

# Phytoremediation of toxic elemental and organic pollutants

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Phytoremediation is the use of plants to extract, sequester, and/or detoxify pollutants. Phytoremediation is widely viewed as the ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced. Plants have many endogenous genetic, biochemical, and physiological properties that make them ideal agents for soil and water remediation. Significant progress has been made in recent years in developing native or genetically modified plants for the remediation of environmental contaminants. Because elements are immutable, phytoremediation strategies for radionuclide and heavy metal pollutants focus on hyperaccumulation above-ground. In contrast, organic pollutants can potentially be completely mineralized by plants.

## Addresses

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## Abbreviations

<b>APS</b>	ATP sulfurase
<b>FRO2</b>	ferric chelate reductase
<b>GCP</b>	glutathione-conjugate transporter
<b>GS</b>	glutathione synthetase
<b>GSH</b>	glutathione
<b>GTN</b>	glycerol trinitrate (or nitroglycerin)
<b>IRT1</b>	<i>IRON TRANSPORTER 1</i>
<b>MT</b>	metallothionein
<b>PAH</b>	polycyclic aromatic hydrocarbon
<b>PC</b>	phytochelatin
<b>PCB</b>	polychlorinated biphenyl
<b>PCS</b>	phytochelatin synthase
<b>PS</b>	phytochelatin synthase
<b>TCE</b>	trichloroethylene
<b>TNT</b>	2,4,6-trinitrotoluene
<b>ZIP</b>	<i>ZINC TRANSPORTER</i>

## Introduction

Phytoremediation is a newly evolving field of science and technology that uses plants to clean-up polluted soil, water, or air [1•–3•]. With the help of genetic engineering plants can be used to extract, sequester, and/or detoxify a wide variety of environmental contaminants. This field is generating great excitement because phytoremediation techniques may offer the only effective means of restoring the hundreds of thousands of square miles of land and water that have been polluted by human activities. Currently, clean-up methods such as physically removing contaminated soil from a site and burying it elsewhere, are generally too costly and environmentally destructive to be applied on the imposing scale that is now required. The principles behind phytoremediation may also improve the utility of traditionally marginal lands for agriculture and forestry.

It is important to distinguish between the phytoremediation of elemental and organic pollutants at the outset. Elemental pollutants are essentially immutable by any biological or physical process short of nuclear fission and fusion, and thus their remediation presents special scientific and technical problems. Elemental pollutants include toxic heavy metals and radionuclides, such as arsenic, cadmium, cesium, chromium, lead, mercury, strontium, technetium, tritium, and uranium. With a few notable exceptions, the best scenarios for the phytoremediation of elemental pollutants involve plants extracting and translocating a toxic cation or oxyanion to above-ground tissues for later harvest; converting the element to a less toxic chemical species (i.e. transformation); or at the very least sequestering the element in roots to prevent leaching from the site.

For organic pollutants, the goal of phytoremediation is to completely mineralize them into relatively non-toxic constituents, such as carbon dioxide, nitrate, chlorine, and ammonia [4]. Organic pollutants that are potentially important targets for phytoremediation include polychlorinated biphenyls (PCBs) such as dioxin; polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene; nitroaromatics such as trinitrotoluene (TNT); and linear halogenated hydrocarbons such as trichloroethylene (TCE). Many of these compounds are not only toxic and teratogenic, but also carcinogenic.

Pollutants can be remediated in plants through several natural biophysical and biochemical processes: adsorption, transport and translocation; hyperaccumulation; or transformation and mineralization. For example, many elemental pollutants enter plants through nutrient transport systems. The degradation of endogenous toxic organics or their sequestration in vacuoles also protects plants from toxic xenobiotics. In many cases, the over-expression of existing plant genes or transgenic expression of bacterial or animal genes is required to enhance these natural properties. My review examines the phytoremediation of elemental pollutants in light of these processes whenever possible, and it addresses the potential for the phytoremediation of organic pollutants.

## Phytoremediation of elemental pollutants

### Adsorption

Root surfaces, which have evolved specifically to adsorb elemental nutrients from soil and pore water, have extraordinarily large surface areas [5] and high-affinity chemical receptors [6–11]. In the process of adsorption, root surfaces bind many elemental pollutants as well as nutrients. For example, Indian mustard (*Brassica juncea*) can rapidly concentrate Cd(II), Ni(II), Pb(II), and Sr(II) into root tissues at levels 500-times greater than those in the liquid medium

in which they are growing [6,7]. Sunflower roots concentrate uranium 30,000-fold from water contaminated with dilute but highly toxic concentrations of this oxyanion [8]. Similarly, tobacco roots exposed to low concentrations (1–5 ppm) of ionic mercury (Hg[II]) in liquid medium lowered the Hg[II] concentration of the medium nearly 100-fold in a matter of hours [9]. In soils, of course, these adsorption processes are orders of magnitude less efficient than in liquid medium [10,11] because root surfaces must compete for nutrients with diverse particulate soil materials (e.g. clays and humic acids).

### Transport

Although research into the molecular physiology of plant transport systems for elemental nutrients and pollutants is still in its infancy, excellent initial information is available on two related subfamilies of ZINC TRANSPORTER (ZIP) proteins that are involved in Zn(II) and Fe(II) uptake [12\*,13\*,14\*\*]. Among other sequence motifs, these two subfamilies share an extramembranal metal-binding motif HXHXH. The ZIP subfamily, represented by the *Arabidopsis* ZIP1, ZIP2, and ZIP3 genes, complement yeast transport mutants that show Zn(II) deficiency. In addition, ZIP1 and ZIP3 are expressed in roots upon induction by zinc deficiency, and these genes undoubtedly play a direct role in zinc uptake from soil [15\*]. The Zn(II) transport activity of these three proteins is inhibited by Mn(II), Co(II), Cd(II), and/or Cu(II), indicating that ZIP proteins may transport potentially toxic metals as well as nutrients. From the other ZIP subfamily, the IRON TRANSPORTER 1 (ITR1) gene of *Arabidopsis* is a good example of an iron (Fe [II]) transporter. ITR1 is expressed in roots, increases expression levels upon iron deficiency, and is required for normal iron utilization [16]. It complements iron-uptake functions in yeast mutants that have deficient iron uptake [16]. The ITR1 protein can actively and efficiently transport Cd(II) and Zn(II) [17\*\*,18]. It has long been recognized that iron-starved plants take up higher levels of other potentially toxic metal ions (e.g. Cu[II], Mn[II] and Zn[II]). Thus, it seems likely that ZIP1, ZIP2, ZIP3, ITR1, and related inducible transporters provide the pathways through which toxic metal ions are actively taken up by plants experiencing nutrient deficiency and stress (as indicated in Figure 1).

The solubility and transport of many heavy metals into roots is increased in acidic soils, which creates special toxicity problems. Even nutrient metals, such as Al(III) and Mn(II), reach toxic levels in plants when the soil pH falls much below 5.0. Plants and soils can, however, be manipulated to increase or decrease the uptake of pollutants under otherwise toxic acidic conditions, and these strategies could form the basis of some phytoremediation techniques. In developed countries, the traditional agricultural approach to reducing cation toxicity is to chemically raise soil pH every few years. On marginal lands, in forests, and in Third World agriculture, however, this strategy is too expensive. As an alternative, plants adapted to accommodate low soil

pH or high metal ion toxicity should grow more efficiently and simultaneously improve these sites. One class of *Arabidopsis* mutant plants selected for Al(III) tolerance is capable of increasing rhizosphere pH [19\*], thereby lowering the availability of Al(III) and other toxic cations to plant roots. Although the cost of this trait in terms of plant yield is unknown, it is likely that appropriate crop and forest species could be used to adjust soil acidity on a larger scale.

The natural variation of maize genotypes in resistance to Al(III) toxicity has been used to identify strategies other than pH adjustment that have broader implications for phytoremediation [20]. On exposure to Al(III) in acidic soils, one naturally resistant maize genotype is stimulated to release citrate from roots into the medium [20]; this does not happen in Al(III) sensitive genotypes. Citrate is a tricarboxylic acid that chelates many metals and specifically complexes with Al(III), preventing its uptake (Figure 1). A second class of Al(III)-resistant *Arabidopsis* mutants helped to confirm the role of this simple organic acid in transport [21\*\*]. These mutants constitutively secrete large quantities of citrate into their medium and again take up less Al(III). The capacity of citrate to block Al(III) uptake has been confirmed independently in transgenic tobacco and papaya plants that over-expressed a bacterial gene for citrate synthetase [22]. Citrate synthetase is a Krebs's cycle enzyme that combines an acetyl group and oxaloacetic acid to form citrate. The excess citrate in these transgenic plants is secreted into the growth medium and provides even greater resistance to Al(III) toxicity.

### Transport and translocation

In contrast to the behavior of citrate, most organic chelators increase metal ion uptake and translocation in plants. In response to nutrient metal ion deficiencies, plants secrete phytosiderophores such as mugenic and avenic acids [23,24]. These metal-chelators increase the bioavailability of metals that are otherwise tightly bound to the soil and help to carry them into plant tissues. Synthetic chelators can mimic these effects. For example, when ethylene diamine tetra-acetic acid (EDTA) is added to lead (Pb[II])-contaminated soils, there is a >100-fold increase in the uptake and transport of the lead-EDTA-chelate (Figure 1) into stems and leaves [25,26,27\*\*]. Thus, in contrast to the role of citric acid in reducing aluminum uptake, plants altered to increase their secretion of particular organic acids will probably demonstrate increased uptake and translocation of metal pollutants (see histidine section below).

### Hyperaccumulation

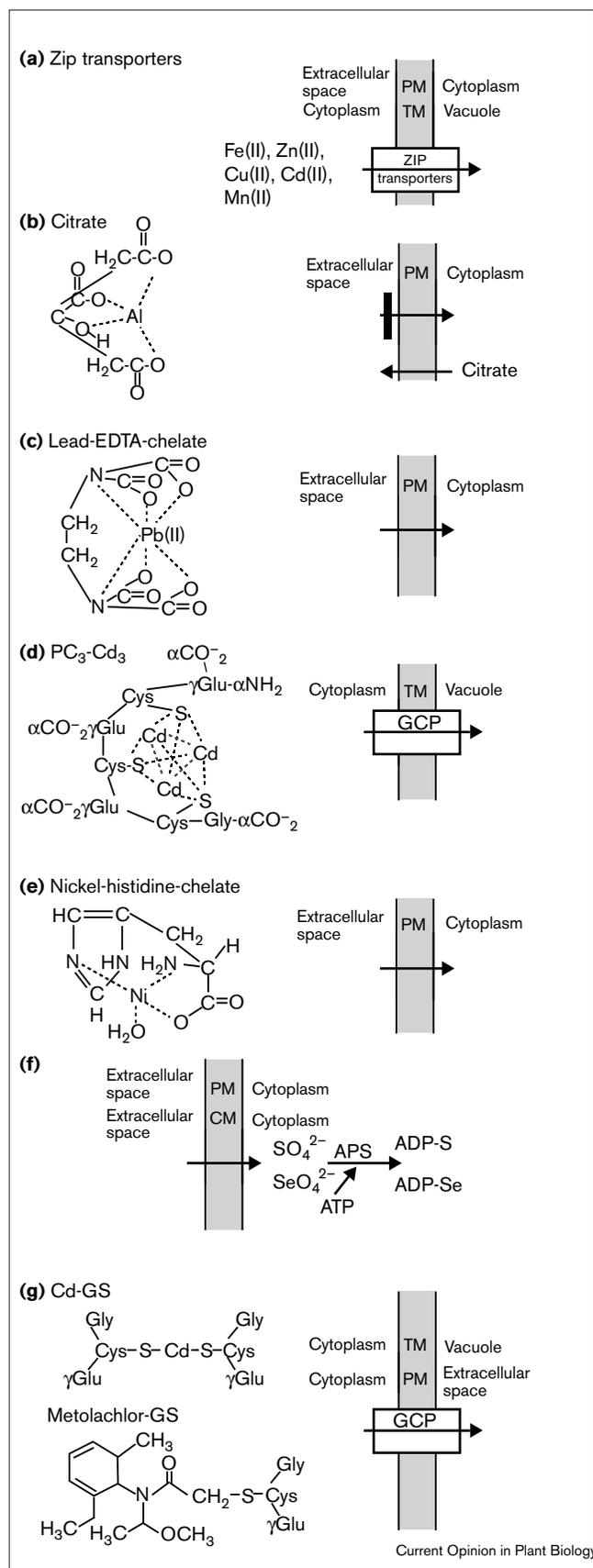
Because elemental pollutants are immutable and cannot be made completely non-toxic, the final goal of most phytoremediation strategies is efficient hyperaccumulation in harvestable above-ground tissues. Hyperaccumulation is usually defined as the concentration of a metal ion to >0.1–1% of the dry weight of the plant [28]. At these concentrations the recovery of metals from the plant tissues is potentially economical [28]. Recovery of even lower

**Figure 1 legend**

Mechanisms and possible ligand complexes that aid the transport and sequestration of toxin pollutants. **(a)** The ZIP transporter families can bring nutrient and toxic metal ions through the plasma membrane (PM) into roots and/or through the tonoplast membrane (TM) into vacuoles. Mutations in the *Arabidopsis* *FERRIC CHELATE REDUCTASE* (*FRO2*) (Figure 2) or other genes that result in iron starvation increase ZIP transporter activity and metal uptake. **(b)** Secreted citrate can form tetrahedral metal ion complexes that block Al(III) and possibly Ni(II) transport into roots. **(c)** The soil additive EDTA can form hexahedral metal ion complexes with metals (Pb(II), Fe(III)) that enhance root uptake and translocation throughout the plant. Naturally secreted organic acids, such as mugenic and avenic acids, may use the same mechanism to scavenge soils for nutrient metals. **(d)** Phytochelatins, in this case a trimeric PC<sub>3</sub>, form tetrahedral complexes with thiol-reactive metals like cadmium (Cd(II)) enhancing tolerance. These structures should aid in the transport into and sequestration of metals in vacuoles via the glutathione S-conjugate pump (GCP). With other metals such as Cu(II) and Zn(II), the  $\alpha$ -carboxyl groups of PCs may participate in forming very different metal-ligand structures (not pictured). **(e)** Histidine (His) can participate in forming tetrahedral metal ion complexes with Ni(II) that aid in uptake, transport, hyperaccumulation, and tolerance. Water forms the fourth ligand in this model. **(f)** Transport of sulfate and selenate through the PM or plastidic membrane (CM) is enhanced by formation of adenosine phosphosulfate (ADP-S) and adenosine phosphoselenate (ADP-Se), respectively, which is catalyzed by ATP sulfurase (APS). **(g)** Toxic metals and large organics can be complexed with glutathione and then pumped by the GCP into vacuoles or out of roots.

hyperaccumulated concentrations of most toxic metals (e.g. As, Cd and Hg) and radionuclides (e.g. isotopes of Ce, U and Te) could be economically viable as an alternative to the extreme expense of physical remediation methods.

Hyperaccumulation of elements may involve all three of the processes outlined above, (i.e. adsorption, transport and translocation) but it also requires large sinks in which to store the pollutant. The most notable mechanisms for sequestering thio-reactive metals involve two classes of cysteine-rich peptides, the metallothioneins (MTs) and phytochelatins (PCs). Metals such as Ag(I), AsO<sub>3</sub>(-III), Cd(II), Co(II), Cu(II), Hg(II), and Ni(II) are sequestered by bonding with organic sulfur (R-SH) on the cysteine residues of these peptides. *In vitro*, MTs form metal-ligands with a specificity correlating with the thiolate series for cation binding (Bi>Hg>Ag>Cu>Cd>Pb>Zn) [29], although the precise specificity of MTs and PCs *in vivo* is not well defined. Plants have a complex family of MT genes [30,31] encoding peptides that are generally composed of 60–80 amino acids and contain 9–16 cysteine residues. The MTs are thought to primarily chaperone nutrient metals to their various necessary roles (e.g. insertion into an enzymatic center during protein folding) [32]. MTs can, however, also protect plants from the effects of toxic metal ions and aid in their accumulation. For example, transgenic over-expression of the 32 amino acid metal-binding  $\alpha$ -domain of mouse MT in tobacco confers moderate levels of Cd(II) resistance and accumulation [33,34]. MT-metal complexes can be glutathionated [35], suggesting that these complexes might be transported into vacuoles for long term sequestration (see below).

**Figure 1**

The PCs are non-ribosomally synthesized peptides with the structure  $(\gamma\text{-Glu-Cys})_n\text{X}$  (Figure 2), where  $n$  is generally 2-11 and  $\text{X}$  is commonly Gly, but can be  $\beta\text{-Ala}$  or Ser. PCs form ligand complexes with nutrient and toxic metals and aid transport into vacuoles [36], where the metals are sequestered. One possible structure of a PC ( $n=3$ ,  $\text{X}=\text{Gly}$ ,  $\text{PC}_3$ ) binding three Cd(II) ions is proposed in Figure 1 [37]. Mutants in the synthesis of PCs or their precursor tripeptide, glutathione (GSH,  $n=1$  and  $\text{X}=\text{Gly}$ ), are hypersensitive to Cd(II) [38,39] and many other sulfur-reactive metals [40], demonstrating the role of PCs in protecting plants from toxic metals. Further evidence of this role comes from the over-expression of a bacterial glutathione synthetase (GS) by *Brassica juncea* [41••]. These transgenic *B. juncea* plants have higher GSH and PC concentrations and increased Cd(II) tolerance and accumulation relative to controls. Plant, fungal, and animal genes encoding phytochelatin synthase (PS) have recently been identified [42•,43•]. Plant PS synthesis is increased several-fold upon exposure to Cd(II) in the medium, suggesting that PS has a direct role in toxic metal metabolism. Over-expression of plant PS in transgenic yeast increases tolerance of and accumulation of Cd(II) [42•]. Clearly, manipulation of GSH and PC concentrations has significant potential for increasing the accumulation of toxic metals by plants.

The study of natural plant hyperaccumulators has a long history. Distinct species with unusual heavy metal requirements or high levels of heavy metal tolerance were widely used as bio-indicators of mineral deposits for more than a century [28]. These natural hyperaccumulators of zinc, nickel, or cadmium come from a number of diverse plant taxa, although the majority occur in the family Brassicaceae [44]. One Brassica species, *Alyssum lesbiacum*, can be grown in Ni(II)-rich medium or soil with only moderate growth reduction [45]. Ni(II) is rapidly transported into the plant, where it accumulates to >3% of the dry weight of above-ground tissues [45]. In contrast, a closely related species, *Alyssum montanum*, is orders of magnitude more sensitive to Ni(II) and does not hyperaccumulate Ni [45]. This suggests that a limited number of genetic loci control tolerance and hyperaccumulation. In three *Alyssum* hyperaccumulator species, exposing roots to increasing levels of Ni(II) or Co(II) reveals a linear relationship between xylem sap metal content and histidine levels. No other small organic molecule shows this positive correlation. Ni-histidine complexes account for most of the Ni in these tissues (Figure 1). Thus, for some hyperaccumulator species, chelation with histidine, uptake, xylem transport, and hyperaccumulation appear to be mechanistically linked and inducible processes [7]. In another Brassica hyperaccumulator species, *Thlaspi goesingense*, histidine may only be involved in the mobilization and transport of Ni(II) and Zn(II) from the rhizosphere into the roots [46,47]. An improved understanding of the genetic basis of natural hyperaccumulation mechanisms should enable their manipulation and enhancement in a wider variety of plant species.

### Transformation of toxic elements

Another natural mechanism that offers exciting phytoremediation possibilities is the transformation of toxic elements into relatively harmless forms. Many elements (e.g. arsenic, mercury, iron, selenium, chromium) can exist in a variety of states, including different cationic and oxyanionic species and thio- and organo-metallics. These forms vary widely in their transport and accumulation in plants and in their toxicity to humans and other life forms. Mercury offers perhaps the best-understood example of the dangers inherent in one particular species of a heavy metal.

Mercury primarily enters the environment either as liquid Hg(0) from industrial and defense-related accidents; or as mercury species (Hg(II)) bound to particulate matter from burning coal and trash or from volcanic activity, and as complex chemical derivatives released in industrial effluents [3•]. Although Hg(II) is relatively toxic, it and Hg(0) have seldom been involved in serious incidents of human mercury poisoning without first being transformed into methylmercury (MeHg) [48]. The world first became aware of the extreme dangers of methylmercury (MeHg) in the 1950s after a large, tragic incident of human mercury poisoning at Minamata Bay, Japan [49]. In aquatic sediments, various mercury species are efficiently converted to MeHg by anaerobic bacteria [50]. Unfortunately, MeHg is biomagnified by several orders of magnitude and has a greater toxicity than any other natural mercury compound [3•]. As a result, the fish-eating predatory animals and humans at the top of the food chain suffer MeHg poisoning [48,51].

Our laboratory made use of two genes from the well-characterized bacterial *mer* operon, *merA* and *merB*, to engineer a mercury transformation and remediation system in plants [2•,3•]. The bacterial *merA* gene encodes an NADPH-dependent mercuric ion reductase that converts ionic mercury (Hg(II)) to elemental, metallic mercury (Hg(0)) as shown in Figure 2. Metallic mercury is nearly two orders of magnitude less toxic than ionic mercury and is readily eliminated because of its volatility. Diverse plant species expressing *merA* constitutively are resistant to at least ten-times greater concentrations of Hg(II) than those that kill non-transgenic controls [3•,52,53••,54]. These plants volatilize and possibly transpire Hg(0) from their tissues, and they accumulate far less mercury than control plants grown in low concentrations of mercury [9]. Transgenic plants expressing *merA* out-perform wild-type plants on mercury contaminated soil [9] as shown in Figure 3. Because MeHg is synthesized at aquatic sites, eliminating all forms of mercury contamination from lakes, rivers, and wetlands should largely prevent MeHg formation. Because plants are autotrophic and have massive root systems, they should be able to increase rate at which mercury is eliminated by orders of magnitude over those catalyzed by endogenous bacteria.

The bacterial *merB* gene encodes an organomercurial lyase that degrades MeHg to methane and Hg(II) (Figure 2). This gene is only expressed in bacteria in conjunction with *merA*

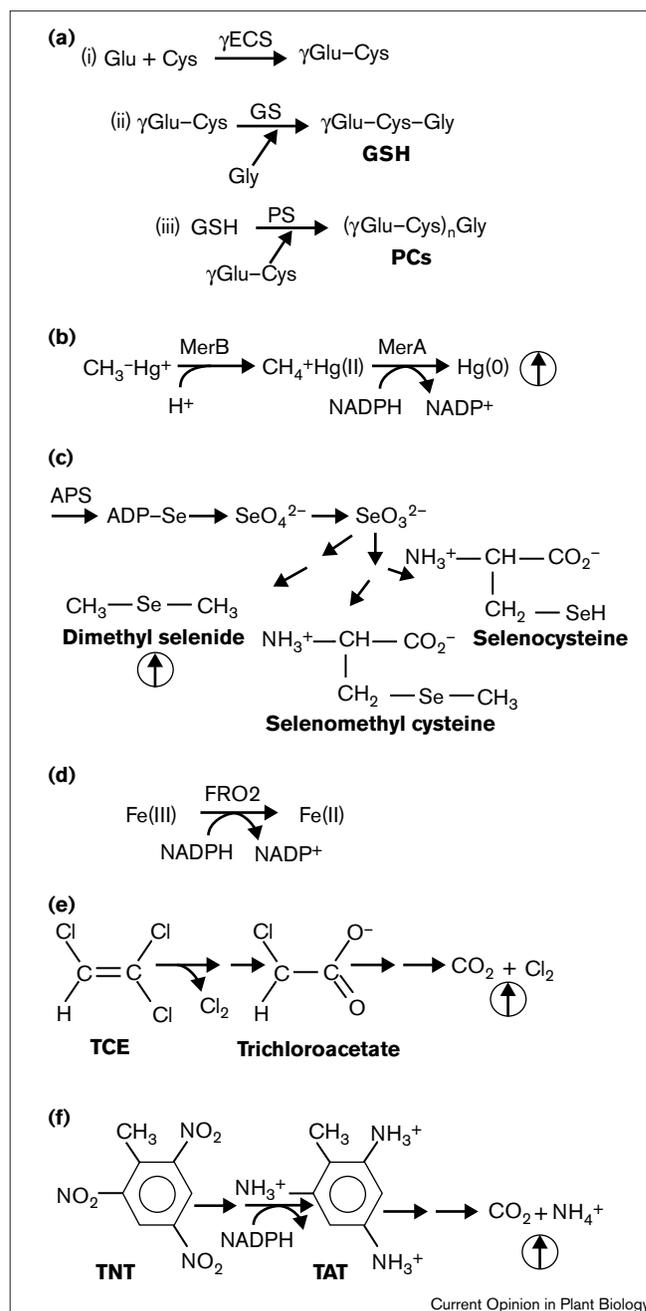
**Figure 2 legend**

Transformation of toxic elemental and organic pollutants.

**(a)** Phytochelatins are peptides that are synthesized biochemically by mechanisms not involving ribosomes. (i) Gamma glutamyl cysteine synthetase ( $\gamma$ ECS) catalyzes the condensation of glutamate (Glu) and cysteine (Cys). The resulting  $\gamma$ Glu-Cys contains a peptide bond between the  $\gamma$ -carboxyl group of Glu and the  $\alpha$ -amino group of Cys. (ii) GS catalyzes the synthesis of GSH and (iii) PS the synthesis of PCs. Transgenic over-expression of the latter two enzymes increases heavy metal resistance in plants and yeast, respectively. **(b)** Methylmercury ( $\text{CH}_3\text{-Hg}^+$ ) is not only the most toxic natural form of mercury, but is biomagnified efficiently in the food chain. Mercury is less toxic as ionic mercury ( $\text{Hg(II)}$ ) and least toxic as reduced and volatile metallic mercury ( $\text{Hg(0)}$ ).  $\text{Hg(0)}$  becomes toxic after reoxidation to  $\text{Hg(II)}$ . The bacterial enzymes MerB and MerA catalyze the detoxification of methyl and ionic mercury, respectively. This pathway has been engineered to work efficiently in plants. **(c)** ATP sulfurylase catalyzes the committed step in sulfate and selenate uptake, forming ADP-S and ADP-Se, respectively. Selenium is most toxic when incorporated into amino acid analogues and least toxic as volatile dimethylselenide. Endogenous enzymes carry out these various reactions to different extents in distinct plant species. The reduced intermediate selenite ( $\text{SeO}_3^{2-}$ ) is more efficiently metabolized into organic forms than selenate ( $\text{SeO}_4^{2-}$ ). **(d)** Toxic ferric iron is reduced to ferrous iron via ferric chelate reductase (FRO2).  $\text{Fe(II)}$  is readily taken up by plants (see Figure 1). **(e)** Chlorinated solvents such as TCE can be mineralized to harmless products by endogenous plant enzymes found at high concentrations in a small percentage of plant species. **(f)** Nitroaromatics like TNT are efficiently broken down through several intermediates such as triamino toluene (TAT) and can be mineralized to harmless products in a few plant species. These reactions can be enhanced by transgenic bacterial gene expression. Volatile products are indicated by an encircled arrow.

so that the final product of the bacterial *mer* operon is always  $\text{Hg(0)}$  [55\*\*]. In plants, however, expression of *merB* alone confers increased MeHg tolerance, probably because of the extreme toxicity of MeHg to most eukaryotic cells [55\*\*]. At environmentally relevant concentrations of MeHg, these transgenic plants accumulate  $\text{Hg(II)}$ , the product of the MerB-catalyzed reaction, and manage to outgrow it. Plant expression of *merA* and *merB* together results in the two-step conversion to volatile  $\text{Hg(0)}$  and produces tolerance of 50-times greater concentrations of MeHg than is required to kill controls plants, and five-times greater than the concentrations that kill *merB* plants [56\*\*]. Methylmercury is such an extreme human health hazard that it is likely that a number of phytoremediation strategies that block its flow into the environment will be adopted.

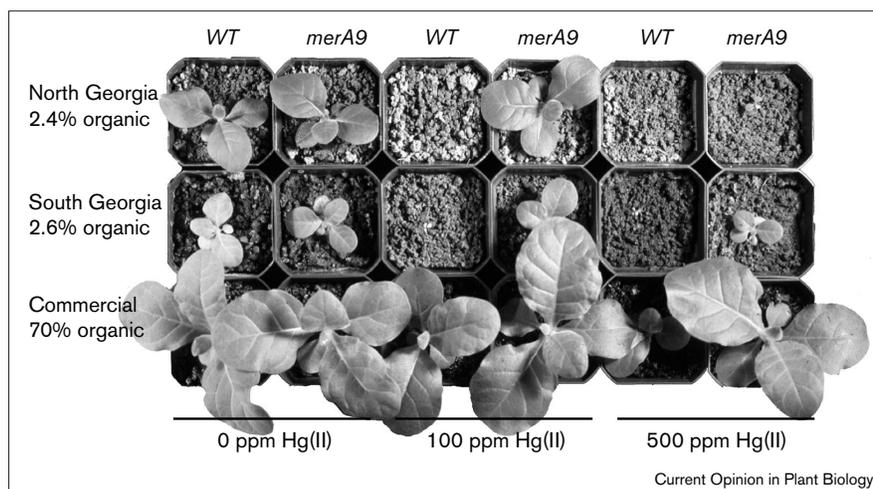
The chemical transformation of other toxic elemental pollutants also leads to their remediation. Selenium builds up in irrigation water and contaminates hundreds of square miles of wetland in the western US. Selenium and sulfur are nutrients with very similar chemical properties and their uptake and assimilation proceed through common pathways. Although sulfur is required by all organisms in relatively large concentrations, high levels of selenium are usually toxic. The assimilation of sulfate and selenate is activated by ATP sulfurylase (Figures 1 and 2). Selenate is converted to adenosine phosphoselenate (ADP-Se), which is subsequently reduced to selenite. Over-expression of

**Figure 2**

the *Arabidopsis* plastidic ATP sulfurylase (*APS1*) in transgenic Indian mustard results in an increased uptake and assimilation of selenate, increased reduction to selenite, and greater tolerance of selenate [57\*\*].

Two plant pathways appear to dominate the natural remediation and/or detoxification of selenium. It should be noted that in most species selenium is most toxic when it is metabolized into analogues of cysteine and methionine and incorporated into proteins. Ironically, one detoxification mechanism found in *Astragalus* [58], a genus with

Figure 3



Transgenic tobacco plants expressing the reconstructed bacterial mercuric ion reductase gene, *merA9*, grow much better on mercury-contaminated soils than wild-type (*WT*) plants [9]. The growth of the *WT* and *merA9* transgenic tobacco plants were compared when growing in two native Georgia soils and one rich commercial potting compost, which ranged in organic content from 2–70%. Each soil was contaminated with ~0, 100 or 500 ppm of ionic mercury ( $\text{Hg(II)}$ ) as calculated on the basis of soil dry weight. Four-week-old *WT* and *merA9* plants were transplanted into these soils and the pots photographed four weeks later. In native soils containing 100 and 500 ppm  $\text{Hg(II)}$ , the transgenic plants all flourished (even if initially delayed in growth) and went on to flower, whereas the *WT* controls died back quickly. Resistance was proportional to the ability of the plants to electrochemically reduce  $\text{Hg(II)}$  to much less toxic metallic  $\text{Hg(0)}$ .

particularly high selenium tolerance levels, involves the hyperaccumulation of large amounts of a seleno-amino acid into proteins. *Astragalus* species have a specific selenocysteine methyltransferase that has a strong specificity for selenate over sulfate (Figure 2). The final metabolic product of this pathway is the amino acid S-methyl selenocysteine, which is incorporated into protein and results in the hyperaccumulation of selenium. It is not known why this genus can tolerate such high concentrations of a normally toxic amino acid analogue. In a second detoxification mechanism, selenate can be converted to dimethylselenide, which is 100-times less toxic than selenate and volatilized from leaves and roots [59,60,61]. As anticipated for many processes of phytoremediation, selenium volatilization is enhanced by additional activities in the bacterial rhizosphere [62].

Chemical transformation is also an essential part of iron metabolism.  $\text{Fe(III)}$  is the major form of iron in typical oxidized soils, but it is essentially unavailable to plants and relatively toxic when it is available. Iron-efficient plants such as *Arabidopsis* utilize a reductive mechanism to extract iron from soils (Figure 2). At the root surface,  $\text{Fe(III)}$  is reduced by ferric chelate reductase (*FRO2*) to less toxic ferrous iron  $\text{Fe(II)}$  [18,63] and transported into plant root cells by a ZIP ferrous ion transporter (see section on transport above). Besides its importance to nutrient uptake, the *FRO2* mechanism suggests an alternative, elegant approach to manipulating the redox state of toxic metals at the root surface. The *FRO2* mutant, *frd-1*, is iron deficient; as with natural iron deficiency, these plants over-accumulate  $\text{Cu(II)}$ ,  $\text{Zn(II)}$ , and  $\text{Mn(II)}$  in their attempt to extract more iron from the soil (Figure 1). In contrast, *Arabidopsis manganese accumulator* (*man1*) mutants appear to be co-ordinately deregulated for  $\text{Fe(II)}$ ,  $\text{Cu(II)}$ ,  $\text{Zn(II)}$ ,  $\text{Mn(II)}$ , and  $\text{Mg(II)}$  and for

*FRO2* activity [64]. These findings suggest that the regulation of other transporters or iron transporters with less specificity are linked in a complex manner to the regulation of *FRO2*.

### Potential for the phytoremediation of organic pollutants

Organic pollutants can potentially be chemically degraded and ultimately mineralized into harmless biological compounds. First, however, they must be efficiently extracted from contaminated sediments and water. The complex physiology and biochemistry of plant roots gives plants great potential as remediators of toxic organic pollutants.

Relatively little is known about the uptake and sequestration of toxic organics in plant roots or their concentration into vacuoles. The best-characterized system involves a family of ATP-binding cassette (ABC) transporters often called the glutathione-S-conjugate pump [65,66] (Figure 1). This system recognizes oxidized diglutathione (GS-SG), glutathione conjugates of organics, conjugates of diverse high-molecular-weight toxic organic xenobiotics, and peptide-metal complexes such as phytochelatins [65,66]. Herbicides and endogenous organic compounds alike can be transported out of cells [67] or into vacuoles [68]. The accumulation of toxic organics in plant vacuoles should favor their subsequent degradation.

Although our basic knowledge of the degradation of organic pollutants by plants lags far behind that of animals and bacteria, plants can transform and mineralize a wide variety of complex organics. These endogenous activities result from the ability of plants to synthesize, rearrange, and detoxify the most complex array of biochemicals and biopolymers of any living organisms (e.g. complex carbohydrates such as cellulose and lignin; flavanol and

flavanoid pigments; aromatic plant protectants; diverse fatty acids; isoprenoids, steroids and carotenoids; and plant hormones). Three exciting examples demonstrate the potential of plant metabolic systems as remediators of toxic xenobiotics.

First, plants contain uncharacterized aliphatic dehalogenases that are capable of degrading TCE [69,70]. Among a long list of industrial solvents that pose a threat to wildlife and humans, TCE is perhaps the most widely distributed environmental pollutant of ground water and soils. Halogenated compounds, such as TCE, are among the most difficult to metabolize and are usually toxic and carcinogenic. Plants grown at polluted sites are known to extract TCE, efficiently transpire it, and enhance the degradation of TCE in the rhizosphere by feeding biodegrading bacteria with root exudates [69,70]. It is only recently becoming clear, however, that plant enzymes play a direct role in the degradative process. Careful mass-balance and isotopic-labeling experiments demonstrate that axenically grown hybrid poplars (*Populus* sp.) actively take up TCE and degrade it to trichloroethanol, chlorinated acetates, and finally CO<sub>2</sub> (Figure 2) [71•]. In one experiment with axenic poplar tissue culture cells, >10% of the TCE was mineralized to CO<sub>2</sub> within 10 d [71•]. These data suggest the presence of an oxidative degradation pathway in plants, that is quite different from the reductive one generally found in bacteria [72].

Second, plants can degrade nitroaromatic compounds that are highly toxic and carcinogenic, and notoriously difficult to metabolize. The explosive TNT (2,4,6-trinitrotoluene) and a large family of related nitro-substituted organic compounds (e.g. hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX] and glycerol trinitrate or nitroglycerin [GTN]) are used in munitions. They contaminate thousands of acres of land and dozens of miles of rivers near production, storage, and disposal facilities. A wide variety of plant species from diverse families appear to degrade TNT (Figure 2), although only a few species do so efficiently [73,74•]. The final products of these plant degradation pathways appear to be CO<sub>2</sub>, and ammonium or nitrate. Although degradation appears to proceed through multiple complex pathways, the products of reductive mechanisms such as triaminotoluene predominate (Figure 2) [74•,75]. For example, axenically grown *Microphyllum aquaticum* plants and *Cartharanthus roseus* hairy root cultures both partially degrade TNT and release degradation intermediates into their growth medium [75]. Axenic cell cultures of sugar beet degrade GTN to the expected intermediates glycerol dinitrate (GDN) and glycerol mononitrate (GMN) [76]. Hairy root cultures of *Cartharanthus roseus* were capable of degrading most of the 25 ppm of TNT added to culture medium within a few days [77•]. The specific activities of these plant enzymes may be no greater than those observed in extracts from bacterial TNT-degrading species, and none of the genes for these plant enzymes has been identified. Nevertheless, plants control most of the

energy in an ecosystem, and usually account for several orders of magnitude greater biomass than any few bacterial species in the soil. Thus, the potential contribution of selected plants to the degradation of TNT in a contaminated ecosystem may be orders of magnitude greater than that of bacteria.

In a striking demonstration of the potential of transgenic plant technologies to enhance the natural capacities of plants, a bacterial NADPH-dependent nitroreductase greatly increased GTN-degrading activity when expressed in tobacco [78••]. Transgenic seedlings were about ten-times more tolerant of GTN and TNT than their wild-type progenitors. Preliminary evidence suggests that these plants were able to break down both GTN and its first degradation product, glycerol dinitrate, twice as fast as the wild-type controls. It is not clear what fraction of these nitro-substituted compounds is completely mineralized. Nevertheless, such enhancements in degradation efficiency increase the feasibility of applying phytoremediation to toxic nitroaromatic pollutants.

Third, PCBs are among the worst pollutants because of their toxicity, carcinogenicity, wide distribution, and slow biodegradation in the environment. Axenic cultures of some plant species have been shown to efficiently degrade several classes of PCBs [79]. For example, sterile cultures of *Solanum nigrum* degrade several PCB congeners relatively efficiently [80]. Wide variation in activity levels is seen among plant species and between developmental stages. PCBs with the most highly chlorinated benzene rings appear to be the most difficult for both bacteria and plants to break down. The metabolic basis for the degradation of PCBs by plants has not been well characterized or quantified. Armed with the dozens of bacterial genes known to enhance PCB degradation, the potential of selected engineered plant species to remediate PCBs should be revealed in the next few years.

## Conclusions

Initial explorations of the natural plant mechanisms effecting the phytoremediation of elemental and organic pollutants suggest great promise for the use of plants in large-scale environmental clean-up efforts. While elegant transport studies have been performed for Cd(II), Fe(II), Ni(II), Selenate, Zn(II) and a few large organic xenobiotics in a few isolated plant species, the function of hundreds of diverse plant transporters that are central to phytoremediation remain uncharacterized. Preliminary data on TCE and TNT degradation suggest that plants can degrade highly toxic and metabolically resistant organics. The over-expression of several plant and bacterial genes in transgenic plants has greatly enhanced these natural plant remediation systems. The small number of laboratories working on these problems at present cannot, however, hope to impact global pollution. Greatly expanded research programs focused on the basic and applied problems effecting each class of

pollutants are needed for significant progress to be made. In particular, more quantitative data from mass-balance studies are needed to determine the rate-limiting steps in the mineralization of organic pollutants. Once the rate-limiting steps in uptake, transport, or transformation have been identified, more informed construction of transgenic plants expressing plant, animal, or bacterial genes will result in dramatic improvements in phytoremediation capabilities.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Salt DE, Smith RD, Raskin I: **Phytoremediation**. *Ann Rev Plant Physiol Plant Mol Biol* 1998, **49**:643-668.  
An excellent review on the uses of plants to clean-up the environment.
2. Rugh CL, Bizily SP, Meagher RB: **Phytoremediation of environmental mercury pollution**. In *Phytoremediation of Toxic Metals: Using Plants to Clean-up the Environment*. Edited by Ensley B, Raskin I. New York: John Wiley and Sons; 1999:151-169.  
This paper examines the phytoremediation of mercury pollution as an alternative to more commonly used physical methods.
3. Meagher RB, Rugh CL, Kandasamy MK, Gragson G, Wang NJ: **Engineered phytoremediation of mercury pollution in soil and water using bacterial genes**. In *Phytoremediation of Contaminated Soil and Water*. Edited by Terry W, Bañuelos G. Berkeley, California: Ann Arbor Press, Inc.; 2000:201-219.  
The integration of a phytoremediation strategy for ionic and methyl mercury with the natural geochemical cycle for mercury is discussed in this paper in the light of concerns about methyl mercury biomagnification into predatory animal populations. The natural physiological properties of plants that make them excellent 'machines' for phytoremediation are outlined.
4. Cunningham SD, Anderson TA, Schwab P, Hsu FC: **Phytoremediation of soils contaminated with organic pollutants**. *Adv Agronomy* 1996, **56**:55-114.
5. Dittmer HJ: **A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*)**. *Am J Bot* 1937, **24**:417-420.
6. Salt DE, Prince RC, Pickering IJ, Raskin I: **Mechanisms of cadmium mobility and accumulation in Indian mustard**. *Plant Physiol* 1995, **109**:1427-1433.
7. Salt DE, Kramer U: **Mechanisms of metal hyperaccumulation in plants**. In *Phytoremediation of Toxic Metals: Using Plants to Clean-up the Environment*. Edited by Raskin I, Ensley BD. New York: John Wiley and Sons; 1999:231-246.
8. Dushenkof S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC, Ensley B: **Removal of uranium from water using terrestrial plants**. *Environ Sci Technol* 1997, **31**:3468-3474.
9. Heaton ACP, Rugh CL, Wang N-J, Meagher RB: **Phytoremediation of mercury and methylmercury polluted soils using genetically engineered plants**. *J Soil Contam* 1998, **7**:497-509.
10. Raskin I, Smith RD, Salt DE: **Phytoremediation of metals: using plants to remove pollutants from the environment**. *Curr Opin Biotechnol* 1997, **8**:221-226.
11. Dushenkof V, Nanda-Kumar PBA, Motto H, Raskin I: **Rhizofiltration: the use of plants to remove heavy metals from aqueous streams**. *Environ Sci Tech* 1995, **29**:1239-1245.
12. Maathuis FJM, Sanders D: **Plasma membrane transport in context making sense out of complexity**. *Curr Opin Plant Biol* 1999, **2**:236-243.  
A review of the known genes and mechanisms effecting the plasma membrane transport of Ca(II), Na(I), K(I), Ba(II), Cd(II), phosphate, and nitrate.
13. Guerinot ML, Eide D: **Zeroing in on zinc uptake in yeast and plants**. *Curr Opin Plant Biol* 1999, **2**:244-249.  
A brief up-to-date examination of the biochemistry, molecular genetics, and known components involved in zinc uptake and transport.
14. Eng BH, Guerinot ML, Eide D, Saier MH Jr: **Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins**. *J Membr Biol* 1998, **166**:1-7.  
This paper publishes the sequence and predicted structural motifs of the ZIP family of heavy metal ion transporters. The Zrt- and Irt-related subclasses are defined.
15. Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D: **Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency**. *Proc Natl Acad Sci USA* 1998, **95**:7220-7224.  
The authors report the cloning of ZIP genes involved in root uptake and intracellular transport of zinc in *Arabidopsis*. The genes are characterized as to sequence, tissue specificity of expression, and mRNA induction during zinc deficiency.
16. Eide D, Broderius M, Fett J, Guerinot ML: **A novel iron-regulated metal transporter from plants identified by functional expression in yeast**. *Proc Natl Acad Sci USA* 1996, **93**:5624-5628.
17. Cohen CK, Fox TC, Garvin DF, Kochian LV: **The role of iron deficiency stress responses in stimulating heavy-metal transport in plants**. *Plant Physiol* 1998, **116**:1063-1072.  
The uptake of Cd(II) in iron-deficient plants is shown to have at least two components: a concentration-dependent uptake into the cell wall and a saturable uptake. The latter has a seven-times greater initial velocity when iron is deficient in comparison to conditions when adequate iron is available. Preliminary data suggest that *Arabidopsis IRT1* could encode this transporter.
18. Yi Y, Guerinot ML: **Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency**. *Plant J* 1996, **10**:835-844.
19. Degenhardt J, Larsen PB, Howell SH, Kochian LV: **Aluminum resistance in the *Arabidopsis* mutant *alr-104* is caused by an aluminum-induced increase in rhizosphere pH**. *Plant Physiol* 1998, **117**:19-27.  
A microelectrode was used to demonstrate a two-fold increase in H<sup>+</sup> influx upon exposure to Al(III) at the root surface of the Al(III) resistant mutant *alr-104*. This influx resulted in an overall increase of 0.1 pH units at the root surface.
20. Pellet DM, Grunes DL, Kochian LV: **Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays L.*)**. *Planta* 1995, **196**:788-795.
21. Larsen PB, Degenhardt J, Tai CY, Stenzler LM, Howell SH, Kochian LV: **Aluminum-resistant *Arabidopsis* mutants that exhibit altered patterns of aluminum accumulation and organic acid release from roots**. *Plant Physiol* 1998, **117**:9-18.  
Aluminum-resistant mutants of *Arabidopsis* mapping to one locus on chromosome I, *alr-108*, *alr-128*, *alr-131*, and *alr-139*, are shown to release greater amounts of citrate, malate, and/or pyruvate than wild-type plants and accumulate less aluminum.
22. de la Fuente JM, Ramirez-Rodríguez V, Cabrera-Ponce JL, Herrera-Estrella L: **Aluminum tolerance in transgenic plants by alteration of citrate synthesis**. *Science* 1997, **276**:1566-1568.
23. Fan TW, Colmer TD, Lane AN, Higashi RM: **Determination of metabolites by <sup>1</sup>H NMR and GC: analysis for organic osmolytes in crude tissue extracts**. *Anal Biochem* 1993, **214**:260-271.
24. Kinnerseley AM: **The role of phytochelates in plant growth and productivity**. *Plant Growth Regul* 1993, **12**:207-217.
25. Huang JW, Chen J, Berti WR, Cunningham SD: **Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction**. *Environ Sci Tech* 1997, **31**:800-805.
26. Chaney RL: **Plants can utilize iron from Fe-N,N'-DI-(2-hydroxybenzoyl)-ethylenediamine-N,N'-Diacetic acid, a ferric chelate with 10<sup>6</sup> greater formation constant than Fe-EDDHA**. *J Plant Nutrition* 1988, **11**:1033-1050.
27. Vassil AD, Kapulnik Y, Raskin I, Salt DE: **The role of EDTA in lead transport and accumulation by Indian mustard**. *Plant Physiol* 1998, **117**:447-453.  
The use of 0.5 mM or more EDTA resulted in plants accumulating >1% w/w lead into shoot tissues in Pb-EDTA complexes; a 75-fold increase over the

Pb concentration in the medium. Shoot Pb concentration is proportional to shoot EDTA levels and xylem sap shows the presence of Pb-EDTA complexes.

28. Baker AJ: **Metal hyperaccumulator plants: a review of the biological resource for possible exploitation in the phytoremediation of metal-polluted soils.** In *Phytoremediation of Contaminated Soil and Water*. Edited by Terry N, Bañuelos GS. Boca Raton, Florida: CRC Press LLC; 1999:85-107.
29. Kägi JH, Schaffer A: **Biochemistry of metallothionein.** *Biochemistry* 1988, **27**:8509-8515.
30. Chatthai M, Kaukinen KH, Tranbarger TJ, Gupta PK, Misra S: **The isolation of a novel metallothionein-related cDNA expressed in somatic and zygotic embryos of Douglas-fir: regulation by ABA, osmoticum, and metal ions.** *Plant Mol Biol* 1997, **34**:243-254.
31. Zhou J, Goldsbrough PB: **Structure, organization and expression of the metallothionein gene family in Arabidopsis.** *Mol Gen Genet* 1995, **248**:318-328.
32. O'Halloran T, O'Halloran SD: **Metal responsive gene regulation and the zinc metalloregulatory model.** In *Metal Ions in Biological Systems*. Edited by Sigel H. New York: Marcel Dekker; 1995:558-578.
33. Pan A, Tie F, Yang M, Luo J, Wang Z, Ding X, Li L, Chen Z, Ru B: **Construction of multiple copies of  $\alpha$ -domain gene fragment of human liver metallothionein I<sup>A</sup> in tandem arrays and its expression in transgenic tobacco plants.** *Protein Eng* 1993, **6**:755-762.
34. Pan A, Tie F, Duau Z, Yang M, Wang Z, Li L, Chen Z, Ru B:  **$\alpha$ -Domain of human metallothionein I<sup>A</sup> can bind to metal in transgenic tobacco plants.** *Mol Gen Genet* 1994, **242**:666-674.
35. Brouwer M, Hoexum-Brouwer T, Cashion RE: **A putative glutathione-binding site in CdZn-metalllothionein identified by equilibrium binding and molecular-modelling studies.** *Biochem J* 1993, **294**:219-225.
36. Salt DE, Rauser WE: **MgATP-dependent transport of phytochelatin across the tonoplast of oat roots.** *Plant Physiol* 1995, **107**:1293-1301.
37. Pickering IJ, Prince RC, George GN, Rauser WE, Wickramasinghe WA, Watson AA, Dameron CT, Dance IG, Fairlie DP, Salt DE: **X-ray absorption spectroscopy of cadmium phytochelatin and model systems.** *Biochem Biophys Acta* 1999, **1429**:351-364.
38. Howden R, Andersen CR, Goldsbrough PB, Cobbett CS: **A cadmium-sensitive, glutathione-deficient mutant of Arabidopsis thaliana.** *Plant Physiol* 1995, **107**:1067-1073.
39. Howden R, Goldsbrough PB, Andersen CR, Cobbett CS: **Cadmium-sensitive cad1 mutants of Arabidopsis thaliana are phytochelatin deficient.** *Plant Physiol* 1995, **107**:1059-1066.
40. Howden R, Cobbett CS: **Cadmium-sensitive mutants of Arabidopsis thaliana.** *Plant Physiol* 1992, **100**:100-107.
41. Zhu YL, Pilon-Smits EAH, Jouanin L, Terry N: **Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance.** *Plant Physiol* 1999, **119**:73-79.  
Expression of the *Escherichia coli gshII* gene in Indian mustard plants resulted in increased GSH and PC synthesis, suggesting glutathione synthetase catalyzes a rate-limiting step in PC synthesis.
42. Vatamaniuk OK, Mari S, Lu YP, Rea PA: **AtPCS1, a phytochelatin synthase from Arabidopsis: isolation and in vitro reconstitution.** *Proc Natl Acad Sci USA* 1999, **96**:7110-7115.  
Suppression cloning in yeast selected for heavy metal tolerance is used to isolate an *Arabidopsis* cDNA, *AtPCS1*, which encodes a 55 kDa putative phytochelatin synthase, gamma-glutamylcysteine dipeptidyl transpeptidase. Yeast expressing *AtPCS1* accumulate excess Cd(II).
43. Clemens S, Kim EJ, Neumann D, Schroeder JI: **Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast.** *EMBO J* 1999, **18**:3325-3333.  
Suppression cloning in yeast selected for heavy metal tolerance is used to isolate an expressed wheat cDNA, *TaPCS1*, encoding a 55 kDa PCS. PCS sequence homologues are identified in *Arabidopsis*, fission yeast, and lower animals; the first two of these also confer metal tolerance upon yeast. Genetic and biochemical studies demonstrate that this sequence in fission yeast encodes a *bona fide* PCS.
44. Baker AJM, Reeves RD, Hajar ASH: **Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl. (Brassicaceae).** *New Phytol* 1994, **127**:61-68.
45. Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC: **Free histidine as a metal chelator in plants that accumulate nickel.** *Nature* 1996, **379**:635-638.
46. Salt DE, Prince RC, Baker AJM, Raskin I, Pickering IJ: **Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray adsorption spectroscopy.** *Environ Sci Tech* 1999, **33**:713-717.
47. Salt DE, Kato N, Kramer U, Smith RD, Raskin I: **The role of root exudates in nickel hyperaccumulation and tolerance in accumulator and non-accumulator species of *Thlaspi*.** In *Phytoremediation of Contaminated Soil and Water*. Edited by Terry W, Bañuelos G. Berkeley, California: Ann Arbor Press, Inc.; 2000:191-202.
48. Keating MH, Mahaffey KR, Schoeny R, Rice GE, Bullock OR, Ambrose RB, Swartout J, Nichols JW: *Mercury Study Report to Congress*. EPA452/R-9-003. US Environmental Protection Agency; 1997:1-51.
49. Harada M: **Minamata disease: methylmercury poisoning in Japan caused by environmental pollution.** *Crit Rev Toxicol* 1995, **25**:1-24.
50. Choi SC, Chase JT, Bartha R: **Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS.** *Appl Environ Microbiol* 1994, **60**:4072-4077.
51. Boischio AA, Henshel DS: **Mercury contamination in the Brazilian Amazon. Environmental and occupational aspects.** *Water Air Soil Pollution* 1996, **80**:109-107.
52. Rugh CL, Wilde D, Stack NM, Thompson DM, Summers AO, Meagher RB: **Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene.** *Proc Natl Acad Sci USA* 1996, **93**:3182-3187.
53. Rugh CL, Senecoff JF, Meagher RB, Merkle SA: **Development of transgenic yellow-poplar for mercury phytoremediation.** *Nat Biotechnol* 1998, **16**:925-928.  
This paper reports the first trees engineered to clean-up a pollutant. Transgenic yellow poplar (*Liriodendron tulipifera*) calli and plants expressing the bacterial *merA* gene are shown to be resistant to Hg(II), which it detoxifies to rapidly volatilized metallic Hg(0).
54. Meagher RB, Rugh CL: **Phytoremediation of heavy metal pollution: ionic and methyl mercury.** In *OECD Biotechnology for Water Use and Conservation Workshop*. Cocoyoc, Mexico: Organization for Economic Co-Operation and Development; 1996:305-321.
55. Bizily S, Rugh CL, Summers AO, Meagher RB: **Phytoremediation of methylmercury pollution: *merB* expression in *Arabidopsis thaliana* confers resistance to organomercurials.** *Proc Natl Acad Sci USA* 1999, **96**:6808-6813.  
Plants expressing a bacterial organomercury lyase gene, *merB*, are resistant to a wide range of toxic levels of the environmental toxin methylmercury. Only a threshold level of MerB enzyme appears to be necessary to confer high level resistance, suggesting that complex kinetic and cell biological parameters effect resistance.
56. Bizily S, Rugh CL, Meagher RB: **Efficient phytodetoxification of the environmental pollutant methylmercury by engineered plants.** *Nat Biotechnol* 2000, in press.  
Plants expressing the two bacterial genes, *merB* and *merA*, are resistant to extremely high levels of the environmental toxin methylmercury. They volatilize 100-1000 times more Hg(0) than wild-type plants or controls expressing either gene alone. MerB enzyme levels appear to be rate limiting, but only account for 40% of the volatilization rate. Other unknown cell biological, biochemical, and kinetic parameters must play a significant role.
57. Pilon-Smits EA, Hwang S, Mel Lytle C, Zhu Y, Tai JC, Bravo RC, Chen Y, Leustek T, Terry N: **Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance.** *Plant Physiol* 1999, **119**:123-132.  
An *Arabidopsis* plastidic ATP sulfurylase gene (*APS1*) was over expressed in Indian mustard. APS1 activates not only sulfate but also selenate for uptake, and this appears to be a rate-limiting step for selenate reduction in plastids and for plant tolerance. Transgenic plants accumulate twice as much Se in shoots than do controls.
58. Neuhiel B, Bock A: **On the mechanism of selenium tolerance in selenium-accumulating plants: purification and characterization of a specific selenocystein methyltransferase from cultured cells of *Astragalus bisculatus*.** *Eur J Biochem* 1996, **239**:235-238.
59. de Souza MP, Pilon-Smits EA, Lytle CM, Hwang S, Tai J, Honma TS, Yeh L, Terry N: **Rate-limiting steps in selenium assimilation and volatilization by Indian mustard.** *Plant Physiol* 1998, **117**:1487-1494.  
The authors show that dimethylselenide is volatilized at two-to-three-times greater rates in plants supplied with selenite than those with selenate. Time-

dependent kinetic studies showed that selenate was taken up twice as fast as selenite. Selenate was rapidly translocated to the shoot, away from the root which is the site of volatilization, ten-times more efficiently than selenite.

60. Terry N, Zayed AM: **Selenium volatilization by plants.** In *Selenium in the Environment*. Edited by Frankenberger WT Jnr, Benson S. New York: Marcel Dekker, Inc.; 1994:343-376.
61. Zayed AM, Terry N: **Selenium volatilization in roots and shoots: effects of shoot removal and sulfate level.** *J Plant Physiol* 1994, **143**:8-14.
62. de Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N: **Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard.** *Plant Physiol* 1999, **119**:565-574.

When compared with axenic controls, plants inoculated with rhizosphere bacteria had five-times greater Se concentrations in roots (the site of volatilization) and four-times greater rates of Se volatilization. The presence of bacteria effected both root surface area and uptake.

63. Robinson NJ, Procter CM, Connolly EL, Guerinot ML: **A ferric-chelate reductase for iron uptake from soils.** *Nature* 1999, **397**:694-697.
- Iron deficiency afflicts > three billion people worldwide, and plants are the principal source of iron in most diets. The *Arabidopsis* *FRO2* gene complements *frd-1* mutations and encodes ferric-chelate reductase, a flavocytochrome that also transports electrons across a membrane. This activity is required for efficient iron uptake in many plants, and may result in the improved Fe-nutrition value of many crops.
64. Delhaize E: **A metal-accumulator mutant of *Arabidopsis thaliana*.** *Plant Physiol* 1996, **111**:849-855.
65. Tommasini R, Vogt E, Fromenteau M, Hortensteiner S, Matile P, Amrhein N, Martinoia E: **An ABC-transporter of *Arabidopsis thaliana* has both glutathione-conjugate and chlorophyll catabolite transport activity.** *Plant J* 1998, **13**:773-780.
- AtMRPs are the *Arabidopsis thaliana* homologues of the human multidrug resistant protein, MRP. The *Arabidopsis* gene *AtMRP3* is a homologue of the yeast genes *MRP1* and yeast cadmium factor 1 (*YCF1*), which are ATP-binding-cassette-type GCPs. A cadmium-sensitive yeast mutant lacking endogenous GCP is complemented by *AtMRP3* and definitive data are presented to show that *AtMRP3* is a GCP.
66. Lu YP, Li ZS, Drozdowicz YM, Hortensteiner S, Martinoia E, Rea PA: **AtMRP2, an *Arabidopsis* ATP binding cassette transporter able to transport glutathione S-conjugates and chlorophyll catabolites: functional comparisons with AtMRP1.** *Plant Cell* 1998, **10**:267-282.
67. Lu YP, Li ZS, Rea PA: **AtMRP1 gene of *Arabidopsis* encodes a glutathione S-conjugate pump: isolation and functional definition of a plant ATP-binding cassette transporter gene.** *Proc Natl Acad Sci USA* 1997, **94**:8243-8248.
68. Li ZS, Alfenito M, Rea PA, Walbot V, Dixon RA: **Vacuolar uptake of the phytoalexin medicarpin by the glutathione conjugate pump.** *Phytochemistry* 1997, **45**:689-693.
69. Anderson TA, Walton BT: **Comparative fate of [<sup>14</sup>C] trichloroethylene in the root zone of plants from a former solvent disposal site.** *Environ Toxicol Chem* 1996, **12**:2041-2047.

70. Anderson TA, Guthrie EA, Walton BT: **Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil.** *Environ Sci Tech* 1993, **27**:2630-2636.
71. Gordon M, Choe N, Duffy J, Ekuon G, Heilman P, Muiznieks I, Ruszaj M, Shurtleff BB, Strand S, Wilmoth J, Newman LA: **Phytoremediation of trichloroethylene with hybrid poplars.** *Environ Health Perspect* 1998, **106**(Suppl 4):1001-1004.
- Axenic tumor culture cells and cuttings of hybrid poplar (*Populus sp.*) transformed TCE to trichloroethanol, di- and trichloroacetic acid, and small amounts of CO<sub>2</sub> after several days of growth. In field trials, 95% of the TCE was removed from the influent water stream.
72. Wackett LP, Sadowsky MJ, Newman LM, Hur HG, Li S: **Metabolism of polyhalogenated compounds by a genetically engineered bacterium.** *Nature* 1994, **368**:627-629.
73. Best EP, Zappi ME, Fredrickson HL, Sprecher SL, Larson SL, Ochman M: **Screening of aquatic and wetland plant species for phytoremediation of explosives-contaminated groundwater from the Iowa Army Ammunition Plant.** *Ann NY Acad Sci* 1997, **829**:179-194.
74. Miskiniene V, Sarlauskas J, Jacquot JP, Cenas N: **Nitroreductase reactions of *Arabidopsis thaliana* thioredoxin reductase.** *Biochim Biophys Acta* 1998, **1366**:275-283.
- Arabidopsis thaliana* NADPH:thioredoxin reductase catalyzed redox cycling of nitroaromatic compounds, including the explosives 2,4,6-trinitrotoluene and tetryl, and the herbicide 3,5-dinitro-o-cresol.
75. Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R: **Transformation of TNT by aquatic plants and plant tissue cultures.** *Environ Sci Tech* 1997, **31**:266-271.
76. Goel A, Kumar G, Payne GF, Dube SK: **Plant cell biodegradation of a xenobiotic nitrate ester, nitroglycerin.** *Nat Biotechnol* 1997, **15**:174-177.
77. Bhadra R, Wayment DG, Hughes JB, Shanks JV: **Confirmation of conjugation processes during TNT metabolism by axenic plant roots.** *Environ Sci Technol* 1999, **33**:446-452.
- The authors use numerous physical and biochemical techniques to examine the products of TNT reduction by cultures of *Catharanthus roseus* (see Figure 2).
78. French CE, Rosser SJ, Davies GJ, Nicklin S, Bruce NC: **Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase.** *Nat Biotechnol* 1999, **17**:491-494.
- This work examines progress in the engineered phytoremediation of explosives. Expression of the bacterial pentaerythritol tetranitrate reductase gene in tobacco enables the growth and degradation of 1 mM glycerol trinitrate (GTN) or 0.05 mM trinitrotoluene (TNT), concentrations that inhibit the germination and growth of wild-type seeds.
79. Kas J, Burkard J, Demnerova K, Kostal J, Macek T, Mackova M, Pazlarova J: **Perspectives in biodegradation of alkanes and PCBs.** *Pure Appl Chem* 1997, **69**:2357-2369.
80. Mackova M, Macek T, Ocenaskova J, Burkhard J, Demnerova K, Pazlarova J: **Biodegradation of polychlorinated biphenyls by plant cells.** *Int Biodeterioration Biodegradation* 1997, **39**:317-324.