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Phytoremediation: novel approaches to cleaning up polluted soils

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Environmental pollution with metals and xenobiotics is a global problem, and the development of phytoremediation technologies for the plant-based clean-up of contaminated soils is therefore of significant interest. Phytoremediation technologies are currently available for only a small subset of pollution problems, such as arsenic. Arsenic removal employs naturally selected hyperaccumulator ferns, which accumulate very high concentrations of arsenic specifically in above-ground tissues. Elegant two-gene transgenic approaches have been designed for the development of mercury or arsenic phytoremediation technologies. In a plant that naturally hyperaccumulates zinc in leaves, approximately ten key metal homeostasis genes are expressed at very high levels. This outlines the extent of change in gene activities needed in the engineering of transgenic plants for soil clean-up. Further analysis and discovery of genes for phytoremediation will benefit from the recent development of segregating populations for a genetic analysis of naturally selected metal hyperaccumulation in plants, and from comprehensive ionomics data – multi-element concentration profiles from a large number of *Arabidopsis* mutants.

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Introduction

Pollution with metals and xenobiotics is a global environmental problem that has resulted from mining, industrial, agricultural and military practices [1]. Many pollutants accumulate in the food chain and threaten human health. In wealthy industrialized countries contamination is often highly localized, and the pressure to use contaminated land and water for agricultural food production or for human consumption, respectively, is minimal. However, soil and water contamination is widespread in Eastern Europe, and is increasingly recognized as dramatic in large parts of the developing world, primarily in India [2] and China [3].

Although the avoidance of pollution should certainly be the primary objective, this principle has not generally been followed in the past. The clean-up of polluted soils and waters is very costly, and for many pollutants no feasible technologies are yet available. Plants possess highly efficient systems that acquire and concentrate nutrients as well as numerous metabolic activities, all of which are ultimately powered by photosynthesis. The term phytoremediation has been coined for the concept that plants could be used for low-cost environmental clean-up and this has attracted considerable attention in the past decade [4–6]. During the 1980s, the US Government initiated a large program for the development of environmental clean-up technologies (The Comprehensive Environmental Response, Compensation, and Liability Act or Superfund), which has accelerated the growth of a new productive research field worldwide. As a result, researchers have come to learn that the development of phytoremediation technologies requires a thorough understanding of the underlying processes at the genetic, molecular, biochemical, physiological and agronomic levels.

This is a review of recent developments in basic and applied research relevant for the plant-based clean-up of soils contaminated with trace metals and metalloids.

Available phytoremediation approaches and technologies

There are two distinct strategies in soil phytoremediation, phytostabilization and phytoextraction [5]. The former is used to provide a cover of vegetation for a moderately to heavily contaminated site, thus preventing wind and water erosion. Plants suitable for phytostabilization develop an extensive root system, provide good soil cover, possess tolerance to the contaminant metals, and ideally immobilize the contaminants in the rhizosphere. Phytostabilization is often performed using species from plant communities occurring on local contaminated sites.

The most effective but also technically the most difficult phytoremediation strategy is phytoextraction. It involves the cultivation of tolerant plants that concentrate soil contaminants in their above-ground tissues. At the end of the growth period, plant biomass is harvested, dried or incinerated, and the contaminant-enriched material is deposited in a special dump or added into a smelter. The energy gained from burning of the biomass could support the profitability of the technology, if the resultant fumes can be cleaned appropriately. For phytoextraction to be worthwhile, the dry biomass or the ash derived from above ground tissues of a phytoremediator crop should

contain substantially higher concentrations of the contaminant than the polluted soil. To achieve this, several bottleneck processes limiting trace element accumulation in plants have to be resolved, including the mobilization of poorly available contaminant trace elements in the soil, root uptake, symplastic mobility and xylem loading, as well as detoxification and storage inside the shoot [7••].

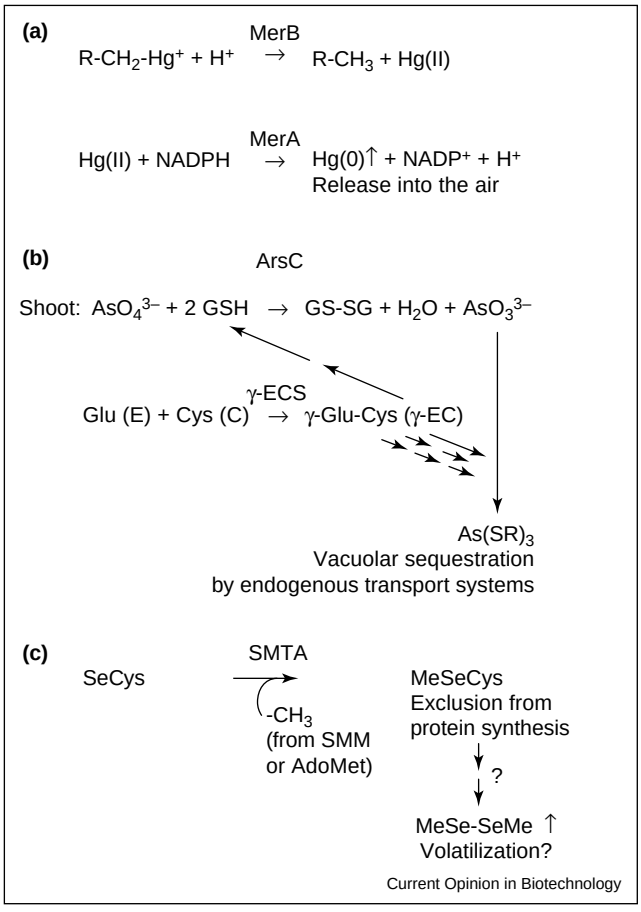
Metal hyperaccumulator plants are naturally capable of accumulating trace elements, primarily Ni, Zn, Cd, As or Se, in their above-ground tissues, without developing any toxicity symptoms [8]. The concentrations of these elements in dry leaf biomass are usually up to 100-fold higher than the concentrations in the soil [4]. Characteristically, the shoot:root ratio of concentrations of the hyperaccumulated trace element is above unity [9,10••,11••]. Although metal hyperaccumulator plants therefore appear to have ideal properties for phytoextraction, most of these plants produce little biomass and are thus primarily used as model organisms for research purposes. The biomass production of a few hyperaccumulator plants has been judged sufficient for phytomining (the use of plants to extract and concentrate inorganic substances of economic value from soils) [12] or phytoremediation; for example, the brake fern *Pteris vittata* accumulated up to 7500 $\mu g\, g^{-1}$ As on a contaminated site [13], without showing toxicity symptoms. One fern cultivar is available commercially for As phytoextraction (http://www.edenspace.com/index.html), and promising field trials have been conducted [14•]. Chelator-assisted phytoremediation is also available commercially. This approach is based on the application of chelators such as EDTA (ethylenediamine tetraacetate) to solubilize poorly available metals (e.g. lead) in the soil, followed by the largely passive accumulation of metal complexes in plant shoots with the transpiration stream [5].

Because of their extensive root system, their high biomass and low-input cultivation, trees are attractive phytoremediators. Metal accumulation is generally poor, however, especially in the wood. Recent genome sequencing, the development of genomics tools, and the ease of genetic transformation of poplar might open up new avenues for the use of trees in phytoremediation [7••].

Breakthroughs in phytoremediation: novel transgenic approaches

A variant of phytoextraction is phytovolatilization, whereby the contaminant is not primarily accumulated in above-ground tissues, but is instead transformed by the plant into a volatile compound that is released into the atmosphere. In some groundbreaking work, the detoxification of highly toxic organomercurial compounds and subsequent volatilization of elemental mercury were successfully engineered in plants. For this purpose, modified bacterial *merA* and *merB* genes were introduced into several plant species including *Arabidopsis*, tobacco and poplar [15,16]. The MerB protein is an organomercurial lyase that catalyzes the removal of Hg(II) from organic mercury compounds (e.g. methylmercury; Figure 1a). The MerA protein, a mercuric ion reductase, reduces Hg(II) to the volatile elemental form Hg(0) using NADPH as an electron donor. Transgenic plants transformed with both *merA* and *merB* were remarkably tolerant to organic mercury compounds and Hg(II). With respect to soil clean-up, the approach is still limited by a generally very low solubility of mercurial compounds in the soil solution. The reaction catalyzed by MerB limited the performance of the plants transformed with *merA* and *merB*. Enhanced specific *in planta* MerB activities were achieved by targeting the MerB protein to the cell wall or to the endoplasmatic reticulum, where the apolar organomercurials are believed to accumulate [17]. Targeting the MerB enzyme to the cell wall is an example for the transfer of a phytoremediation-active compound to the

Figure 1



Chemical reactions in transgenic phytoremediation. **(a)** The detoxification and volatilization of organomercurials. **(b)** Arsenate detoxification and immobilization. **(c)** Selenite detoxification. Gene names are explained in the text. AdoMet, *S*-adenosylmethionine; GSH, glutathione (reduced); GS-SG, oxidized glutathione; Me, methyl; SMM, *S*-methylmethionine.

extracytoplasmic surface of plant cells. Similar approaches may help to solve the problem of low uptake rates of contaminants by plant cells, which still limits all emerging phytoextraction technologies. In order for transgenic phytoremediation to become more widely accepted, the development and implementation of biological encapsulation strategies will be of high value. Biological encapsulation is a term to describe procedures that dramatically decrease the probability of the spread of a transgene from a genetically modified crop to natural plant populations, for example by introducing the transgene into the chloroplast genome instead of the nuclear genome [18].

A second example further illustrates how well-conceived plant engineering strategies have been designed based on microbial detoxification pathways. In plants, As is taken up as arsenate (AsO_4^{3-}) by phosphate uptake systems [19]. *Arabidopsis* plants were generated that overexpress an *Escherichia coli* arsenate reductase ArsC (Figure 1b), which reduces arsenate to arsenite (AsO_3^{3-}) using glutathione as the electron donor [20••]. Because arsenite possesses a high affinity for thiol groups and is thus likely to be bound and immobilized inside the cells where it is formed, the *arsC* gene was introduced downstream of the soybean *SRS1* (small subunit of Rubisco 1) promoter, which confers shoot-specific, light-induced expression. The rationale behind this was to keep As immobilization in the root to a minimum in order not to interfere with the translocation of As to the shoot. The resulting transgenic plants were hypersensitive to arsenate, which was attributed to the depletion of the glutathione pool and to the high affinity of arsenite for binding to protein thiol groups. *Arabidopsis thaliana* plants transformed with *arsC* and an additional second transgene, which encoded the *E. coli* γ-glutamylcysteine synthetase (γ-ECS) expressed under a constitutive actin (*ACT2*) promoter, were more tolerant to arsenate than the wild-type or single γ-*ECS* transformants. Double-transformant lines accumulated up to 3.4-fold higher shoot As concentrations than wild-type plants on agarose-based media (Table 1). The efficiency of this approach in As phytoextraction remains to be tested on contaminated soils. The expression of ArsC in plants also increases Cd tolerance and accumulation [21••].

Alternatively, strategies to engineer plants for phytoextraction can be designed based on naturally selected metal hyperaccumulation mechanisms. Transgenic *A. thaliana* plants expressing a selenocysteine methyltransferase (SMTA) isolated from the Se hyperaccumulator *Astragalus bisulcatus* accumulated methylselenocysteine and contained up to eightfold higher Se concentrations than wild-type plants, when grown on a soil supplemented with selenite (SeO_3^{2-}; see Figure 1c and Table 1) [22•]. However, the transgene did not confer tolerance to or enhanced accumulation of Se when the metalloid was provided in the predominant chemical form present in soils, selenate (SeO_4^{2-}). The next important step will be to enhance the rate-limiting conversion of selenate to selenite by the plants. In *Brassica juncea* seedlings expressing the same SMTA protein, another research group observed slight selenate tolerance and an approximately fivefold increase in Se accumulation when plants were exposed to selenate [23•]. They report slightly enhanced dimethyl diselenide volatilization from mature transgenic *B. juncea* plants exposed to selenite or selenate.

Novel insights from basic research

In the past few years, substantial progress has been made in elucidating the mechanistic basis of the homeostasis and detoxification of metals and metalloids in plants. Insights into the mechanisms of naturally selected plant trace element tolerance and hyperaccumulation have also been gained. On the basis of this work, improved engineering strategies can now be devised. For example, the current transgenic approach in As phytoremediation (described above) could be combined with the expression of phosphate transporters, which appear to be the major uptake pathway for the chemically similar arsenate anions

Table 1

Summary of genes introduced into plants and the effects of their expression on trace element accumulation in shoots.

Gene(s)	Product/function	Source	Target	Expression	Maximum effect[a]	Root medium
arsC	Arsenate reductase	*E. coli*	Tobacco	Shoot	1.4-fold Cd	Hydroponic
arsC and γ-*ECS*	Arsenate reductase and γ-EC synthetase	*E. coli* *E. coli*	*Arabidopsis*	Shoot and constitutive	Threefold As from AsO_4^{3-}	Agar
SMTA	Selenocysteine Methyltransferase	*A. bisulcatus*	*Arabidopsis*	Constitutive	Eightfold Se from SeO_3^{2-}	Amended soil
SMTA	Selenocysteine methyltransferase	*A. bisulcatus*	*Brassica juncea*	Constitutive	[b]Fivefold from SeO_4^{2-} [c]Volatilization	Agar Sand
YCF1	Vacuolar sequestration of GSH–conjugates	*S. cerevisiae*	*Arabidopsis*	Constitutive	1.4-fold Pb, 1.5-fold Cd	Gravel/hydroponic
HMA4	Cellular metal efflux	*A. thaliana*	*Arabidopsis*	Constitutive	[d]Twofold Zn, 1.4-fold Cd	Hydroponic

[a]The 'maximum effect' is the maximum concentration increase observed in shoot dry biomass, relative to control plants not expressing the transgene. For a summary of earlier data see [6]. [b]Value likely to refer to concentration in whole seedlings. [c]The volatilized compound is dimethyl diselenide (MeSe-SeMe). [d]Data were from a single transformant line only.

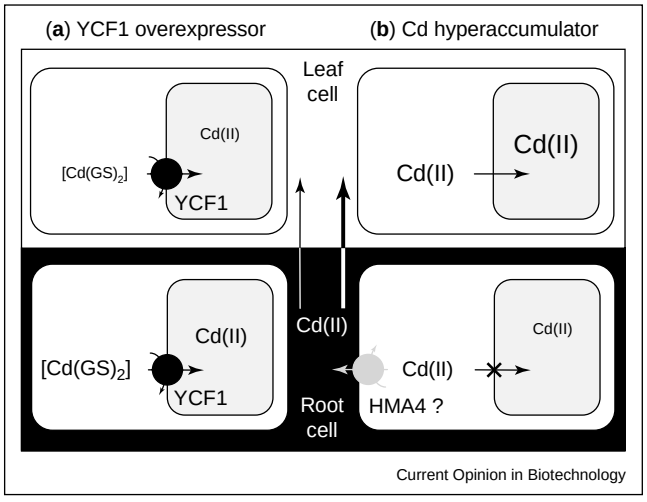
in the roots. There may even be the possibility to isolate mutant or variant phosphate uptake systems with an enhanced affinity for arsenate. The root systems of the As hyperaccumulating fern *P. vittata* possess a higher affinity for arsenate uptake than those of a related non-accumulator fern species [24•]. A suppression of endogenous arsenate reduction in roots may serve to enhance root-to-shoot translocation of As [20••,25], and the overexpression of a glutathione–conjugate pump in the leaves could increase the capacity for detoxification of As(III)–glutathione complexes in the vacuole. Finally, phytochelatins [26] — metal-chelating molecules of the general formula $(\gamma\text{-GluCys})_n\text{Gly}$ (where $n = 2$ to 11) synthesized by the ubiquitous plant enzyme phytochelatin synthase [27–29] — are known to contribute to As detoxification in As-sensitive [30], As-tolerant [31], and As hyperaccumulator plants [32•,33•]. It is interesting to note that the ability to synthesize phytochelatins reduces the extent of cellular As tolerance conferred by expression of the cellular arsenite efflux transporter ScAcr3p in *Schizosaccharomyces pombe* [34•].

Genomics approaches are increasingly being employed in phytoremediation-related research. Recently, powerful 'ionomics' screens have been initiated [35••,36]. These involve unbiased multi-element profiling in *A. thaliana* mutant populations to identify mutants with altered elemental composition of rosette leaves. These and similar screens will serve to identify novel genes with a key role in metal accumulation. Using *A. thaliana* oligonucleotide microarrays, cross-species transcript profiling was employed to compare *A. thaliana* and a closely related Zn- and Cd-tolerant Zn hyperaccumulating accession of *Arabidopsis halleri* [10••,11••]. This study confirmed on a genome-wide scale what had been observed earlier for single genes in several different hyperaccumulator species [37–39,40••,41•]: several metal homeostasis genes are constitutively expressed at very high levels in metal hyperaccumulators, when compared with closely related non-accumulators. In *A. halleri*, these include genes encoding several membrane transporter proteins of the ZRT-IRT-related protein (ZIP) family (zinc-regulated transporter, iron-regulated transporter) [42], which are likely to mediate zinc influx into the cytoplasm, and two isoforms of the enzyme nicotianamine synthase. These genes are expressed at low levels or only upregulated under conditions of zinc deficiency in *A. thaliana*. Other genes found to be constitutively expressed at high levels in the hyperaccumulator species *A. halleri* encode membrane transport proteins of the HMA (heavy metal P-type ATPase) family of P_{1B}-type metal ATPases, which are potentially involved in metal export into the apoplast for metal detoxification or for root-to-shoot metal translocation in the xylem. Finally, the transcript analyses implicated a metal tolerance protein 1 (MTP1)-like protein of the so-called cation diffusion facilitator family, which contributes to the sequestration of Zn ions primarily in leaf vacuoles (see below). Global transcript analyses of metal hyperaccumulation and associated tolerance in *A. halleri* are thus consistent with an involvement of several major genes, as supported by preliminary genetic analyses [43•,44,45•]. Collating the available data shows that in several cases homologues of the same *A. thaliana* gene are overexpressed in various hyperaccumulator species: *A. halleri* (hyperaccumulating primarily Zn), *Thlaspi caerulescens* (Zn), a different accession of *T. caerulescens* (Cd, Zn), and *Thlaspi goesingense* (Ni). Together with site-directed and random mutagenesis approaches, the comparison of amino acid sequences of metal transporters from several hyperaccumulator species might be a starting point in the identification of determinants of differential metal specificities [46]. For example, a sequence comparison of the IRT1 protein from *A. thaliana* and related proteins from *T. caerulescens* accessions differing in their Cd accumulation might help to identify regions and amino acids that specify Cd transport or exclusion [47•].

Recent work (see also above) suggests a role for nicotianamine as a chelator conferring cellular tolerance to Ni [48•] and Zn [10••,11••] in a Zn hyperaccumulating accession of *T. caerulescens* and in *A. halleri*, respectively. Nicotianamine synthase catalyzes the biosynthesis of nicotianamine from three molecules of *S*-adenosylmethionine, releasing three molecules of *S*-methyladenosine as a by-product [49–51]. Nicotianamine is a non-proteinogenic amino acid metal chelator that is ubiquitous in higher plants. Each nicotianamine molecule is able to chelate a metal ion with high affinity via three carboxylate groups and the free electron pairs of a maximum of three amino groups [52]. In the past, nicotianamine was identified as a chelator essential for the intercellular and long-distance transport of Fe, Zn and Cu in plants. It is also an intermediate in the biosynthesis of phytosiderophores, which participate in the mobilization of rhizosphere Fe(III) by roots of graminaceous plants [53].

As the molecular inventory of metal hyperaccumulation is gradually reaching completion, it is vital to determine the site of action and the precise function of the implicated proteins. Researchers have begun to generate the tools required for a genetic analysis of metal hyperaccumulation. For example, an F2 and a backcross 1 population segregating for metal tolerance and hyperaccumulation, respectively, have been generated by crossing *A. halleri* with the closely related non-tolerant non-accumulating *Arabidopsis lyrata* ssp. *petraea* [44,45•]. One such population has been used to demonstrate that two genes of *A. halleri*, which are closely related to the *A. thaliana MTP1* gene and encode almost identical membrane transport proteins capable of transporting Zn^{2+} into the vacuole (see above), co-segregate with zinc tolerance [40••]. This highlights the major importance of the central vacuole of plant cells as a compartment for metal detoxification

Figure 2



Model of Cd partitioning. **(a)** Cd partitioning in YCF1-overexpressing transgenic *A. thaliana*. Cd^{2+} enters plant cells primarily through non-specific transport systems (not shown). Inside the cell a fraction of Cd(II) binds to thiol groups of glutathione (GSH) molecules as ligands. The complex is a substrate for YCF1. In root vacuolar membranes, YCF1 partitions Cd(II) into the vacuole and thus generates a Cd(II) sink in root cells. This may restrict the amount of Cd(II) that is available for movement into the shoot (thin arrow). Consequently, Cd(II) influx into leaf cells is reduced, and vacuoles in the shoot may not be the primary Cd(II) sink in the transgenic plants. **(b)** Cd partitioning in plants exhibiting naturally selected Cd hyperaccumulation (e.g. *T. caerulescens*; accession Ganges). In root cells of Cd hyperaccumulator plants, Cd(II) transport into the vacuole is likely to be suppressed. Instead, Cd(II) is likely to be exported by the metal pump HMA4, and possibly other transporters, for transport into the shoot via the xylem. A larger proportion of Cd(II) than in (a) is translocated from the root into the shoot (thick arrow). In leaf cells, efficient transporters exist for the sequestration of Cd(II) in the vacuoles, thus generating a strong sink for Cd(II) in the central vacuoles of leaf cells.

[54,55] and as a compartment that can be used to generate artificial metal sinks (see below and Figure 2). Segregating populations have also been obtained by crossing accessions of the hyperaccumulator *T. caerulescens* differing in Zn, Ni and Cd tolerance and accumulation [43•,56,57]. Finally, metal hyperaccumulator species have been evaluated systematically with respect to their suitability for molecular genetic approaches [58••,59].

The characterization of single genes involved in metal homeostasis has yielded important insights into their functions and potential use in phytoremediation [60•]. Among these, some of the most notable studies address the high-affinity iron uptake system IRT1 of *A. thaliana* [46,61,62••,63,64] and the two P_{1B}-type Zn^{2+}/Cd^{2+}-ATPases HMA2 and HMA4 [41•,65,66•,67,68], which function in the export of Zn^{2+} and Cd^{2+} from cells in root-to-shoot metal transport.

Important insights have been gained from transgenic plants overexpressing microbial and plant metal homeostasis proteins. Transgenic *A. thaliana* plants were generated that overexpress the yeast YCF1 (yeast cadmium factor 1 [69]) membrane transport protein of the ATP-binding cassette (ABC) transporter family [70••]. This protein is capable of transporting Cd–glutathione conjugates into the vacuole of yeast and plant cells. Transgenic plants were less metal-sensitive and accumulated up to 1.5-fold and 1.4-fold higher shoot concentrations of Cd and Pb, respectively (Table 1; Figure 2). The overexpression of *A. thaliana* HMA4 (see above) was reported to increase the tolerance of transgenic plants to Cd and Zn, and leaf Zn and Cd concentrations were twofold and 1.4-fold higher than in the wild type, respectively (Table 1) [66•]. All of these transgenic plants remain to be tested for shoot metal accumulation on metal-contaminated soils. Lee *et al.* [71••] successfully expressed a bacterial homologue of *At*HMA4, the *E. coli* ZntA protein, in *A. thaliana*. The protein was shown to localize to the plasma membrane of *A. thaliana* cells. The transgenic lines were more tolerant to Pb and Cd than wild-type plants, but accumulated less Cd and Pb in the leaves [71••]. In addition to increasing the rate of metal removal from the cytoplasm, metal tolerance can also be generated by producing metal-chelating molecules. One low molecular weight chelator implicated in Ni hyperaccumulation and tolerance is free histidine [9,72]. Expression in *A. thaliana* of a bacterial gene encoding ATP phosphoribosyl transferase, which catalyzes the rate-limiting, first committed step in histidine biosynthesis, increased free histidine levels and conferred enhanced Ni tolerance [73•], but did not increase leaf Ni accumulation. Reported results on the overexpression of phytochelatin synthase in plants with the aim to overproduce metal chelating phytochelatins have so far been contradictory [74,75]. Although phytochelatins are necessary for basal metal tolerance in most plants [76], inhibitor studies suggest that phytochelatins are not required in naturally selected Zn and Cd hyperaccumulation and hypertolerance [77,78•].

Conclusions

As far as can be inferred from the published data, the performance of transgenic plants generated so far is not yet sufficient for commercial phytoextraction (Table 1). Improvements could be made by using tissue- or cell-type-specific promoters. In roots, it is desirable to maintain trace element contaminants in a mobile chemical form, and their export into the xylem should be maximized (Figure 2). It is important to avoid the generation of metal sinks in the roots and to create sinks for metals and metalloids, either by tight binding or by sequestration, in the above-ground tissues. The engineering of transgenic plants suitable for phytoextraction will probably require a change in the expression levels of several genes. Beyond a certain number of genes, this could render transgenic approaches impractical, unless regulatory factors can be identified that control several target genes in concert. Enhancing contaminant mobilization and uptake into

the roots will require more attention in the future, especially with respect to the competition between nutrient ions and target trace element contaminant ions.

Metal and metalloid contaminations seriously threaten the health of a large number of people worldwide and require novel, low-cost, flexible and effective phytoremediation technologies. Despite considerable and rapid progress in recent years, a lack of basic understanding of metal handling in plants is still limiting the design of phytoremediation approaches. This can only be overcome by concerted multilateral and more widespread national research programs, as well as through facilitated access to biological resources such as seeds and an open exchange of information, tools and materials.

Update

Kobae *et al*. [79] have shown that the *A. thaliana* T-DNA insertion line *mtp1-1*, which is disrupted in *MTP1* expression, is hypersensitive to Zn. This suggests that MTP1 contributes to basal Zn tolerance in *A. thaliana*.

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• Wood KV, Harris HH, Pickering IJ, Salt DE: **Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase**. *BMC Plant Biol* 2004, **4**:1.
The analysis of Se speciation in wild-type and transgenic plants was performed in much detail using high pressure liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry and HPLC coupled to electrospray ionization quadrupole time-of-flight mass spectrometry, as well as X-ray absorption spectroscopy. Unfortunately, no potential Se-containing volatiles were analyzed. Concerning tolerance to and accumulation of Se from soil selenate, the results found here and in the article by LeDuc *et al*. [23] are somewhat contradictory.

23. LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP,
• AbdelSamie M, Chiang CY, Tagmount A, deSouza M *et al.*: **Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation**. *Plant Physiol* 2004, **135**:377-383.
The physiological analysis of transgenic plants was carried out somewhat more carefully and on more different substrates in this study (compare with Ellis *et al*. [22•]). A mechanistic explanation for the high accumulation of Se from selenate by the transgenic *B. juncea* plants is lacking.

24. Poynton CY, Huang JW, Blaylock MJ, Kochian LV, Elless MP:
• **Mechanisms of arsenic hyperaccumulation in *Pteris* species: root As influx and translocation**. *Planta* 2004, **219**:1080-1088.
Using ^{73}As-labelled arsenate, the authors compare root uptake in As hyperaccumulator and non-accumulator fern species. Although somewhat noisy, the data indicates that root arsenate uptake in the As hyperaccumulator *P. vittata* displays a higher affinity for arsenate (K_M ninefold lower than in the non-accumulator fern). In both types of ferns arsenate uptake is inhibited by phosphate.

25. Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE: **Reduction and coordination of arsenic in Indian mustard**. *Plant Physiol* 2000, **122**:1171-1177.

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• McGrath SP: **The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata***. *New Phytol* 2003, **159**:403-410.
This study shows that the inhibitor of γ-EC synthetase, L-buthionine-sulfoximine (BSO), significantly decreases reduced glutathione, phytochelatin and As concentrations in *P. vittata* exposed to arsenate, and also reduces arsenate tolerance. However, control (-As) plants are also strongly inhibited by the chosen BSO concentration, and the additional effect of BSO under exposure to arsenate is not well resolved.

33. Raab A, Feldmann J, Meharg AA: **The nature of arsenic-**
• **phytochelatin complexes in *Holcus lanatus* and *Pteris cretica***. *Plant Physiol* 2004, **134**:1113-1122.
The authors investigate As-phytochelatin complexes in two species: *Holcus lanatus* and *P. cretica*. They report that in above-ground vegetative tissues most As is present in inorganically bound form in both species (at least 78% in *H. lanatus* and 99% in *P. cretica*). Based on high pressure liquid chromatography electrospray ionisation mass spectrometry (HPLC-ESI-MS) analyses they report that As(III)-$(\gamma EC)_3$-G is the dominant phytochelatin complex in the As-tolerant non-accumulating *H. lanatus,* accounting for 10% of the total As. By contrast, mixed GS-As(III)-$(\gamma EC)_2$-G complexes are dominant in the As hyperaccumulating fern *P. cretica*, accounting for approximately 0.25% of the total As.

34. Wysocki R, Clemens S, Augustyniak D, Golik P, Maciaszczyk E,
• Tamas MJ, Dziadkowiec D: **Metalloid tolerance based on phytochelatins is not functionally equivalent to the arsenite transporter Acr3p**. *Biochem Biophys Res Commun* 2003, **304**:293-300.
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•• Guerinot ML, Harper JF, Ward JM, McIntyre L *et al.*: **Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana***. *Nat Biotechnol* 2003, **21**:1215-1221.
In an ionome screen (see text) the authors identify novel mutants and novel alleles of known mutants altered in leaf elemental concentration profiles. Novel alleles of the *man1* (*frd3*) mutant, which accumulates very high concentrations of Mn, Co and other metals, are identified.

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•• Meyer RC, Saumitou-Laprade P, Krämer U: **Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high *MTP1* transcript levels**. *Plant J* 2004, **39**:425-439.
Three distinct copies of *MTP1* genes are shown to be present at independently segregating loci in the genome of the Zn-tolerant *A. halleri*. By contrast, *MTP1* is a single-copy gene in the closely related, Zn-sensitive species *A. thaliana* and *A. lyrata*. Two *A. halleri MTP1* gene copies co-segregate with Zn tolerance in the backcross 1 (BC1) generation of a cross between *A. halleri* and *A. lyrata*. In an allele-specific RT-PCR, these two copies are shown to contribute high amounts of *MTP1* transcripts, whereas the third *A. halleri MTP1* locus is expressed at low background levels and does not co-segregate with Zn tolerance. The MTP1 protein is shown to mediate cellular Zn detoxification and is localized to the vacuolar membrane.

41. Papoyan A, Kochian LV: **Identification of *Thlaspi caerulescens***
• **genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase**. *Plant Physiol* 2004, **136**:3814-3823.
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• McGrath SP: **Co-segregation analysis of cadmium and zinc accumulation in *Thlaspi caerulescens* interecotypic crosses**. *New Phytol* 2004, **163**:299-312.
The authors analyze the segregation of Zn and Cd tolerance and shoot accumulation in parents and 234 seedlings of the F2 generation of a cross between a Cd and Zn hyperaccumulating accession of *T. caerulescens* (Ganges) and a Zn hyperaccumulating accession (Prayon). The distribution of Cd hyperaccumulation in F2 progeny suggests that Cd hyperaccumulation is controlled by more than one gene, but phenotypic analysis is complicated by competition between Cd and Zn and by Cd

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