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Phytoremediation of toxic trace elements in soil and water

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Abstract Toxic heavy metals and metalloids, such as cadmium, lead, mercury, arsenic, and selenium, are constantly released into the environment. There is an urgent need to develop low-cost, effective, and sustainable methods for their removal or detoxification. Plant-based approaches, such as phytoremediation, are relatively inexpensive since they are performed in situ and are solar-driven. In this review, we discuss specific advances in plant-based approaches for the remediation of contaminated water and soil. Dilute concentrations of trace element contaminants can be removed from large volumes of wastewater by constructed wetlands. We discuss the potential of constructed wetlands for use in remediating agricultural drainage water and industrial effluent, as well as concerns over their potential ecotoxicity. In upland ecosystems, plants may be used to accumulate metals/metalloids in their harvestable biomass (phytoextraction). Plants can also convert and release certain metals/metalloids in a volatile form (phytovolatilization). We discuss how genetic engineering has been used to develop plants with enhanced efficiencies for phytoextraction and phytovolatilization. For example, metal-hyperaccumulating plants and microbes with unique abilities to tolerate, accumulate, and detoxify metals and metalloids represent an important reservoir of unique genes that could be transferred to fast-growing plant species for enhanced phytoremediation. There is also a need to develop new strategies to improve the acceptability of using genetically engineered plants for phytoremediation.

Keywords Phytoremediation · Phytovolatilization · Selenium · Constructed wetlands · Hyperaccumulators

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Introduction

Heavy metals and metalloids, such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), and selenium (Se), are released into the environment by mining, industry, and agriculture, threatening environmental and human health [36, 50]. In the United States alone, more than 50,000 metal-contaminated sites await remediation, many of them Superfund sites [24]. Due to the acute toxicity of these contaminants, there is an urgent need to develop low-cost, effective, and sustainable methods to remove them from the environment or to detoxify them. Plant-based approaches, such as phytoremediation, are relatively inexpensive since they are performed in situ and are solar-driven [57–59]. In this review, we discuss specific advances in plant-based approaches for the remediation of contaminated water and soil. Dilute concentrations of trace element contaminants can be removed from large volumes of wastewater by constructed wetlands. Plants play an important but indirect role in that they supply fixed carbon and other nutrients to rhizosphere microbes responsible for the uptake and detoxification of contaminants. We discuss the potential of constructed wetlands for use in remediating agricultural drainage water and industrial effluent, as well as concerns over their potential ecotoxicity.

Plants play a more direct role in remediation of upland soil. Plants may be used to accumulate metals/metalloids in their harvestable biomass (phytoextraction). Plants can also convert and release certain metals/metalloids in a volatile form (phytovolatilization) [23]. We discuss how genetic engineering has been used to develop plants with enhanced efficiency for phytoextraction and phytovolatilization. Metal-hyperaccumulating plants [6] and microbes with unique abilities to tolerate, accumulate, and detoxify metals and metalloids represent an important reservoir of unique genes that could be transferred to fast-growing plant species for enhanced phytoremediation [18]. Improved analytical techniques are being used to elucidate the mechanisms by which plants detoxify trace element contaminants. This knowledge is crucial for optimizing new genetic engineering strategies. Finally, new strategies are required to improve the acceptability of using genetically engineered plants for remediation projects.

Advances in plant-based approaches to trace element remediation

Constructed wetlands

Constructed wetlands have been used as a low-cost treatment to remove a wide-range of waterborne contaminants from polluted waters such as municipal wastewater and effluents from electricity generating facilities and oil refineries in the United States and Europe [7, 34, 76]. They comprise a complex ecosystem of plants, microbes, and sediment that together act as a biogeochemical filter, efficiently removing dilute contaminants from very large volumes of wastewater. The anoxic environment and organic matter production in wetlands promote biological and chemical processes that transform contaminants to immobile or less toxic forms [28]. Plants support microbially mediated transformations of contaminants by supplying fixed-carbon as an energy source for bacteria and by altering the chemical environment in their rhizosphere [51, 64]. Plants also take up and accumulate metals and metalloids in their tissues [53, 75, 79]. At this point, some metals and metalloids can be metabolized to non-toxic and/or volatile forms, which may escape the local ground ecosystem by release to the atmosphere [31, 41].

In 1996, an experimental wetland was constructed at the Tulare Lake Drainage District (TLDD) in the San Joaquin Valley (Calif.). Its purpose was to evaluate the potential of constructed wetlands for the removal of Se from agricultural irrigation drainage water. Ten individual cells were tested, either unvegetated or vegetated singly or with a combination of sturdy bulrush [*Schoenoplectus robustus* (Pursh) M.T. Strong], Baltic rush (*Juncus balticus* Willd.), smooth cordgrass (*Spartina alterniflora* Loisel.), rabbitfoot grass [*Polypogon monspeliensis* (L.) Desf.], saltgrass [*Distichlis spicata* (L.) Greene], cattail (*Typha latifolia* L.), tule [*Schoenoplectus acutus* (Muhl. ex Bigelow) Á. Löve& D. Löve], and widgeon grass (*Ruppia maritima* L.) [28]. On average, the wetland cells removed 69% of the total Se mass from the inflow. Vegetated wetland cells removed Se more efficiently than the unvegetated cell, without significant differences among vegetated cells [41]. An important objective of the TLDD wetland project was to determine if Se concentrations in drainage water could be reduced to less than 2 μg/L before disposal into evaporation ponds, the overall goal being to minimize toxic effects of Se on aquatic biota and waterfowl in the ponds. Although the Se concentrations in the outflow were significantly lower than those in the inflow for all cells, the goal of 2 μg Se per liter in the outflow was not reached [28].

Microcosm experiments provide an initial means of evaluating the remediation potential of a constructed wetland with a greater degree of experimental control, less cost, and substantially reduced environmental risk than a study of the wetland itself. Such a microcosm study was used to evaluate the potential of constructed wetlands to remediate effluent containing highly toxic selenocyanate (SeCN), As, and boron (B) generated by a coal gasification plant [70]. The concentrations of these contaminants were several orders of magnitude higher than those normally treated by constructed wetlands. The microcosms removed 79% Se, 67% As, 57% B, and 54% CN mass, significantly reducing the toxicity of the effluent. Because cattail (*Typhia latifolia* L.), *Thalia dealbata* Fraser ex Roscoe, and rabbitfoot grass (*P. monspeliensis* L. Desf.) showed no growth retardation when supplied with the contaminated wastewater, constructed wetlands planted with these species show particular promise for remediating this highly toxic effluent.

Although constructed wetlands offer a less expensive alternative to other water-treatment methods, the approach needs to be optimized to enhance efficiency and reproducibility, and reduce ecotoxic risk. Most of the contaminants removed from the waste-stream are immobilized in the sediment. For example, in the microcosm experiment discussed above, the sediment contained 63% of the Se, 51% of As, and 36% of B, while only 2–4% was accumulated in plant tissue [70]. In the TLDD wetland, 41% of the supplied Se left the wetland; the remaining 59% was retained in the wetland cell, partitioned between the surface sediment (0–20 cm; 33%), organic detrital layer (18%), fallen litter (2%), standing plants (< 1%), and standing water (< 1%) [28]. The Se in the agricultural drainage water entering the TLDD wetland was predominantly in the form of selenate (95% [28]); it was reduced in sediment to a mixture of elemental Se (45%), organic Se (40%), and selenite (15% [41]). Although elemental Se is essentially non-toxic, some selenite and some species of organic Se are more toxic than selenate. There is concern that, since Se concentrations in the organically rich surface sediments increased over time, that this Se could eventually enter the aquatic food chain and exert ecotoxic effects.

Biological selenium volatilization

One very important way of increasing the efficiency of Se removal and decreasing Se ecotoxicity of wetlands is to enhance Se volatilization by plants and microbes. Because of the chemical similarity of sulfur (S) and Se, plants and microbes are able to take up inorganic and organic forms of Se and metabolize them to volatile forms via the S assimilation pathway. Biological volatilization has the advantage of removing Se from a contaminated site in relatively non-toxic forms, such as dimethylselenide (DMSe), which is 500–700 times less toxic than SeO_4^{-2} or SeO_3^{-2} [42, 69]. Although the volatilized Se may eventually be redeposited in other areas, this is not a problem in California where much of the state is deficient in Se with respect to the nutrition of animals, which require Se in low concentrations [55].

The extent of Se volatilization is highly dependent upon a number of environmental factors, such as the composition of the microbial community, choice of macrophytes, Se speciation, organic matter amendment, and other physiochemical conditions [3, 26, 62, 74, 75]. Selenium volatilization rates increase with increasing ambient temperature [41]. Not only do higher temperatures increase the vapor pressure of volatile DMSe, they also stimulate the metabolic activity of plants and microbes. The chemical form of Se present in the inflow also affects the extent of Se volatilization [41]. This is because biological metabolism of Se from inorganic forms, the predominant Se forms in most waste streams, to volatile DMSe is slowed by certain rate-limiting enzymatic steps. For example, Se-removal is more efficient from selenite-dominated water than selenate-dominated water [31, 75], because the reduction of selenate to selenite is often a rate-limiting step. Certain plant and microbe species may not have the same rate-limitations on Se metabolism as others. For instance, microbes living in the rhizosphere of rabbitfoot grass, the highest volatilizing cell, appear to efficiently metabolize Se such that 77% of the Se was present in organic forms. Selenium volatilization may be enhanced through managing hydrological conditions, judicious choice of plant species, altering carbon availability to promote microbial activity, or seeding with microbes and microalgae [41]. Another possible approach is to genetically manipulate microbes, algae, or plants to increase their output of volatile Se.

Genetic modification of plants to enhance phytoremediation

Recent research in our laboratory has shown that genetic modification of plants can increase their phytoremediation efficiency [37, 56]. Identifying candidate genes for transfer and/or overexpression is critical. One useful approach is to overexpress enzymes catalyzing rate-limiting steps; for example, ATP sulfurylase (APS), which facilitates the reduction of selenate to selenite, is rate limiting with respect to the production of reduced, organic Se compounds [18]. Indian mustard plants overexpressing APS have increased tolerance and accumulation of selenium [53]. However, APS Indian mustard does not volatilize more Se than wild type [66]. This is likely due to additional downstream rate-limiting steps in the S/Se assimilation pathway. Indeed, Se volatilization rates from Indian mustard are similar from selenocysteine (SeCys) and selenite, while volatilization from selenomethionine (SeMet) is many-fold faster [18]. This suggests the involvement of a rate-limiting step in the synthesis of SeMet from SeCys. To test this hypothesis, Indian mustard plants overexpressing cystathionine-γ-synthase (CGS) were developed. The CGS Indian mustard had enhanced tolerance to selenite and volatilized Se two to three times faster than wild type, while at the same time accumulating less Se in roots and shoots [66].

Multiple metals

Most metal-polluted sites contain marginal, aged soil polluted with mixtures of metals. An optimal phytoremediation strategy would be to use plants with enhanced phytoextraction capacity for an array of metals. Such plants would necessarily have a broad ability to take up and tolerate metals using a general protective mechanism. Wangeline et al. [68] recently evaluated the ability of APS Indian mustard to tolerate and accumulate 12 metals. Mature APS Indian mustard successfully removed more Cd, chromium (Cr), copper (Cu), manganese (Mn), Pb, and zinc (Zn) from polluted soil than wild type by accumulating higher shoot concentrations. APS seedlings, exposed to the contaminants continuously from seed, tolerated arsenate [As(V)], arsenite [As(III)], Cd (2-fold better), Hg, and Zn significantly better than wild type. The APS seedlings also had up to 2.5-fold higher shoot concentrations of As(V), As(III), Hg, molybdenum, Pb, and vanadium.

The APS Indian mustard may tolerate metals better because it has higher glutathione (GSH) concentrations than wild type [53]. Glutathione (γ-Glu-Cys-Gly) plays an important role in heavy-metal detoxification. The GSH can directly form GSH-metal complexes [40] and, as part of the active oxygen-scavenging system [46], can protect the plant cell from oxidative stress [27, 67]. The GSH is also the direct precursor of phytochelatins (PCs), which bind, detoxify, and sequester metal ions to the vacuole [54, 61, 73].

Previously, the overexpression of γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS) in Indian mustard was shown to confer increased tolerance to Cd in solution culture [77, 78]. This tolerance was correlated with 1.5–2.5 higher levels of GSH and PCs. Transgenic APS, ECS, GS, and WT Indian mustard, as well as a mixture of wild grasses, were evaluated for their ability to phytoextract contaminants from a multiple-metal contaminated soil collected at a USEPA Superfund site near Leadville (Co.) [4]. The APS, ECS, and GS lines removed significantly more metals from soil than WT. The ECS and GS are the best candidates for phytoextraction as they had the highest concentrations of metals in their harvestable, aboveground biomass. Both ECS and GS had 1.5- to 2-fold higher concentrations of Cd and Zn in their shoots than wild type. The ECS also successfully accumulated 2.4- to 3-times as much Cr, Cu, and Pb as wild type. Metal concentrations in APS shoots were not higher than in wild type; it is possible that the APS lines stored more metals in their roots. Similarly, grasses appear to be better for metal phytostabilization since the majority of the metals are in the roots rather than in the easily harvestable shoots.

Thus far, genetic engineering approaches have resulted in 2- to 3-fold higher metal concentration per plant. Some strategies have focused on improving metal uptake by overexpressing metal-transporter proteins [1, 33, 59, 65]. Other researchers have developed transgenic plants that overproduce metal-chelators such as citrate [17], metallothioneins [25, 32], and ferritin [29]. Other strategies to improve the efficiency of phytoextraction have practical limitations. Mixing contaminated soils with clean soils or compost, or altering pH, reduces metal toxicity, allowing for more vigorous plant growth, but at the same time decreases metal bioavailability and, in turn, metal concentrations in plant shoots [44]. Although adding metal-chelating agents, such as EDTA, can greatly increase metal uptake by plants [5], the increased bioavailability may lead to enhanced metal leaching to groundwater [43].

Hyperaccumulators

Some plants naturally hyperaccumulate metals, meaning that they are able to accumulate metals to ppm levels in the order of thousands in their shoots. Hyperaccumulating plants have been identified for a number of metals [9, 43]. The phytoremediation efficiency of most metal hyperaccumulators is limited by their slow growth rate and low biomass. For example, *Thlaspi caerulescens*, a Cd and Zn hyperaccumulator, successfully removed 43% Cd and 7% Zn from an industrially contaminated soil, but it took 391 days [43]. Using genetic engineering we should be able to enhance phytoremediation potential by transforming fast-growing host plants with key genes from natural hyperaccumulators.

One such gene is selenocysteine methyltransferase (SMT), cloned from the Se hyperaccumulator *Astragalus bisulcatus* [48]. SMT converts the amino acid SeCys to the non-protein amino acid (MetSeCys). By doing so, it diverts the flow of Se from the Se amino acids that may otherwise be incorporated into protein, leading to alterations in enzyme structure and function and toxicity [48]. Transgenic plants overexpressing SMT show enhanced tolerance to Se, particularly selenite, and produced 3- to 7-fold more biomass than wild type and 3-fold longer root lengths [37]. The SMT plants accumulated up to 4-fold more Se than wild type, with higher proportions in the form of MetSeCys. Additionally, SMT Arabidopsis and SMT Indian mustard volatilized Se two to three times faster when treated with SeCys and selenate, respectively.

Use of microorganisms in the remediation of toxic metals

The diversity and adaptability of microorganisms allows them to thrive in harsh, toxic environments where higher plants are unable to grow. As such, microbes represent a potential reservoir of important genes involved in metal detoxification. Highly efficient phytoremediating plants could be generated that overexpress microbial genes [19]. Many such microorganisms have been found, but much remains to be learned at the molecular level.

One promising strategy to elucidate microbial hypertolerance and hyperaccumulation mechanisms is to compare natural cultures with adapted cultures. *Euglena gracilis*, a free-living, unicellular, fast-growing protist, is such an organism, able to hyperaccumulate Cd [20, 46] and tolerate relatively high concentrations of other heavy metals [8, 16, 21]. Interestingly, pretreatment of *E. gracilis* cultures with 1.5 $HgCl_2$ for 60 generations led to a culture with permanently increased Cd tolerance and accumulation [2]. The genetic and biochemical basis for this adaptation is an interesting target for genetic engineering. The Hg-pretreated population has higher concentrations of Cd (43–79%) along with citrate, cysteine, and GSH in the mitochondria, suggesting that compartmentation of Cd bound to Cd-binding metabolites could be an important aspect of the resistance mechanism. However, since the levels of PCs were the same in both cultures, the additional resistance and accumulation observed in the Hg-pretreated cultures did not involve PCs [2]. The Hg-pretreated culture may also have altered expression and activity levels of key transcription factors, metal transporters, and other enzymes involved in Cd detoxification. Although the mechanism of Cd tolerance and accumulation in the Hg-pretreated *E. gracilis* has not been fully elucidated, it remains a worthy bioremediation candidate for removing Cd and other metals from polluted waters. Promisingly, lactate-grown Hg-pretreated cells removed 45% Cd from the culture medium [2].

In another example, a single-celled freshwater microalgae (*Chlorella* sp.) is interesting because of its ability to efficiently reduce selenate [49]. In fact, in just 24 h, 87% of the selenate accumulated had been converted to intermediate organic compounds. This capacity to efficiently reduce Se may have evolved in microalgae because their large surface to volume ratio means that their Se uptake rates can be relatively high while space available for storage of toxic Se compounds is small. Since high rates of accumulation have toxic effects on long-term development [26], the ability to convert selenate to DMSe could be a big advantage. The potential of these microalgae for bioremediation is limited, however, by the fact that uptake of selenate is strongly inhibited by the presence of sulfate in the medium. Without sulfate, the *Chlorella* sp. was able to remove 90% of supplied selenate through accumulation and volatilization. These high rates were not observed in the presence of 1 mM sulfate, where only 1.8% of Se was volatilized. Without sulfur, the *Chlorella* had 2.6 times higher sulfate transporter activity, which most likely leads to the higher rates of selenate uptake. It has previously been observed that sulfate deprivation can lead to increased activity of enzymes involved in sulfate uptake and reduction [39, 63, 71, 72]. However, since the action of selenate reduction does not appear rate-limiting in this microalga, transforming plants with the *Chlorella* ATP sulfurylase gene may be a useful means to increase Se volatilization rates in higher plants.

Analytical techniques

The successful use of genetic engineering to optimize plants for phytoremediation depends on a thorough knowledge of the uptake and metabolism of trace element contaminants of interest. Elucidating the genetic and biochemical basis for metal/metalloid tolerance and accumulation strategies is often hampered by the difficulty in determining the levels of, and positively identifying, intermediate metabolites and complexes [26]. Fortunately, technologies are being developed and improved that should shed new light on these metabolic pathways. For example, recent work with HPLC-ICP-MS and HPLC-ESI-MS has identified selenomethylmethionine (SeMM) as the predominant Se species in *Brassica juncea* roots supplied with SeMet [30]. This work provides chemical evidence for the view that SeMet is methylated to SeMM [62]. Since roots are the primary site of Se volatilization [72], cleavage of SeMM appears to directly produce volatile DMSe. Similar techniques have also shown promise in elucidating the fate of As in plants, which is less well understood. As(V), an analog of phosphate, is readily taken up by plants and reduced to the more toxic and less bioavailable form, As(III) [52]. However, As(III) is more readily detoxified because of its affinity for thiols [60]. Indeed, co-expression of two bacterial genes, an arsenate reductase and ECS, to produce more GSH and PCs, in Arabidopsis resulted in plants with greater tolerance to As(V) [22]. Although thiol-PC complexes have been identified in As-treated plants, direct evidence for As-PC complexes is lacking. In a recent study, HPLC-ICP-MS was used to analyze As metabolites in As-treated Indian mustard. Arsenic species were found bound to thiols. The ESI-Q-TOF results strongly suggest the presence of As bound to PC2, PC3, and PC4 [47].

Chloroplast engineering

After all the work involved in identifying key genes, transforming plants, and evaluating their phytoremediation potential in laboratory and greenhouse experiments, there are still regulatory barriers to overcome in getting transgenic plants in the field, remediating contaminated sites. Such constraints have spurred researchers to innovate new methods of creating transgenic plants that will be more palatable to the public and pose less potential risk of hybridizing with nearby plants or adversely affecting wildlife. One such technique is the use of chloroplast transformation, the use of which prevents the escape of transgenes via pollen to related weeds and crops [10, 11, 12]. This method was recently used to stably integrate the bacterial merAB operon into the chloroplast genome of tobacco. The resulting plants were substantially more resistant to highly toxic organic mercury, in the form of phenylmercuric acetate, than wild type [56]. Previously, all attempts to genetically engineer plants with improved phytoremediation had been based on transformation of the nuclear genome. Other important advantages of chloroplast transformation include the fact that codon optimization is not required to improve expression of bacterial transgenes [15, 35, 38], very high levels of transgene expression (up to 46% w/w of total protein) [15], absence of gene silencing [38], absence of positioning effect [14], ability to express multiple genes in a single transformation event [13, 15], and sequestration of foreign proteins in the organelle, preventing adverse interactions with cytoplasm [14, 45].

Conclusions

Recent research has shown that phytoremediation can be an effective method for removing and detoxifying heavy metals and metalloids such as Cd, Se, and As from contaminated soil and water. The identification of unique genes from natural Se hyperaccumulators and their subsequent transfer to fast-growing species is another promising approach as demonstrated by our recent success with SMT transgenic plants. Microbial genomes may provide another reservoir of candidate genes for use in genetic engineering strategies. Advances in optimizing plants for phytoremediation will depend on gaining new knowledge about the fate and transport of metals/metalloids in plants and innovative technologies to improve the acceptability of transgenic organisms for phytoremediation.

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