23].

How do hyperaccumulators achieve this remarkable bioaccumulation of soil metals? Research has identified several characteristics that are important:

1. The plant must be able to tolerate high levels of the element in root and shoot cells: hypertolerance is the key property that makes hyperaccumulation possible. Such tolerance is believed to result from vacuolar compartmentalization and chelation [24,25••]. The most direct demonstration used isolated vacuoles from the protoplasts of tobacco cells that had accumulated high levels of Cd and Zn [24]. Whether hypertolerance in the known hyperaccumulators is due to an enhancement of these mechanisms is not yet known. However, electron microprobe analysis [26] supports this conclusion for Zn in leaves of *Thlaspi caerulescens*.

2. A plant must have the ability to translocate an element from roots to shoots at high rates. Normally, root Zn, Cd or Ni concentrations are 10 or more times higher than shoot concentrations, but in hyperaccumulators, shoot metal concentrations can exceed root levels [27,28••,29••]. Krämer *et al.* [29••] recently found that although the chemical forms of Ni found in extracts of leaves of *Alyssum* hyperaccumulators are chelates with malate and citrate, in the xylem exudate histidine chelates about 40% of the total Ni present; nearly all of the histidine in exudate is chelated with Ni. Whether Ni(histidine)₂, Ni²⁺ or a mixed chelate such as Ni(histidine, malate) is pumped into the xylem by a membrane transporter remains unknown. Additions of histidine to nutrient solution increased Ni tolerance and transport to shoots by *Alyssum montanum*, a nonhyperaccumulator species.

3. There must be a rapid uptake rate for the element at levels that occur in soil solution. Here, quite different patterns have been observed in different groups of hyperaccumulators. Brown *et al.* [27] found that *T. caerulescens* accumulated Zn and Cd from nutrient solution only about as well as tomato and *Silene vulgaris* did, but tomato was severely injured at 30 μM Zn, *S. vulgaris*

at 320 μM Zn, and *T. caerulescens* only at 10 000 μM Zn. Because this species can keep tolerating and accumulating Zn and Cd at high soil solution levels, it is found in nature with 1–4% Zn whereas surrounding plants are <<500 mg Zn kg⁻¹ (Zn excluders). Further, studies have shown that highly Zn-tolerant genotypes of *T. caerulescens* require much higher solution Zn²⁺ (10⁴-fold) and leaf Zn concentrations (100–300 mg kg⁻¹ versus 10–12 mg kg⁻¹ in normal plants) to grow normally than do related nonaccumulator species [28••]. By implication, the highly effective compartmentalization to reduce the toxicity of Zn and Cd appears to require the plant to accumulate much more Zn to have an adequate supply. In contrast, the Ni-hyperaccumulator *Alyssum* species accumulate remarkably higher shoot Ni levels compared to other species grown at the same Ni²⁺ activity in solution [29••,30], and the Se-accumulating species similarly accumulate higher shoot Se levels and many can volatilize Se at high rates growing beside plants with more normal levels and slow volatilization [31,32].

What evolutionary advantage does metal hyperaccumulation give these species? Boyd *et al.* [33,34] have demonstrated that high (but not low) Ni levels in the leaves of hyperaccumulators can reduce herbivory by chewing insects and reduce the incidence of bacterial and fungal diseases. Similar results were found for Zn in *T. caerulescens* (AJ Pollard, AJM Baker, unpublished data).

For the effective development of phytoremediation, each element must be considered separately because of its unique soil and plant chemistry. Both agronomic management practices and plant genetic abilities need to be optimized to develop commercially useful practices. Some elements can be accumulated by plant roots and converted to a volatile species such as dimethylselenide [32] or Hg⁰ [35••]. Although many plants can volatilize dimethylselenide (or dimethyldiselenide in the case of the Se-hyperaccumulators) [36], co-contaminating sulfate and salinity in Se-contaminated soils commonly inhibit this process [36,37]; very high B or salinity can kill most plants. So growing species in normal crop rotations that can phytovolatilize soil Se or accumulate Se into the forage biomass for sale as an Se supplement for livestock feeds are alternative approaches to treating irrigation drainage waters, which are much higher in B and sulfate than the water used for irrigation [36,38].

Whether metal hyperaccumulation in shoots or high shoot biomass is more important in the phytoremediation of soil metals has been debated [2••,3••,7••]. A quantitative example may provide clarity: presume that a high-biomass crop plant is grown on a contaminated soil with the pH adjusted to attain a 50% yield reduction (*Z. mays* and *Brassica juncea* are examples of such annual crops). Under favorable conditions, these plants can reach 20 tons dry biomass/ha. In the case of the usual Zn and Cd co-contamination at 100 mg Zn:1 mg Cd, crop plants suffer a significant yield reduction when the shoots have

about 500 mg Zn kg⁻¹ at harvest, because Cd is not 100 times more toxic than Zn: soil Zn phytotoxicity is the factor controlling plant yield. At a 50% yield reduction (10 tons ha⁻¹), dry biomass contains 500 mg kg⁻¹ (500 g Zn ton⁻¹); one removes only 5 kg of Zn ha⁻¹ year⁻¹. *T. caerulescens*, which can remove both soil Zn and Cd, has a low yield compared with the above species, but can tolerate up to 25 000 mg Zn kg⁻¹ (25 kg ton⁻¹) [39] without yield reduction. Even with a low yield of 5 tons ha⁻¹ at the point of incipient yield reduction, Zn removal would be 125 kg ha⁻¹. We conclude that the ability to hyperaccumulate and hypertolerate the metals to be phytoremediated is of greater importance than high biomass. Some authors have suggested that the yield of a crop would be two orders of magnitude higher than that for hyperaccumulators such as *T. caerulescens*, but pot and field studies show that such perennial species grown as a crop can attain as high as 5 tons ha⁻¹ before breeding to increase the combination of yield and shoot metal concentration [27,28••]. Further, the recycling of shoot metals in commerce may provide value for the ash from metal hyperaccumulators, such that there is no need to pay for safe disposal. Continuing the above model, biomass ash contains 20–40% Zn for *T. caerulescens*, but only 0.5% for *Z. mays*; the former is a rich ore, whereas the latter is a phytotoxic waste requiring disposal. Increasing the yield of a crop could give a linear increase in phytoremediation capacity with increasing yield. But increasing from ‘normal’ tolerance to ‘hypertolerance’ and hyperaccumulation increases the potential annual removal of the soil contaminant 25–400-fold. Even for elements that have little value in the biomass, the higher the concentration, the less expensive the disposal of the phytoremediation crop residue or ash (e.g. ¹³⁷Cs, As and U) will be. Thus, we have emphasized the importance of the domestication of metal hyperaccumulator plants and the breeding of improved cultivars [7••,28••], the characterization of the mechanisms used by hyperaccumulators to accumulate, translocate and tolerate metals, and, eventually, the cloning and use of these genes to convert high biomass agronomic plants into special phytoremediation cultivars if this is required for some elements [7••].

The remediation of other elements (e.g. As, Cu, Cs, Sr, U) from soils by hyperaccumulator crops has not been demonstrated, but is expected to be possible if creative research is applied [2••,3•,7••,35••]. In some cases, the phytoremediation of an element may require soil amendments such as chelating agents because soil or plant chemistry reduces element uptake or translocation to shoots [18•,19••].

Use of biotechnology to improve phytoremediation

Biotechnology approaches to develop phytoremediation plants have been examined. Traditional plant breeding can only use the available genetic diversity within a species

to combine the characteristics needed for successful phytoremediation. Researchers expected that increasing the concentrations of metal-binding proteins or peptides in plant cells would increase metal-binding capacity and tolerance. Although plant cell cultures expressing mammalian metallothioneins (MTs) [40] or phytochelatins (PCs) [41••] are more tolerant of acute Cd toxicity, the transfer of mammalian MT genes to higher plants appear to provide no benefit for phytoremediation. Further, when natural metal-tolerant plants were examined, the concentration of PCs showed no difference, suggesting that hypertolerance to Cd and Zn in these plants were not due to the hyperaccumulation of PC peptides [42,43]. The evidence for the role of PCs is that their presence does correlate with normal levels of metal tolerance, since mutations that abolished PC production in *Arabidopsis* and fission yeast resulted in hypersensitivity to Cd [41••,44•,45•]. Cd-sensitive (hypotolerant) single gene mutants *cad1* [44•] and *cad2* [45•] of *Arabidopsis thaliana* have been identified and studied (blocked in glutathione synthesis or PC synthesis). For a plant species with normal tolerance (*A. thaliana*), PCs were essential for the normal level of tolerance.

Interestingly, when these researchers tested genotypes with and without effective PC biosynthesis, the outcome was a surprise in that the sensitive mutants (low PCs) had a significantly lower degree of transport of Cd to shoots than the wild type [45•]. A similar result was observed in corn inbreds that differed substantially in shoot Cd; higher levels of PCs were associated with higher shoot Cd [46].

Although these studies have allowed the cloning of new genes and the characterization or confirmation of metabolic pathways, the environmental relevance of findings from such acute Cd exposure has not been established. An alternative view of Cd-catalyzed PC biosynthesis is that the chelation of PCs with Cd alienates the feedback inhibition of the γ -glutamyl-cysteine transferase: as long as Cd activity in the cytoplasm is high, an enzyme supports more transfer to form more PCs and longer PCs. Because the level of Zn present in nearly all environments is 100 times higher than that of Cd, if the acutely toxic Cd dose is provided, the plants would be killed by Zn. Even the formation of the sulfide-stabilized high molecular weight Cd-PC complex in vacuoles [4••,25••,41••] may result from the acutely toxic Cd supply without Zn. Further, the finding that the *hmt1* vacuolar membrane pump protein (which restored Cd tolerance to mutant fission yeast) transported both Cd-PCs and PCs without Cd, raises questions about how the pump works to induce Cd hypertolerance *in vivo*. Cd phytotoxicity in soil is a recent anthropogenic effect, whereas Zn phytotoxicity and coaccumulation of trace levels of Cd are normal biogeochemical phenomena. We believe that scientists should be more suspicious of ‘Cd tolerance’ in plants. It seems increasingly likely that Cd tolerance mechanisms are incidental biochemical phenomena. Although Cd-PCs can be found at low levels in plants in the environment,

they account for only a small fraction of the tissue Cd [47,48,49•].

Another goal of developing transgenic plants with increased metal-binding capacity was to use these metal-binding factors to keep Cd in plant roots, thus reducing Cd movement to the food chain or into tobacco [50–53]. Vacuolar compartmentation of Cd only in roots may reduce Cd translocation to shoots; the expression in plants of the *hmt1* vacuolar pump for Cd-PCs from fission yeast [25••] has not yet been successful, and the modification of codons will be required before its effectiveness can be tested (similar to the mercury reductase gene changes [35••]). The expression of MT as the whole protein, the Cd-binding 'α-domain' part of the protein, or a fusion protein with β-glucuronidase (GUS), under several promoters [50–53] increased Cd tolerance of tobacco and other plants, but had little effect on Cd transport to shoots. Recently, the use of the improved 35S² promoter may have increased the ability of MT to keep Cd in roots [53]; tests have not yet progressed to soil studies which must be the important measure of success. Many of the studies noted here have used acutely toxic levels of Cd, such that the study results do not model-metal contaminated soils in the environment. Rauser and Meuwly [49] used nontoxic levels of Cd (3 μM, 30 times the level generally found in soil solution) to study PC physiology in *Z. mays*, and found that, in the short term, PCs bound only a small fraction of cell Cd, but, over time, over 90% of root Cd was bound to PCs. McKenna and Chaney [54•] used chelator-buffered [55••] Cd to grow lettuce at Cd levels relevant to foodchain safety and found no evidence of Cd-PCs in lettuce leaves.

Possible use of 'metallophores' to aid phytoextraction of soil metals

Because *Poaceae* species secrete mugineic acid family phytosiderophores (chelating agents) to solubilize soil Fe, and accumulate the intact chelate into root cells [56••], Raskin [5•] suggested that transgenic plants could be developed to secrete metal-selective ligands into the rhizosphere which could specifically solubilize elements of phytoextraction interest. Although this approach holds promise, phytosiderophores obtain their specificity not by chelation specifically only of Fe in soils, but from their uptake of nearly only Fe phytosiderophores by a membrane carrier [55••,56••,57•]. Finding other simple biosynthetic molecules with selective chelation ability that plants can make and secrete into the rhizosphere at adequate concentrations and simultaneously creating a selective transport protein for the metal chelate seems difficult, but worth examination to develop unique phytoextraction tools. Regulatory control of phytosiderophore secretion in barley was induced by Fe-deficiency, but not Mn, Zn, or Cu deficiency [58•] in contrast with other reports, which indicated that Zn deficiency also induced the biosynthesis and secretion of phytosiderophores.

Lastly, extensive progress has recently been achieved in identifying genes and proteins involved in the uptake of

Fe by yeast and plants [59••,60••,61•,62]; high affinity Zn [63] and Cu membrane transporters have also been found in yeast. A fundamental understanding of both uptake and translocation processes in normal plants and metal hyperaccumulators, regulatory control of these activities, and the use of tissue-specific promoters offer great promise that the use of molecular biology tools can give scientists the ability to develop effective and economic phytoextraction plants for soil metals.

Conclusion

Extensive progress has been made in characterizing the soil chemistry needed for phytoextraction, and physiology of plants that hyperaccumulate and hypertolerate metals. It is increasingly clear that hypertolerance is fundamental to hyperaccumulation, and high rates of uptake and translocation are observed in hyperaccumulator plants. Fundamental characterization of mechanisms, and cloning of genes required for phytoextraction has begun with the mercuric ion reductase [35••], and *hmt1* [25••] expression in higher plants is expected soon. Improved hyperaccumulator plants and agronomic technology, to increase the annual rate of phytoextraction and to allow recycling of toxic soil metals accumulated in plant biomass is very likely to support commercial environmental remediation, which society can afford in contrast with present practices. Although most phytoextraction systems are still in development, or in plant breeding to improve the cultivars for field use, application for Se phytovolatilization has already begun. Many opportunities have been identified for research and development to improve the efficiency of phytoextraction. Progress has been hindered in the 12 years since the first report on the model for phytoextraction [8] by limited funds for research and development. New commercial firms are moving into this field and phytoextraction technologies will be increasingly applied commercially in the near term.

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