POTENTIAL OF PLANT GENETIC ENGINEERING FOR PHYTOREMEDIATION OF TOXIC TRACE ELEMENTS

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Keywords: phytoremediation, phytoextraction, heavy metals, chelation, metallothionein, phytochelatin, nicotianamine, glutathione, absorption, uptake, translocation, transporter, vacuolar storage, HMA, CDF, MTP, ZIP, OPT, YSL, ABC, MATE, field study

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Summary

Successful phytoremediation of toxic trace elements depend on many factors: plant genotype, the environment and the interactions between the plant genotype and its environment. Potential of plant genetic engineering is here discussed primarily in the framework of improving phytoextraction efficiency. Efforts to optimize remediation of Cd and Hg, two of the most harmful heavy metals and environmental threats, are

emphasized. Potential targets that have been or might be manipulated to improve transport, sequestration or detoxification processes are reviewed. Targets of genetic engineering include genes involved in the uptake of trace elements from the soil to the root, translocation from the root to the shoot, vacuolar sequestration, and chelation. Successes in genetically modifying plants to phytoextract Se or Hg are detailed.

1. General Introduction

The term phytoremediation simply means using plants and their associated microbes to clean up toxic substances in the environment (Terry et al., 2000). The many possible ways in which plants can achieve this include phytoextraction, phytovolatilization, detoxification, and sequestration. Phytoextraction and phytovolatilization are particularly attractive in that their use has the potential of moving the contaminants from the local ecosystem altogether. In phytoextraction, plants take up the contaminant of interest from soil, sediment, or water, and accumulate it. In order to achieve this, the plant may need some mechanism of protecting itself from the toxic effects of the contaminant. As such, phytoextraction often involves phytodetoxification – the conversion of a contaminant from a more toxic to less toxic form – or a means of internal sequestration to a less sensitive part of the plant, such as the vacuole. Phytoextraction is most successful when the plant is able to transport the contaminant from the roots to the shoots. In such a case, harvesting the aboveground biomass succeeds in removing the contaminant from the local ecosystem. Phytovolatilization is relevant to those trace element contaminants that can be metabolized to a volatile form. Well-studied examples are selenium (Se), volatile forms of which include dimethylselenide and dimethyldiselenide amongst several others, and mercury (Hg), which is volatile in its elemental form (Terry et al., 2000; Rugh et al., 1996). The advantage of phytovolatilization is clear – the plant does the work of synthesizing the volatile form and releases it to the atmosphere where it is diluted and joins the global cycle of the corresponding element.

It is important to remember that these processes occur naturally in plants, with wide variation observed depending on plant species (and ecotype), trace element contaminant, and geologic and environmental conditions. The primary advantages of phytoremediation are its low cost and low management requirements (Bañuelos et al., 2002). Growing plants is much cheaper than the excavating, removing, soil-washing, or capping technologies often used (Cunningham et al., 2000). Management of a phytoremediation plot becomes a matter of farming – fertilization, perhaps, and irrigation when needed, whereas bacteria-based bioremediation (biopiles) requires constant amendments (Cookson, 1995). The disadvantage is the long timescale that would be necessary for natural, phytoremediation processes to remediate a local environment.

Spurred on by the potential benefits of widespread implementation of phytoremediation, researchers have sought plant species that could remove contaminants at especially fast rates. Plants suitable for phytoextraction should possess exceptional qualities such as high capacities for absorption, root/shoot translocation, and detoxification of the metals or non-metals to be extracted and, last but not least, a high biomass and preferably a rapid growth rate.

High levels of tolerance to specific metals are known to occur but the majority of metal tolerant species are excluders. The combination of high tolerance and high accumulation of heavy metals can be found in a rare class of plants called hyperaccumulators. These species (more than 400 have been identified) can accumulate exceptional concentrations of heavy metals in their aerial parts without visible toxicity symptoms. Brooks defined a hyperaccumulator as a plant able to accumulate high concentrations of heavy metals in the shoot. The concentrations accumulated are about 100 times those that occur in non-accumulator plants growing in the same substrates (Brooks, 1998). Levels to define hyperaccumulators are 1% of shoot dry weight (SDW) for zinc (Zn), 0.1 % SDW for nickel (Ni), lead (Pb), copper (Cu), and cobalt (Co); and 0.01% SDW for cadmium (Cd). Hyperaccumulators of non-metal toxic trace elements like Se have also been identified (> 0.1% SDW). However, most hyperaccumulators do not develop sufficient biomass, even in the absence of high concentrations of heavy metals to be useful in phytoextraction and/or are not amenable to agronomic practices (mechanical harvest). However this argument does not apply to all hyperaccumulator species. Some of the 300 Ni hyperaccumulators identified, such as *Alyssum murale*, seem to develop sufficient biomass and consequently important mineral mass (Li et al. 2003, Chaney et al. 1997, 2005). *Stanleya pinnata* is a selenium hyperaccumulator that could also be used in phytoremediation as well (Feist and Parker 2001; Parker et al. 2003).

For other metals, hyperaccumulating plants have not yet been identified or seem to be very rare. With respect to Cd, for instance, only three low biomass, slow growing *Brassicacae* species (*Thlaspi caerulescens, Thlaspi praecox* and *Arabidopsis halleri*) have been described as hyperaccumulators. Moreover, their development of leaves in rosettes at the ground level impedes mechanical harvest. Nevertheless, some authors believed in the potential of the polymetallic hyperaccumulator, *Thlaspi caerulescens,* for direct use in phytoremediation (Chaney et al. 2005) because of its exceptional capacity of heavy metal (Zn, Cd, and Ni) accumulation.

To create suitable plants for phytoremediation, one possibility is the selection of high biomass plants like poplar, *Brassica juncea,* or tobacco with the highest tolerance and accumulation capacities, provided that the species displays sufficient genetic variation for those characteristics. Another avenue of recent research is the association of metal tolerant micro-organisms like bacteria or fungi to enhance tolerance. In this alternative, the genetic engineering of plant-associated bacteria seems promising (Barac et al. 2004; Taghavi et al. 2005 ; van der Lelie et al. 2005). Improvement of hyperaccumulator biomass is also envisaged by the genetic manipulation of plant hormone levels (Eapen and D'Souza 2005). However, no concrete example has been published up to now.

Finally, genetic engineering of plants can substantially improve phytoremediation efficiency of plants. This review will focus on the genetic engineering of plants to improve the efficiency of phytoextraction of heavy metals and Se (which is a non-metal) from contaminated soils. Of major concern with respect to health and environment concerns are the metalloids, arsenic and selenium, and the metals, cadmium and mercury. Genetic manipulation has aimed to improve accumulation, tolerance and detoxification capacities of high biomass and rapid growing plants in order to optimise the phytoextraction process.

2. General Consideration in Choosing Target Genes for Phytoremediation

Heavy metal tolerance is not a monogenic trait. Some researchers have proposed that there is no correlation between metal accumulation and tolerance and that these characters are independent, which means that several genes may have to be transferred to optimize phytoremediation performance (Baker and Walker 1990; Bert et al. 2003; Macnair et al. 1999). The choice of genes to be transferred is difficult. Many genes are involved in metal uptake, translocation, and sequestration. The genes to be transferred depend on the heavy metal to be extracted from the soil. At least three categories of genes are generally considered: transporters, proteins involved in metal chelation, and metabolic enzymes involved in detoxification. Transgenic plants with improved metal uptake and/or sequestration have been developed for cadmium, zinc, lead, mercury, arsenic and selenium.

The genes that have been transferred, thus far, are mainly cloned from micro-organisms for which the tolerance or detoxification mechanisms were known or from the non-tolerant, non-accumulator model plant species, *Arabidopsis thaliana* but usually not from hyperaccumulators. A lack of understanding of the mechanisms involved and responsible genes has impeded rapid progress. A common strategy to identify genes necessary to tolerate heavy metals or non-metals is to study plants that are naturally adapted to those toxic trace elements. Hyperaccumulators constitute an exceptional biological material and gene reservoir to understand adaptation to extreme metallic environments. Recently transcriptomic studies on hyperaccumulators have provided novel insights into the molecular mechanisms underlying metal tolerance and accumulation as well as access to the identification of a large array of genes which are constitutively (in the absence of excess of metallic ions) overexpressed and are thought to be involved in the hyperaccumulation trait (Weber et al. 2004; Weber et al. 2006; Becher et al. 2004; Talke et al. 2006; Craciun et al. 2006; van de Mortel et al. 2006, Filatov et al. 2006). Classic genetic studies and recent QTL analysis of hyperaccumulators support the existence of only few genes that are responsible for tolerance or accumulation (Assunção et al. 2006; Deniau et al. 2006, Courbot et al. 2007, Willems et al. 2007). Only one gene co-localizing with a QTL for Cd tolerance and Zn tolerance has been published (Courbot et al. 2007, Willems et al. 2007). Not understood is the origin of constitutive gene overexpression in hyperaccumulators. Interestingly, constitutive expression of existing genes seems to be a common process in the adaptation of plants to extreme environments (Taji et al. 2004).

Here we will focus on genes encoding for transporters or involved in metal chelation whose overexpression already conferred higher tolerance or accumulation to heavy metals and some non-metals or are thought to be good candidate genes to improve those traits in high biomass plants. Many proof of concept studies have been published with the model plant *Arabidopsis thaliana,* awaiting validation in plant species more amenable for phytoremediation. Up to now, the constitutive 35S CaMV promoter was extensively used. However to fine-tune toxic elements accumulation, targeted expression of genes encoding transporters or enzymes involved in chelation will be necessary in order to avoid futile cycles. For example, the constitutive overexpression (in all tissues of the plant), of a plasma membrane pump or transporter involved in the efflux of a toxic trace element may result in futile cycling inside the plant without

substantial accumulation in harvestable parts. Novel powerful tools to control the timing, the location and the level of transgene expression are expected from the characterization of native promoters of hyperaccumulators.

3. Genetic Engineering of Metal Transporters

Transport mechanisms of metal or metalloid ions are extremely complex and diverse. Metal uptake, metal partitioning to plant organs and cell types, and metal delivery in sub-cellular localizations including metal storage in vacuoles require metal transporters. For simplicity, the processes to be considered are either localized at the soil – root passage, root to shoot translocation, or at the cellular level, the storage to the vacuole from the cytoplasm. Depending on the limiting steps for the envisaged metals, one, two, or all three processes may need to be enhanced. Metal transporters that we will consider here in light of their relevance to phytoremediation are involved in

(1) uptake of metal from the soil to the root
(2) translocation from the root to the shoot
(3) detoxification by storage in the vacuoles

3.1. Uptake of Metal from the Soil to the Root

Several genes encoding transporters at the plasma membrane of root epidermal cells have been identified. There is to our knowledge no published example of genetic manipulation within the framework of phytoremediation to enhance that transport system in order to increase absorption of toxic metals or metalloids from the soil solution to the roots.

Toxic trace elements that we consider in this review (e.g. Cd, Hg, As, and Se) enter plant cells via uptake systems for essential ions. Some of the transporters of these essential ions have been identified, for example, by virtue of their induction upon mineral deficiency conditions, like Zn or Fe deficiency. For example, several ZIP (zinc-regulated transporter, iron-regulated transporter protein) transporters are induced upon deficiency and are involved in the cellular uptake of Zn across plasma membrane. Interestingly, several ZIP members are overexpressed in *A. halleri* and *T. caerulescens* (ZIP 3, 4, 6, 9, and 10 and IRT3) and are thought to play a role in the enhanced root metal uptake of those Zn hyperaccumulators (Kramer et al 2007). IRT1, one of the first discovered members of the large ZIP family, is involved in the uptake of Fe^{2+} from the soil. IRT1 is induced upon Fe deficiency and also is responsible for the uptake of heavy metals such as zinc, manganese, cobalt, and cadmium. Plants overexpressing IRT1, engineered to improve Fe nutrition, also accumulated more cadmium (Connolly et al. 2002).

Unless specific modifications of the transporters would increase their affinity towards the pollutants, overproduction of uptake systems for essential ions might deplete the soil in those nutrients. Nevertheless, there are several examples of enhanced absorption of metals as a result of increased capacity to sequester them in the vacuoles (see point 3 of this section).

3.2. Translocation from the Root to the Shoot

Translocation from the root to the shoot is a major step for phytoextraction, as above-ground biomass will be harvested. Efficient translocation of metal ions to the shoot requires radial symplastic passage, an active loading into the xylem, and the availability of suitable ligands in the xylem (Clemens, 2006).

The study of Zn and Cd hyperaccumulators has allowed the identification of P-ATPase as an important player for the passage from the cytosol of root cells into the vascular tissues.
-P-ATPase-HMA

The P_{1B}-type ATPases, also known as the Heavy Metal transporting ATPases (HMAs), play an important role in transporting transition metal ions against their electrochemical gradient using the energy provided by ATP hydrolysis. They have been identified in all living organisms from archaea to humans, including yeast and plants. The HMAs cluster into two classes: those transporting monovalent cations (Cu/Ag group) and those transporting divalent cations (Zn/Co/Cd/Pb group: Axelsen and Palmgren 2001). Although all classes of P_{1B}-type ATPases have been detected in prokaryotes (Rensing et al. 1999), in non-plant eukaryotes only Cu/Ag P_{1B}-type ATPases have been identified to date. *A. thaliana* and *Oryza sativa* contain eight *HMA* genes (Baxter et al. 2003), suggesting that these enzymes play important roles in the transport of metals in plants. In *A. thaliana*, analysis of the complete genome sequence predicts the distribution of these eight HMAs in two classes: HMA1-4 for the transport of Zn/Co/Cd/Pb, and HMA5-8 for Cu/Ag.

HMA4 was the first plant P_{1B}-type ATPase of the divalent transport group to be cloned and characterized in *A. thaliana* (Mills et al. 2003). A role for HMA4 in Zn homeostasis, Cd detoxification, and in the translocation of these metals from the root to the shoot has been demonstrated in *A. thaliana* (Hussain et al. 2004; Mills et al. 2003, 2005; Verret et al. 2004, 2005). In the Cd/Zn hyperaccumulators, *A. halleri* and *T. caerulescens, HMA4* is more highly expressed in both roots and shoots compared with Cd/Zn-sensitive close relatives (Bernard et al. 2004, Papoyan et al. 2004, Talke et al. 2006, Courbot et al. 2007). The elevated expression of *HMA4* in two different Zn/Cd hyperaccumulator species that evolved independently strongly supports the idea that HMA4 plays an important role in tolerance to both metals. In *A. halleri* at least, this hypothesis is reinforced by the QTL analysis of Cd and Zn tolerance performed, indicating the co-localization of major QTLs of Zn and Cd tolerance with the *HMA4* gene (Willems et al. 2007; Courbot et al. 2007). *HMA4* is currently the only gene for which there is genetic evidence for a role in both Zn and Cd tolerance. HMA4 would be involved in the detoxification of roots by translocating Cd and Zn to the shoot. As observed in AtHMA4-overexpressing lines (Verret et al. 2004), which translocate more Cd and Zn to the shoot and are also more tolerant to Cd and Zn excess, the increased expression of *HMA4* in *A. halleri* or *T. caerulescens* might be related to the increased Cd and Zn tolerance and hyperaccumulation phenotype of these species (Bernard et al. 2004, Courbot et al. 2007, Popayan et al. 2004, Talke et al. 2006 and Willems et al. 2007).

The study of hyperaccumulators has also revealed the overexpression of metal ligands or metal-ligand complexes transporters, which are thought to play a role in metal long-distance transport

MATE

MATE is a large family of multi-drug and toxin extrusion (or efflux) membrane proteins. Some members of the family were shown to function as drug/cation antiporters that remove toxic compounds and secondary metabolites from the cytosol by exporting them out of the cell or sequestering them to the vacuole (Delhaize et al. 2007). FRD3 is a member of the MATE subfamily, which is thought to efflux citrate in the root vasculature. Citrate is necessary for the transport of Fe and most likely also that of Zn (Durrett et al. 2007). Interestingly *FRD3* is constitutively overexpressed in *A. halleri* compared to its close relative *A. thaliana* and may play a role in Zn translocation (Talke et al. 2006). Few MATE members have been characterized in plants up to now. MATE members other than FRD3 might be involved in metal transport.

OPT:

OPT is a superfamily of oligopeptide transporters including the yellow-stripe 1-like (YSL) subfamily. The AtYSL gene family was identified in Arabidopsis following cloning and characterization of maize YS1. YS1 is a plasma membrane-localized transporter protein that is necessary for acquisition of Fe by uptake of Fe(III)-phytosiderophores (Curie et al., 2001). Dicotyledons do not synthesize phytosiderophores, but the structurally related non-protein amino acid nicotianamine (NA). Some YSL transporters are involved in the loading and unloading of nicotianamine-metal chelates. There is evidence for a role of YSL transporters in the Zn and Ni hyperaccumulation of *Thlaspi caerulescens*, especially for TcYSL3 and TcYSL7 expressed around the root vascular tissues (Gendre et al. 2007). YSL3 was shown to transport Ni-NA chelates.

Other OPT members may be involved in the transport of metal chelates, such as AtOPT6, which is able to transport glutathione derivatives and Cd-glutathione complexes (Cagnac et al. 2004). However no demonstration of a clear role relevant to phytoextraction has been published up to now.

3.3. Vacuolar Transporters

For metals that cannot be volatilised, vacuolar storage in the leaves is thought to be the main pathway of detoxification, and this process is usually limiting in non-tolerant species.

CDF-MTP:

The family of Cation Diffusion Facilitators also named in plants Metal Transporter Proteins contains members involved in the transport of Zn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} not only from cytoplasm to organelles or apoplasm, but also from the cytoplasm to the endoplasmic reticulum (Peiter et al. 2007). ZAT (Zinc transporter of *Arabidopsis thaliana*) recently renamed AtMTP1 encodes a Zn transporter suggested to be involved

in vacuolar sequestration in *Arabidopsis thaliana*. Overproduction of the Zn transporter ZAT in *A. thaliana* resulted in higher Zn tolerance and a 2-fold higher Zn accumulation in roots (Van der Zaal et al. 1999). ShMTP is involved in the vacuolar storage of Mn in the Mn tolerant *Stylosanthes hamata*, which is a tropical legume. *ShMTP* conferred higher tolerance and accumulation of Mn when overexpressed in *Arabidopsis thaliana* (Delhaize et al. 2003).

CAX:

CAX is the acronym of CAtion eXchanger. It is a large family of membrane proteins, which was recently subdivided in "true" CAX and CCH (calcium cation exchanger). Some of the CAX members of *A. thaliana* have been characterized, and all seem to be involved in metal vacuolar sequestration up to now. *In vitro* studies have shown a Cd^{2+}/H^+ antiport activity in tonoplast-enriched vesicles from oat roots with a K_m of 5.5 µM (Salt and Wagner 1993). In his review, Clemens questioned the availability of Cd^{2+} ions in the micromolar range (Clemens, 2006). Still, there is experimental evidence that AtCAX2 and AtCAX4 can transport Cd^{2+} in the vacuoles. Under the activity of the constitutive 35S CaMV promoter, expression of *AtCAX2* and *AtCAX4* resulted in higher accumulation of Cd in the root vacuoles (Korenkov et al. 2007). It would be interesting to test overexpression of CAX vacuolar transporters using a leaf specific promoter to see whether it can also increase Cd accumulation in the leaves.

ABC:

The superfamily of ABC (ATP-Binding Cassette) transporters is involved in many physiological processes. Some of the ABC transporters are involved in vacuolar sequestration of various metals or xenobiotics. In two subfamilies, MRP and PRD, members are involved in the transport of chelated heavy metals or in the organic acid necessary for the transport of heavy metals.

Overexpression of the YCF1 MRP member of *Saccharomyces cerevisiae* conferred higher capacity of lead and cadmium accumulation in *Arabidopsis thaliana* by enhanced transport of Cd-GS2 in the vacuoles (Song et al. 2003).

Hmt1 is the transporter (ABC half-size family) of PC-Cd complexes in the vacuoles of *S. pombe* (Ortiz et al. 1995). Enhancing this process may be relevant in the framework of optimizing phytoremediation efficiency. However, there is no true homolog of Hmt1 identified in any plant up to now, and overexpression of *Hmt1* in plants has not been reported thus far. The closest homolog in plant is the mitochondrial ATM3, also involved in Cd tolerance (Hanikenne et al. 2005; Kim et al. 2006)

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Bibliography

Andres-Colas N., Sancenon V., Rodriguez-Navarro S., Mayo S., Thiele D.J., Ecker J.R., Puig S., Penarrubia L. (2006) The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. *Plant J.* 45: 225–236. [This article presents the characterization of a heavy metal ATPase, including results supporting the interaction of the transporter with the copper metallochaperone, CCH.]

Arisi A.C.M., Noctor G., Foyer C.H., Jouanin L. (1997) Modification of thiol contents in poplars (*Populus tremula* x *P. alba*) overexpressing enzymes involved in glutathione synthesis. *Planta* 203: 362–372. [The characterization of transgenic poplars overexpressing the E. coli gene for γ-glutamyl-cysteine synthetase in the cytosol and containing enhanced foliar levels of glutathione is presented.]

Assunção A.G.L., Pieper B., Vromans J., Lindhout P., Aartsand M.G.M., Schat H. (2006) Construction of a genetic linkage map of *Thlaspi caerulescens* and quantitative trait loci analysis of zinc accumulation. *New Phytologist* 170: 21–32. [The paper presents the identification of 2 significant QTL for Zn accumulation in the Zn hyperaccumulation Thlaspi caerulescens.]

Axelsen K.B., Palmgren M.G. (2001) Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. Plant Physiol 126: 696–706. *This review presents the 5 subfamilies of P-Type ATPases in Arabidopsis thaliana.*

Baker A.J.M., Walker P.L. (1990) Ecophysiology of metal uptake by tolerant plants. In AJ Shaw, ed, *Heavy Metal Tolerance in Plants: Evolutionary Aspects.* CRC Press, Boca Raton FL: 155–177 [This book chapter summarizes the relationships between uptake, translocation and accumulation of metals. The three basic strategies of metal uptake by plants in relation to substrate concentrations (excluder, indicator, accumulator) are presented].

Balogh S.J., Huang Y., Offerman H.J., Meyer M.L., Johnson D.K. (2002) Episodes of elevated methylmercury concentrations in prairie streams. *Environ. Sci. Technol.* 36: 1665-1670. [This paper describes how leaves from trees growing on mercury-contaminated sediment transfer methylmercury to the aquatic environments in which they fall.]

Bañuelos G.S., Schrale G. (1989) Crop selection for removing selenium from the soil. *Calif. Agric.* 43: 19-20. [This paper demonstrates that secondary accumulators with fast growth rates and higher biomass, such as Brassica juncea, are effective for phytoremediation.]

Bañuelos G.S., Ajwa H.A., Mackey B., Wu L., Cook C., Akohoue S., Zambrzuski S. (1997) Evaluation of different plant species used for phytoremediation of high soil selenium. *J. Environ. Qual.* 26: 639-646. [This paper demonstrates that secondary accumulators with fast growth rates and higher biomass, such as Brassica juncea, are effective for phytoremediation.]

Bañuelos G.S., Lin Z.Q., Wu L., Terry N. (2002) Phytoremediation of selenium-contaminated soils and waters: fundamentals and future prospects. *Rev. Environ. Health* 17:291-306. [This review defines phytoremediation and explores the future potential of this low-cost and environmentally friendly technology.]

Bañuelos G., Terry N., LeDuc D.L., Pilon-Smits E.A.H., Mackey B. (2005) Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. *Environ. Sci. Technol.* 39: 1771-1777. [This report is the first successful phytoremediation field trial using genetically-engineered plants.]

Barac T., Taghavi S., Borremans B., Provoost A., Loeyen J.V., Colpaert J., Vangronsveld J., van der Lelie D. (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble volatile organic pollutants. *Nature* Biotechnol. 22: 583-588. [The authors show the genetic manipulation of lupine endophyte to degrade toluene and the successful use of the engineered endophytic bacteria-lupine system in phytoremediation of these toxic organic xenobiotics.]

Baxter I., Tchieu J., Sussman M.R., Boutry M., Palmgren M.G., Gribskov M., Harper J.F., Axelsen K.B. (2003) Genomic comparison of P-type ATPase ion pumps in *Arabidopsis* and rice. *Plant Physiology* 132: 618–628. [This manuscript presents the high diversity of P-Type ATPases in plants and the high conservation between a monocot and a dicot in all five major sub-families of P-type ATPases.]

Becher M., Talke I.N., Krall L., Kramer U. (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. *Plant J* 37: 251–268. [Arabidopsis thaliana gene chips were here used to compare the transcriptional profile of the Zn hyperaccumulator Arabidopsis halleri with the non-tolerant, non-accumulator, A. thaliana, in different Zn conditions. Results indicate high constitutive expression of metal homeostasis genes in A. halleri.]

Bennett L.E., Burkhead J.L., Hale K.L., Terry N., Pilon M., Pilon-Smits E.A.H. (2003) Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *J. Environ. Qual.* 32:432-440. [This paper can be seen as a precursor to the first field trial using genetically engineered plants in that three transgenic Indian mustard lines were evaluated with respect to their tolerance and phytoextraction capabilities on metal-contaminated sediment.]

Bernard C., Roosens N., Czernic P., Lebrun M., Verbruggen N. (2004) A novel CPx-ATPase from the cadmium hyperaccumulator *Thlaspi caerulescens*. *FEBS Letters* 569: 140-148. [This article reports on the functional screen of a cDNA library of the Cd hyperaccumulator Thlaspi caerulescens in Saccharomyces cerevisiae on toxic cadmium concentration and the identification of a pump able to transport Cd^{++}.]

Bert V., Meerts P., Saumitou-Laprade P., Salis P., Gruber W., Verbruggen N. (2003) Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant Soil* 249: 9–18. [This article presents a first genetic analysis of Cd accumulation and Cd tolerance characteristics in a Cd hyperaccumulator ecotype of Arabidopsis halleri.]

Bizily S.P., Rugh C.L., Summers A.O., Meagher R.B. (1999) Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. *Proc. Natl. Acad. Sci. USA* 96: 6808-6813. [This manuscript demonstrates that the overexpression of merB in plants results in increased tolerance to organomercurials.]

Bizily S., Rugh C.L., Meagher R.B. (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. *Nat. Biotechnol.* 18: 213-217. [This represents the first construction and characterization of plants overexpressing both merA and merB from the bacterial mer operon.]

Bizily S.P., Kim T., Kandasamy M.K., Meagher, R.B. (2003) Subcellular targeting of methylmercury lyase enhances its specific activity for organic mercury detoxification in plants. *Plant Physiology* 131: 463-471. [This paper suggests that judicious localization of protein expression through modification of the transgene can result in huge gains in metal detoxification.]

Brandle J. E., Labbe H., Hattori J., Miki B.L. (1993) Field performance and heavy metal concentrations of transgenic flue-cured tobacco expressing a mammalian metallothionein-beta-glucuronidase gene fusion. *Genome* 36: 255-260. [This reference reports on results of a field test in Canada with tobacco plants expressing a mammalian metallothionein fused to β-glucuronidase under the activity of a constitutive 35S promoter. The paper highlights the need to test performance of transgenic plants in field conditions.]

Brooks R.R. (1998) Plants that hyperaccumulate heavy metals. CAB International Wallingford 379. [Brooks has been one of the major botanists to work on hyperaccumulation. This book is an overview of the phenomenology, biology and applications of hyperaccumulation]

Burnell J.N. (1981) Selenium metabolism in *Neptunia amplexicaulis*. *Plant Physiology* 67: 316-324. [This paper suggested that selenium metabolism parallels, in many instances, that of sulfur such that selenate can also serve as a substrate for the enzyme ATP sulfurylase.]

Cagnac O., Bourbouloux A., Chakrabarty D., Zhang M.Y., Delrot S. (2004) AtOPT6 transports glutathione derivatives and is induced by primisulfuron. *Plant Physiology* 135: 1378-1387. [Results presented in this manuscript support a role for the OPT6 Arabidopsis transporter in the uptake of glutathione derivatives and metal complexes, in addition to peptide transport.]

Chaney R., Malik M., Li Y. M., Brown S.L., Brewer E.P., Angle J.S., Baker A.J.M. (1997) Phytoremediation of soil metals. *Curr. Opin. Biotechnol.* 8: 279–284. [This review highlights the unique properties of metal hyperaccumulators and the biotechnology approaches to develop phytoremediation plants.]

Chaney R.L., Angle J.S., McIntosh M.S., Reeves R.D., Li Y.M., Brewer E.P., Chen K.Y., Roseberg R.J.,

Perner H., Synkowski E.C., Broadhurst C.L., Wang S., Baker A.J. (2005) Using hyperaccumulator plants to phytoextract soil Ni and Cd. *Z. Naturforsch* 60:190-198. [This review explains the phytoremediation assays of Cd with efficient Southern France populations of Thlaspi caerulescens and of Ni with Alyssum murale.]

Che D., Meagher R. B., Heaton A. C. P. , Lima A., Rugh C. L., Merkle S. A. (2003) Expression of mercuric ion reductase in Eastern cottonwood (Populus deltoides) confers mercuric ion reduction and resistance. *Plant Biotechnology Journal* 1 (4) , 311-319. [This paper explains the genetic engineering and analysis of merA cottonwood plants.]

Chen A., Komives E.A., Schroeder I.J. (2006) An improved grafting technique for mature Arabidopsis plants demonstrates long-distance shoot-to-root transport of phytochelatins in Arabidopsis. *Plant Physiology* 141: 108–120. [The article shows the analysis of the phytochelatin deficient cad1 mutant expressing the wheat phytochelatin synthase 1 gene in the shoot. Results support a role for PC in long-distance shoot-to-root transport.]

Chen Y., Leustek T., Lee S. (1997) Analysis of ATP sulfurylase overexpression in *Arabidopsis thaliana* (abstract no. 702). *Plant Physiology* 117: 1487-1494. [This seminal abstract demonstrating how ATP sulfurylase expression varied under conditions such as sulfur deficiency provided the motivation for overexpressing ATP sulfurylase to improve plants' phytoremediation potential]

Clark D.L., Weiss A.A., Silver S. (1977) Mercury and organomercurial resistances determined by plasmids in *Pseudomonas. J. Bacteriol*. 132: 186-196. [This manuscript demonstrates that mercury resistance in Pseudomonas is due to the presence of plasmids encoding mercury-detoxification enzymes.]

Clarkson T.W. (1994) In *The Toxicology of Mercury and Its Compounds*, eds. Watras, C.J. and Huckabee, J.W. (Lewis, Ann Arbor, MI): 631-642. [This book provides a review and comparison of the extent and mechanism of toxicity of different mercury compounds.]

Clemens S., Kim E.J., Neumann D., Schroeder J.L. (1999) Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. *EMBO J*. 18: 3325–3333. [This article reports on the cloning of a wheat phytochelatin synthase 1 gene by a screen in Saccharomyces cerevisiae on cadmium.]

Clemens S. (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 88: 1707–1719. [This review discusses the molecular mechanisms of toxic metal accumulation and tolerance with a main emphasis on cadmium.]

Compeau G.C., Bartha R. (1985) Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol*. 50: 498-502. [This paper is the first to identify sulfate-reducing bacteria, in this case, Desulfovibrio desulfuricans, as responsible for methylating mercury.]

Cobbett C.S. and Goldsbrough P.B. (2000) Mechanisms of metal resistance: Phytochelatins and metallothioneins. p. 247-271. In I. Raskin and B.D. Ensly (ed.) *Phytoremediation of toxic metals – Using plants to clean up the environment*. John Wiley & Sons, New York. [This book chapter reviews how metal toxicity is decreased through chelation of the thiol-containing phytochelatins and metallothioneins.]

Connolly E.L., Fett J.P., Guerinot M.L. (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell* 14: 1347–1357. [The results of the Fe root transporter IRT overexpression highlight the complexity of IRT1 expression regulation.]

Cookson J.T. (1995) *Bioremediation Engineering, Design and Application*. New York, McGraw-Hill, Inc. [This book describes the advantages, disadvantages, and potential applications of bacteria-based bioremediation strategies.]

Courbot M., Willems G., Motte P., Arvidsson S., Roosens N., Saumitou-Laprade P., Verbruggen N. (2007) A major quantitative trait locus for cadmium tolerance in *Arabidopsis halleri* colocalizes with HMA4, a gene encoding a heavy metal ATPase. *Plant Physiology* 144: 1052–1065. [This article shows the identification of 3 QTL for Cd tolerance in Arabidopsis halleri and the functional characterization of a gene encoding a Cd/Zn pump, co-localizing with the peak of the major QTL.]

Craciun A., Courbot M., Bourgis F., Salis P., Saumitou Laprade P., Verbruggen N. (2006) Comparative cDNA-AFLP analysis of Cd-tolerant and -sensitive genotypes derived from crosses between the Cd hyperaccumulator *Arabidopsis halleri* and *Arabidopsis lyrata ssp. petraea. Journal of Experimental Botany* 57: 2967-2983. [This article reports on a global transcriptomic analysis of a cross segregating for

Cd tolerance, between the Cd hyperaccumulator, Arabidopsis halleri, and the non-tolerant, non-accumulator, Arabidopsis lyrata ssp petrae. The results support constitutive (under normal growth conditions) expression of many members of gene families, not only those directly involved in metal homeostasis.]

Cunningham S.D. and Berti W.R. (2000) Phytoextraction and phytostabilization: technical, economic and regulatory considerations of the soil-lead issue. In *Phytoremediation of Contaminated Soil and Water*, CRC Press: Boca Raton, FL. [This book chapter compares phytoremediation with other remediation strategies in terms of cost, requirements, and potential for effective toxin clean up.]

Curie C., Panaviene Z., Loulergue C, Dellaporta S.L., Briat J.F., Walker E.L. (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature* 409: 346-349. [The manuscript presents the cloning of the first iron-phytosiderophore transporter in a monocotyledon.]

Czakó M., Feng X., He Y., Liang D., Márton L. (2006) Transgenic *Spartina alterniflora* for phytoremediation. *Environ. Geochem. Health* 28: 103-110. [This paper reports the successful transformation of the wetland plant, Spartina alterniflora, with the merA/merB genes which is especially critical since wetlands environments are in most need of mercury remediation.]

Daniell H., Muthukumar B., Lee, S.B. (2001) Marker free transgenic plants: engineering the chloroplast genome without the use of antibiotic selection. *Curr. Genet.* 39: 109-116. [This paper discusses progress made in creating chloroplast-engineered plants without using antibiotic markers, eliminating one of the primary public concerns about transgenic plants.]

Daniell H., Khan K.S., Allison L. (2002) Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology. *Trends Plant Sci.* 7: 84-89. [This paper reviews how the use of chloroplast engineering to make transgenic plants may limit potential negative impacts, such as the spread of transgenes.]

Dhankher O.P., Li Y., Rosen B.P., Shi J., Salt D., Senecoff J.F., Sashti N.A., Meagher R.B. (2002) Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression. *Nat Biotechnol*. 20:1140-1145. [Arabidopsis thaliana transgenic plants, expressing the Escherichia coli arsenate reductase (ArsC) gene, which catalyzes the glutathione (GSH)-coupled reduction of arsenate to arsenite and the E. coli gene encoding gamma-glutamylcysteine synthetase (gamma-ECS) showed arsenic tolerance. When grown on arsenic, these plants accumulated 4- to 17-fold greater fresh shoot weight and accumulated 2- to 3-fold more arsenic per gram of tissue than wild type or plants expressing gamma-ECS or ArsC alone.]

Delhaize E., Kataoka T., Hebb D.M., White R.G., Ryan P.R. (2003) Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *The Plant Cell* 15: 1131–1142. [The paper describes the identification of a member of the cation diffusion facilitator family in the Mn^{++} tolerant tropical legume Stylosanthes hamata that is able to increase Mn^{++} tolerance by sequestering Mn^{++} into vacuoles.]

Delhaize E., Gruber B.D., Ryan P.R. (2007) The roles of organic anion permeases in aluminium resistance and mineral nutrition. *FEBS Letters* 581: 2255-2262. [This review highlights the roles of ALMT and MATE transporters in aluminium resistance and mineral nutrition.]

Deniau A., Pieper B., Ten Bookum W.M., Lindhout P., Aarts M.G.M., Schat H. (2006) QTL analysis of cadmium and zinc accumulation in the heavy metal hyperaccumulator *Thlaspi caerulescens. Theor. Appl. Genet*. 113: 907–920. [The identification of 2 QTL for Cd accumulation in root, 3 QTLs for Zn accumulation in shoot and one QTL for Cd accumulation in shoot are reported in this study.]

de Souza M.P., Pilon-Smits E.A.H., Lytle C.M., Hwang S., Tai J., Honma T.S.U., Yeh, L., Terry N. (1998) Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. *Plant Physiology* 117: 1487-1494. [This paper identified the enzymatic step catalysed by ATP sulfurylase as being rate-limiting for selenium assimilation in Indian mustard, spurring on the construction and characterization of APS transgenic plants.]

Dilworth G.L. and Bandurski R.S. (1977) Activation of selenate by adenosine 5'-triphosphate sulfurylase from *Saccharomyces cerevisiae. Biochem. J*. 163: 521-529. [This paper reports that selenate, in addition to sulfate, serves as a substrate for ATP sulfurylase.]

Dominguez-Solis J.R., Lopez-Martin C., Ager F.J., Ynsa D., Romero L.C., Gotor C. (2004) Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana*. *Plant Biotechnology Journal* 2: 469-476. [This manuscript reports on the enhancement of cysteine synthesis.]

Douchkov D., Gryczka C., Stephan U.W., Hell R., Baümlein H. (2005) Ectopic expression of nicotianamine synthase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant, Cell & Environment* 28: 365–374. [The paper explains the genetic engineering of tobacco plants to produce elevated levels of nicotianamine and reports on their analysis.]

Durrett T.P., Gassmann W., Rogers E.E. (2007) The FRD3-Mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiology* 144: 197–205. [FRD3 is a member of the MATE (multidrug and toxin efflux) family of transporters. The analysis of a frd3 mutant and of the FRD3 gene shows that FRD3 is involved in the efflux of citrate and the long-distance transport of Fe as ferric-citrate complex from the root to the shoot.]

Eapen S., D'Souza S.F. (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnology Advances* 23: 97-114. [This is a review about the potential of plant genetic engineering in the remediation of metallic and non-metallic toxic elements in soils.]

Elmayan T., Tepfer M. (1994) Synthesis of a bifunctional metallothionein/β-glucuronidase fusion protein in transgenic tobacco plants as a means of reducing leaf cadmium levels. *The Plant Journal* 6: 433-440. [This paper reports on the engineering and analysis of tobacco plant expressing a mammalian metallothionein-β-glucuronidase fusion under the activity of a constitutive strong promoter.]

Ernst W.H.O., Krauss G.-J., Verkleij J. A.C., Wesenberg D. (2008) Interaction of heavy metals with the sulphur metabolism in angiosperms from an ecological point of view. *Plant Cell and Environment* 31: 123-143. [The metabolism of sulfur in angiosperms is reviewed under the aspect of exposure to ecologically relevant concentrations of sulphur, heavy metals and metalloids.]

Evans K.M., Gatehouse J.A., Lindsay W.P., Shi J., Tommey A.M., Robinson N.J. (1992) Expression of the pea metallothionein like gene Ps MTA in *Escherichia coli* and *Arabidopsis thaliana* and analysis of trace metal ion accumulation: implications of Ps MTA function. *Plant Mol Biol* 20: 1019-1028. [This manuscript shows that a metallothionein gene from pea, overexpressed in Escherichia coli cells and in Arabidopsis thaliana plants, can increase Cu accumulation in bacterial cells in roots of transgenic plants compared to their respective controls.]

Farago S. and Brunold C. (1990) Regulation of assimilatory sulfate reduction by herbicide safeners in *Zea mays* L. *Plant Physiology* 94: 1808-1812. [In this paper, the increase in cysteine and glutathione concentrations induced by certain herbicide safeners is correlated with increased ATP sulfurylase expression.]

Feist L.J., Parker D.R. (2001) Ecotypic variation in selenium accumulation among populations of *Stanleya pinnata*. *New Phytol.* 149: 61-69. [This manuscript reports on selenium hyperaccumulation among the Brassicaceae Stanleya pinnata and on perspectives in phytoremediation.]

Filatov V., Dowdle J., Smirnoff N., Ford-Lloyd B., Newbury H.J., Macnair M.M. (2006) Comparison of gene expression in segregating families identifies genes and genomic regions involved in a novel adaptation, zinc hyperaccumulation. *Mol Ecol* 15: 3045–3059. [The article presents a combination of genomics (transcriptional profile) and classical genetics (QTL analysis) to identify genes involved in the Zn hyperaccumulation character of Arabidopsis halleri.]

Foster T.J. (1983) Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. *Microbiol. Rev.* 47: 361-409. [This comprehensive review discusses the information known about plasmid-located enzymes in bacteria and their role in resistance to antibiotics and metals, with a section specifically about mercury.]

Foster T.J. and Brown N.L. (1985) Identification of the merR gene of R100 by using mer-lac gene and operon fusions. *J. Bacteriol.* 163:1153-1157. [This paper corrects the reading frame and sequence of the merR gene in the pBR322 plasmid.]

Fox B. and Walsh C.T. (1982) Mercuric reductase. Purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. *J. Biol. Chem.* 257: 2498-2503. [This paper reports the first purification of a mercuric reductase, in this case from Pseudomonas aeruginosa PAO9501 carrying the plasmid pVS1.]

Gallego S.M., Benavides M.P., Tomaro M.L. (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Sci.* 121: 151-159. [This paper studies the effect of Fe^{2+}, Cu^{2+}, and Cd^{2+} on superoxide dismutase, other antioxidant enzymes, chlorophyll content, and glutathione demonstrating that these metals induce oxidative stress in plants.]

Gendre D., Czernic P., Conéjéro G., Pianelli K., Briat J.-F., Lebrun M., Mari S. (2007) TcYSL3, a member of the YSL gene family from the hyper-accumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. *The Plant Journal* 49:1–15. [YSL transporters are involved in the loading and unloading of nicotianamine-metal chelates. This manuscript provides strong support for a role for YSL transporters in the overall scheme of NA and NA-metal, particularly NA-Ni, circulation in the Zn, Ni hyperaccumulator plant, Thlaspi caerulescens.]

Gilmour C.C., Henry E.A., Mitchell R. (1992) Sulfate stimulation of mercury methylation in freshwater sediments. *Environ. Sci. Technol.* 26: 2281-2287. [This paper demonstrates that experimentally added sulfate stimulated mercury methylation by sulfate-reducing bacteria.]

Gorinova N., Nedkovska M., Todorovska E., Simova-StoilovaL., Stoyanova Z., Georgieva K., Demirevska-Kepova K., Atanassov A., Herzig R. (2007) Improved phytoaccumulation of cadmium by genetically modified tobacco plants *(Nicotiana tabacum* L.). Physiological and biochemical response of the transformants to cadmium toxicity. Environmental Pollution 145: 161-170. *The article presents the analysis of Cd tolerance and Cd accumulation of tobacco plants overexpressing a metallothionein gene from Silene vulgaris.*

Gray D.H. and Sotir R.B. (1996) *Biotechnical and soil bioengineering stabilization.* John Wiley & Sons, New York. [This book includes a relevant discussion about the inherent advantages trees possess in phytoremediation applications.]

Grill E., Löffler S., Winnacker E.L., Zenk M.H. (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific gamma glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase), *Proc. Natl. Acad. Sci. USA* 86: 6838–6842. [This is a study of the activity of phytochelatin synthase in Silene cucubalus cell suspension cultures upon different metallic treatments. It shows that Cd^{++} is by far the best metal activator of the enzyme.]

Grill E., Winnacker E.L., Zenk M.H. (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science* 230: 674–676. [The manuscript is a landmark in the study of phytochelatins and reports on the isolation of novel heavy-metal complexing peptides, which are induced upon heavy metal treatment.]

Ha S.B., Smith A.P., Howden R., Dietrich W.M., Bugg S., O'Connell M.J., Goldsbrough P.B., Cobbett C.S. (1999) Phytochelatin synthase genes from *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. *Plant Cell* 11: 1153–1163. *This is one of the first papers to report cloning of a phytochelatin synthase gene from plants.*

Hamlett N.V., Landale E.C., Davis B.H., Summers A.O. (1992) Roles of the Tn21 merT, merP, and merC gene products in mercury resistance and mercury binding. *J. Bacteriol.* 174: 6377-6385. [This paper defines roles in mercury binding and transport for the merT and merP gene products but leaves the function of merC undetermined.]

Hanikenne M., Krämer U., Demoulin V., Baurain D. (2005) A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae*. *Plant Physiology* 137: 428–446. [This article is an overview of plant metal transporters, with an inventory of putative metal transporters in two unicellular algal models, the green alga Chlamydomonas reinhardtii and the red alga Cyanidioschizon merolae.]

Hansen D., Duda P., Zayed A.M., Terry N. (1998) Selenium removal by constructed wetlands: role of biological volatilization. *Environ. Sci. Technol.* 32: 591-597. [The authors studied a wetland filled with effluent from a Chevron oil refinery containing selenite and found that a significant portion of the incoming selenium ends up being volatilized.]

Harada M. (1995) Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit. Rev. Toxicol.* 25: 1-24. [This review describes the classic story of methylmercury poisoning in Japan, the study of which has likely contributed the most to our understanding of its toxicity in the environment and man.]

Hatzfeld Y., Cathala N., Grignon C., Davidian J.-C. (1998) Effect of ATP sulfurylase overexpression in bright yellow 2 tobacco cells. *Plant Physiology* 116: 1307-1313. [This paper examines the effects of ATP sulfurylase expression in a culture of tobacco cells and finds, in contrast to later work in Brassica juncea, no increase in sulfate uptake or selenate tolerance.]

Hatzfeld Y., Lee S., Lee M., Leustek T., Saito K. (2000) Functional characterization of a gene encoding a fourth ATP sulfurylase isoform from *Arabidopsis thaliana*. *Gene* 248: 51-58. [The authors describe a fourth plastid-localized ATP sulfurylase isoform from Arabidopsis thaliana whose expression is not induced by sulfur starvation or treatment with O-acetylserine.]

Heiss S., Schäfer H.J., Haag-Kerwer A., Rausch T. (1999) Cloning sulfur assimilation genes of *Brassica juncea* L.: cadmium differentially affects the expression of a putative low-affinity sulfate transporter and isoforms of ATP sulfurylase and APS reductase. *Plant Mol. Biol.* 39:847-857. [The authors report the cloning of genes encoding a sulfate transporter, ATP sulfurylase, and APS reductase from Brassica juncea and demonstrated that expression of the latter two are induced by cadmium.]

Hussain D., Haydon M.J., Wang Y., Wong E., Sherson S.M., Young J., Camakaris J., Harper J.F., Cobbetta C.S. (2004) P-Type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *The Plant Cell* 16: 1327–1339. [This paper demonstrates the essential role of HMA2 and HMA4 in Zn translocation from the root to the shoot in Arabidopsis thaliana.]

Hussein H. S., Ruiz O.N., Terry N., Daniell H. (2007) Phytoremediation of mercury and organomercurials in chloroplast transgenic plants: enhanced root uptake, translocation to shoots, and volatilization. *Environ. Sci. Technol.* 41: 8439–8446. [The authors investigate Hg uptake and translocation in chloroplast transgenic tobacco plants expressed the merA,B operon.]

Ingle R.A., Mugford S.T., Rees J.D., Campbell M.M., Smith J.A.C. (2005) Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. *The Plant Cell* 17: 2089–2106. [This study further demonstrates the importance of histidine for the Ni hyperaccumulation in Alyssum lesbiacum and the effect of enhancing histidine biosynthesis by genetic transformation in the non- accumulator Arabidopsis thaliana.]

Jensen S. and Jernelov A. (1969) Biological methylation of mercury in aquatic organisms. *Nature* 223:753. [This paper reports the discovery that methylation of mercury was due to anaerobic microorganisms.]

Kim D.Y, Bovet L., Kushnir S., Noh E.W., Martinoia E., Lee Y. (2006) AtATM3 is involved in heavy metal resistance in *Arabidopsis*. *Plant Physiology* 140: 922–932. [Results of the paper support the idea that ATM3 is an ABC transporter in the inner mitochondrial membrane that is able to export glutathione-cadmium complexes.]

Klonus, D., Höfgen, R., Willmitzer, L., and Riesmeier, J.W. (1994) Isolation and characterization of two cDNA clones encoding ATP-sulfurylases from potato by complementation of a yeast mutant. *Plant J.* 6: 105-112. [Two cDNA sequences encoding potato ATP-sulfurylases were cloned and found to be expressed in roots, stems, and leaves.]

Klonus D., Riesmeier J.W., Willmitzer L. (1995) A cDNA clone for an ATP-sulfurylase from *Arabidopsis thaliana*. *Plant Physiology* 107: 653-654. [The authors report the cloning of the third discovered ATP sulfurylase in Arabidopsis thaliana with similarities to the first two cloned.]

Kondo N., Imai K., Isobe M., Goto T., Murasugi A., Wada- Nakagawa A., Hayashi Y. (1984) Cadystin A and B, major unit peptides comprising cadmium binding peptides induced in a fission yeast—separation, revision of structures and synthesis. *Tetrahed Lett* 25: 3869–3872. [This is the first report of phytochelatins in Schizosaccharomyces pombe.]

Korenkov V., Park S.H., Cheng N.H., Sreevidya C., JLachmansingh J., Morris J., Hirschi K., Wagner G.J. (2007) Enhanced Cd^{2+} selective root-tonoplast-transport in tobaccos expressing *Arabidopsis* cation exchangers. *Planta* 225: 403-411. [This paper is a study of several CAtion eXchangers (CAX) that are involved in the vacuolar sequestration of Cd^{2+}.]

Kramer U., Cotter-Howells J.D., Charnock J.M., Baker A.J.M., Smith J.A.C. (1996) Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379: 635–638. [The manuscript is the first demonstration of the role of histidine in nickel hyperaccumulation.]

Krämer U., Talke I., Hanikenne M. (2007) Transition metal transport. *FEBS Letters* 581: 2263-2272. [This review summarizes current knowledge on transition metal transporters and on the adaptation of metal hyperaccumulator plants to extreme metalliferous environment.]

Lappartient A.G. and Touraine B. (1997) Glutathione-mediated regulation of ATP sulfurylase activity, SO_4^{2-} uptake, and oxidative stress response in intact canola roots. *Plant Physiology* 114:177-183. [The authors experiment with hydrogen peroxide challenge and glutathione treatments to conclude that expression of ATP sulfurylase is distinct from oxidative stress but related to glutathione status.]

LeDuc, D.L., Tarun, A.S., Montes-Bayón, M., Meija, J., AbdelSamie, M., Wu, C.P., Malit, M.F., Chang, C.-Y., Tagmount, A., de Souza, M., Neuhierl, B., Böck, A., Caruso, J., and Terry, N. (2004) Overexpression of selenocysteine methyltransferase in *Arabidopsis thaliana* and *Brassica juncea* increases selenium tolerance and accumulation. *Plant Physiology* 135:377-383. [Using genetic engineering, the authors demonstrated the important role that selenocysteine methyltransferase plays in selenium hyperaccumulation.]

LeDuc D.L., AbdelSamie M., Móntes-Bayon M., Wu C.P., Reisinger S.J., Terry N. (2006) Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. *Environ. Poll.* 144: 70-76. [The authors constructed and characterized transgenic Brassica juncea overexpressing both ATP sulfurylase and selenocysteine methyltransferase to combine increased selenate uptake with increased selenocysteine detoxification.]

Lee J., Donghwan S., Song W. Y., Hwang N. Lee Y. (2004) *Arabidopsis* metallothioneins 2a and 3 enhance resistance to cadmium when expressed in *Vicia faba* guard cells. *Plant Molecular Biology* 54: 805–815. [The experiments presented here constitute a strong support for the in vitro capacity of 2 Arabidopsis thaliana metallothioneins to chelate Cd^{++}. Two Arabidopsis thaliana metallothioneins were expressed in guard cells of Vicia faba after biolistic bombardment and transformed cells were analyzed after Cd^{++}-treatment.]

Leggett J.E. and Epstein E. (1956) Kinetics of sulfate adsorption by barley roots. *Plant Physiology* 31: 222-226. [This paper provides experimental evidence for the role of sulfate transporters in plant sulfate uptake.]

Leustek T., Murillo M., Cervantes M. (1994) Cloning of a cDNA encoding ATP sulfurylase from *Arabidopsis thaliana* by functional expression in *Saccharomyces cerevisiae. Plant Physiology* 105: 897-902. [The authors report the first cloning of a plant ATP sulfurylase and predict the existence of at least three more isoforms in Arabidopsis thaliana.]

Leustek T. and Saito K. (1999) Sulfate transport and assimilation in plants. *Plant Physiology* 120: 637-643. [This review describes sulfate transport and assimilation including detailed information about the role of ATP sulfurylase.]

Li Y.M., Chaney R.L., Brewer E.P. Roseberg R.J., Angle J.S., Baker A.J.M., Reeves R.D., Nelkin J. (2003) Development of a technology for commercial phytoextraction of nickel: Economics and technical considerations. *Plant Soil* 249: 107-115. [This paper presents the developmental of the commercial exploitation of Ni phytoextraction capacity of Alyssum ecotypes in which genetic variation allows further improvement of efficiency. Moreover, the paper explains the necessary fertility management to apply this phytoextraction technology.]

Li Y.M., Dankher O.P., Carreira L., Lee D., Schroeder J.I., Balish R.S., Meagher R.B. (2004) Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. *Plant Cell Physiology* 45: 1787-1797. [The paper presents the analysis of Arabidopsis thaliana plants overexpressing phytochelatin synthase 1, which are more sensitive to cadmium stress while being more resistant to arsenic.]

Logan H.M., Cathala N., Grignon C., Davidian J.C. (1996) Cloning of a cDNA encoded by a member of the *Arabidopsis thaliana* ATP sulfurylase multigene family: expression studies in yeast and in relation to plant sulfur nutrition. *J. Biol. Chem.* 271: 12227-12233. [This paper reports the cloning of an ATP sulfurylase cDNA sequence in Arabidopsis thaliana that is highly similar to a sequence in Brassica napus with increased expression upon sulfur starvation.]

Lund P.A. and Brown N.L. (1987) Role of the merT and merP gene products of transposon Tn501 in the

induction and expression of resistance to mercuric ions. *Gene* 52: 207-214. [This paper describes the roles of merT and merP gene products in mercury binding and transport.]

Macnair M.R., Bert V., Huitson S.B., Saumitou-Laprade P., Petit D. (1999) Zinc tolerance and hyperaccumulation are genetically independent characters. *Proc Biol Sci* 266: 2175–2179. [This paper supports the independence of Zn tolerance and hyperaccumulation characteristics in Arabidopsis halleri. Results also support the existence of a single major gene for Zn tolerance.]

Maitani T.H., Kubota H., Sato K., Yamada Y. (1996) The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorum*. *Plant Physiology* 110: 1145-1150. [This manuscript reports on the ionic forms of metals that induce phytochelatins and metallothioneins in plants.]

Mari S., Gendre D., Pianelli K., Ouerdane L., Lobinski R., Briat J.-F., Lebrun M. Czernic P. (2006) Root-to-shoot long-distance circulation of nicotianamine and nicotianamine–nickel chelates in the metal hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany* 57: 4111–4122. [The manuscript provides evidence for a role of nicotianamine in the circulation of Ni in the metal hyperaccumulator Thlaspi caerulescens.]

Marschner H. (1995) *Mineral nutrition of higher plants*. Academic Press, San Diego, CA. [This book summarizes the uptake and assimilation of minerals, including sulfate, in plants and describes the role of the ATP sulfurylase enzyme in the sulfur assimilation pathway.]

Mason R.P., Reinfelder J.R., Morel F.M.M. (1996) Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* 30: 1835-1845. [The authors demonstrate, using a diatom as a model, that methylmercury compounds have a greater transfer efficiency than inorganic mercury compounds, leading to methylmercury accumulation in fish.]

McConnell K.P. and Portman O.W. (1952) Toxicity of dimethyl selenide in the rat and mouse. *Proc. Soc. Exp. Biol. Med.* 79: 230-231. [This paper establishes that selenium in the form of volatile dimethyl selenide is over 500 times less toxic to rodents than the inorganic forms, selenate and selenite.]

Meagher R.B. (2000) Phytoremediation of toxic elemental and organic pollutants. *Curr. Opin. Plant Biol.* 3: 153-162. [This review discusses the potential of phytoremediation for metals, radionuclides, and organic contaminants.]

Mills R.F., Francini A., da Rocha P.S.C.F., Baccarini P.J., Aylett M., Krijger G.C., Williams L.E. (2005) The plant P-1B-type ATPase AtHMA4 transports Zn and Cd and plays a role in detoxification of transition metals supplied at elevated levels. *FEBS Lett* 579: 783–791. [Using both knock-out mutants of Arabidopsis and heterologous expression in Saccharomyces cerevisiae, this paper provides evidence for the role of AtHMA4 as an Zn and Cd efflux pump.]

Mills R.F., Krijger G.C., Baccarini P.J., Hall J.L.,Williams L.E. (2003) Functional expression of AtHMA4, a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass. *Plant J* 35: 164–176. [This is the first report about a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass in plants.]

Misra S., Gedamu L. (1989) Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. *Theor Appl. Genet.* 78: 16-18. [This paper supports the role of metallothionein II (here a human MT II) in Cd^{++} sequestration. The paper discusses the possibility of genetic engineering Brassica napus for better partitioning of Cd^{++} in non-consumed plant tissues.]

Misra T.K., Brown N.L., Haberstroh L., Schmidt A., Goddette D., Silver S. (1985) Mercuric reductase structural genes from plasmid R100 and transposon Tn501: functional domains of the enzyme. *Gene* 34: 253-262. [The coding regions following merC from R100 and Tn501 are compared to show high sequence similarity, and amino acids in the mercuric reductase active site and substrate binding positions are shown to be highly conserved.]

Moffat A.S. (1995) Plants proving their worth in toxic metal cleanup. *Science* 269: 302-303. [This Science news article discusses early work in phytoremediation and potential concerns of its field use including transgene spread and how risk-benefit analysis must be done to ensure that harm to the ecosystem is reduced in the accumulation and volatilization of toxins via plants.]

Moore B. (1960) A new screen test and selective medium for the rapid detection of epidemic strains of

Staphylococcus aureus. Lancet ii: 453-458. [This paper describes the discovery of mercury-resistant bacteria.]

Murillo M. and Leustek T. (1995) Adenosine-5'-triphosphate-sulfurylase from *Arabidopsis thaliana* and *Escherichia coli* are functionally equivalent but structurally and kinetically divergent: nucleotide sequence of two adenosine-5'-triphosphate-sulfurylase cDNAs from *Arabidopsis thaliana* and analysis of a recombinant enzyme. *Arch. Biochem. Biophys*. 323:195-204. [This manuscript reports on the cloning of the second and third discovered ATP sulfurylase cDNAs in Arabidopsis thaliana and describes specific activities of their enzyme products.]

Mutoh N., Hayashi Y. (1988) Isolation of mutants of *Schizosaccharomyces pombe* unable to synthesize cadystin, small cadmium-binding peptides. *Biochem. Biophys. Res. Commun.* 15:32-39. [This paper describes the isolation of the first mutant of Schizosaccharomyces pombe in its capacity to synthesize phytochelatins.]

Neuhierl B. and Böck A. (1996) On the mechanism of selenium tolerance in selenium-accumulating plants. Purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragalus bisulcatus. Eur. J. Biochem*. 239: 235-238. [This manuscript describes the purification of selenocysteine methyltransferase, which plays a primary role in selenium tolerance in the selenium hyperaccumulator, Astragalus bisulcatus.]

Noctor G., Strohm M., Jouanin L., Kunert K.J., Foyer C.H., Rennenberg H. (1996) Synthesis of glutathione in leaves of transgenic poplar overexpressing γ-glutamylcysteine synthetase. *Plant Physiology* 112: 1071-1078. [This paper is an example of how glutathione content can be increased in plants through the overexpression of the limited γ-glutamylcysteine synthetase and also describes how cysteine is the limiting reactant in the production of glutathione.]

Nussbaum S., Schmutz D., Brunold C. (1988) Regulation of assimilatory sulfate reduction by cadmium in *Zea mays* L. *Plant Physiology* 88: 1407-1410. [Here it is shown that ATP sulfurylase and adenosine 5'-phosphosulfate sulfotransferase activities were upregulated in plants by cadmium challenge, but activities decreased once a certain threshold cadmium concentration was surpassed.]

Ogawa H.I., Tolle C.L., Summers A.O. (1984) Physical and genetic map of the organomercury resistance (Omr) and inorganic mercury resistance (Hgr) loci of the IncM plasmid R831b. *Gene* 32: 311-320. [This early work demonstrated that although the loci responsible for organomercury and inorganic mercury resistance are coordinately regulated, they are physically separated.]

Ohlendorf H.M., Hothem R.L., Aldrich T.W., Krynitsky A.J. (1987) Selenium contamination of the grasslands, a major California waterfowl area. *Sci. Total Environ*. 66: 169-183. [This paper describes how agricultural drainage water containing selenium was disposed of in areas of California's Central Valley used by waterfowl and eventually led to embryonic deformities and bird deaths.]

O'Halloran T.V. and Cizewski Culotta V. (2000) Metallochaperones, an intracellular shuttle service for metal ions. *J. Biol. Chem*. 275: 25057-25060. [This review explains the role of copper metallochaperones in the intracellular trafficking.]

Ortiz D.F., Ruscitti T., McCue K.F., Ow D.W. (1995) Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. *J. Biol. Chem*. 270: 4721–4728. [This paper provides evidence for the role of the ABC transporter HMT1 in vacuolar sequestration of phytochelatin-Cd^{++}-complexes.]

Parker D.R., Feist L.J., Varvel T.W., Thomason D.N., Zhang Y. (2003) Selenium phytoremediation potential of *Stanleya pinnata. Plant and Soil* 249: 1573-5036. [This is a report on Se hyperaccumulation by Stanleya pinnata.]

Peiter E., Montanini B., Gobert A., Pedas P., Husted S., Maathuis F.J.M., Blaudez D., Chalot M., Sanders D. (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *PNAS* 104: 8532-8537. [This article reports on the study of a CDF member of Arabidopsis thaliana MTP 11. Results support the idea that Golgi-mediated exocytosis is an important mechanism for Mn tolerance.]

Persans M.W., Yan X., Patnoe J.-M. M.L., Krämer U., Salt D.E. (1999) Molecular dissection of the role of histidine in nickel hyperaccumulation in *Thlaspi goesingense* (Halacsy). *Plant Physiology* 121:1117–1126. [This is a demonstration that Ni hyperaccumulation phenotype in T. goesingense is not determined

by the overproduction of His in response to Ni.]

Phartiyal P., Kim W.-S., Cahoon R.E., Jez J.M., Krishnan H.B. (2006) Soybean ATP sulfurylase, a homodimeric enzyme involved in sulfur assimilation, is abundantly expressed in roots and induced by cold treatment. *Arch. Biochem. Biophys.* 450: 20-29. [This paper reports the first cloning of an ATP sulfurylase cDNA from soybean whose expression was most abundant in roots and induced by cold treatment.]

Pianelli K., Mari S., Marquez L., Lebrun M., Czernic P. (2005) Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. *Transgenic Res*. 14: 739-748. [This manuscript explores the potential of genetic engineering of nicotianamine in phytoremediation. Arabidopsis plants overexpressing a nicotianamine synthase cDNA from Thlaspi caerulescens and grown on nickel-contaminated soil are analyzed].

Pich A., Scholz G., Stephan U.W. (1994) Iron-dependent changes of heavy metals, nicotianamine, and citrate in different plant organs and in the xylem exudate of two tomato genotypes. Nicotianamine as possible copper translocator. *Plant Soil* 165: 189–196. [The paper supports the key role of NA to translocate Cu from the root to the shoot. The paper also explores the correlation between Fe supply and NA concentration.]

Pich A. and Scholz G. (1996) Translocation of copper and other micronutrients in tomato plants (*Lycopersicon esculentum* Mill.): nicotianamine-stimulated copper transport in the xylem. *Journal of Experimental Botany* 47: 41-47. [The paper reports on the analysis of a tomato mutant chloronerva deficient in nicotianamine. Results support an important role of NA in Cu translocation.]

Pickering I.J., Prince R.C., George M.J., Smith R.D., George G.N., Salt D.E. (2000) Reduction and coordination of arsenic in Indian mustard. *Plant Physiology* 122: 1171-1177. [Using, among other techniques, X-ray absorption spectroscopy, the authors determine that arsenate is reduced to arsenite, which is then chelated by thiolates.]

Pilon-Smits E., Hwang S., Lytle C.M., Zhu Y., Tai J.C., Bravo R.C., Chen Y., Leustek , T., Terry N. (1999) Over-expression of ATP sulfurylase in *Brassica juncea* leads to increased selenate uptake, reduction and tolerance. *Plant Physiology* 119: 123-132. [This key paper demonstrates the potential of overexpressing ATP sulfurylase in Brassica juncea for phytoremediation.]

Presser T.S. and Ohlendorf H.M. (1987) Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. *Environ. Manage.* 11: 805-821. [This paper describes the effects of the Kesterson Reservoir disaster in which selenium-containing drainage water was emptied into a wildlife refuge from 1978 to 1982, resulting in bird deaths and deformities first observed in the 1983 nesting season.]

Pomponi M., Censi V., Di Girolamo V., De Paolis A., di Toppi L.S., Aromolo R., Costantino P., Cardarelli M. (2006) Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances Cd^{++} tolerance and accumulation but not translocation to the shoot. *Planta* 223: 1432-2048. [This paper support the importance of phytochelatins for Cd^{++} tolerance but not for Cd^{++} translocation from the root to the shoot.]

Papoyan A. and Kochian L.V. (2004) Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase1. *Plant Physiology* 136: 3814–3823. [This paper reports on the screen of a cDNA library of the Cd/Zn hyperaccumulator Thlaspi caerulescens in Saccharomyces cerevisiae and on the identification of a Cd and Zn pump.]

Rauser W.E. (1995) Phytochelatins and related peptides. *Plant Physiology* 109: 1141-1149. [This review discusses the chemical nature of phytochelatins (and related peptides) and their biosynthesis, induction by metal stress, manner of cadmium complexation and localization, and importance in metal tolerance.]

Rensing, C., Ghosh M., Rosen B.P. (1999) Families of soft-metal-ion-transporting ATPases. *J. Bacteriol*. 181: 5891-5897. [This review explains the evolution of genes, in particular those encoding pumps, involved in resistance to soft metals.]

Roosens N.H., Bernard C., Leplae R., Verbruggen N. (2004) Evidence for copper homeostasis function of metallothionein (MT3) in the hyperaccumulator *Thlaspi caerulescens*. *FEBS Letters* 577: 9–16. [The paper presents the first study of a metallothionein in a hyperaccumulator species.]

Roosens N.H., Leplae R., Bernard C., Verbruggen N. (2005) Variations in plant metallothioneins: the heavy metal hyperaccumulator *Thlaspi caerulescens* as a study case. *Planta* 222 :716 – 729. [The role of modifications and expression of metallothioneins in the heavy metal hyperaccumulator is analyzed here.]

Rüegsegger A., Schmutz D., Brunold C. (1990) Regulation of glutathione synthesis by cadmium in *Pisum sativum* L. *Plant Physiology* 93: 1579-1589. [In this work, the authors conclude that glutathione is a precursor of phytochelatins and that cadmium increases the activity of enzymes in the sulfur assimilation pathway to make more cysteine, the limiting reagent in glutathione synthesis.]

Rugh C.L., Wilde D., Stack N.M., Thompson D.M., Summers A.O., Meagher R.B. (1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a bacterial *merA* gene. *Proc. Natl. Acad. Sci. USA* 93: 3182-3187. [This is the first paper in which a plant overexpressing a gene from a bacterial mer operon was constructed and characterized.]

Rugh C.L., Senecoff J.F., Meagher R.B., Merkle S.A. (1998) Development of transgenic yellow poplar for mercury phytoremediation. *Nature Biotechnol.* 16: 925-928. [The authors extend their previous pilot study with merA Arabidopsis thaliana and overexpress a modified version of merA in poplar, a host with phytoremediation potential.]

Rugh C.L., Bizily S.P., Meagher R.B. (2000) Phytoremediation of environmental mercury pollution: In *Phytoremediation of Toxic Metals – Using Plants to Clean up the Environment* (Raskin, I. and Ensley, B.D., eds). New York. [This review discusses the mercury problem and the possibilities of using phytoremediation to solve it, including engineering plants with the bacterial mer operon.]

Ruiz O.N., Hussein H.S., Terry N., Daniell H. (2003) Phytoremediation of organomercurial compounds via chloroplast genetic engineering. *Plant Physiology* 132: 1344-1352. [The authors refine the merA/merB operon overexpression strategy by specifically engineering the chloroplasts of tobacco to construct a plant with protein expression localized to a mercury toxicity target, greater protein copy number, and less chance of transgene spread.]

Salt D.E. and Wagner G.J. (1993) Cadmium transport across tonoplast of vesicles from oat roots. Evidence for a Cd^{2+}/H^+ antiport activity. *J. Biol. Chem.* 268: 12297-12302. [This article presents support for Cd^{++} transport via Cd^{++}/H^+ antiport activity into tonoplasts.]

Schat H., Llugany M., Vooijs R., Hartley-Whitaker J., Bleeker P.M. (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and nonhyperaccumulator metallophytes. *Journal of Experimental Botany* 53: 2381-2392. [This article demonstrates that tolerance of heavy metal hyperaccumulators does not depend on phytochelatins, however, as tolerance is dependent on PC-based sequestration.]

Schmöger M.E.V., Oven M., Grill E. (2000) Detoxification of arsenic by phytochelatins in plants. *Plant Physiology* 122: 793-801. [This paper reviews the role of phytochelatins in arsenic detoxification including knowledge of how phytochelatin production is induced by arsenic and evidence for the existence of arsenic-phytochelatin complexes isolated from plants.]

Schottel J., Mandal A., Clark D., Silver S., Hedges R.W. (1974) Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature* 251: 335-337. [This manuscript reports the first identification of organomercurial-reducing E. coli and determines the resistance is due to plasmid-borne loci.]

Schottel J.L. (1978) The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of *Escherichia coli*. *J. Biol. Chem.* 253: 4341 – 4349. [This paper reports the early purification and characterization of a mercuric reductase and organomercurial hydrolase, including information on their cofactors, K_m's, molecular weight, isoelectric point, and optimal pH.]

Setya A., Murillo M., Leustek T. (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5'-adenylylsulfate reductase. *Proc. Natl. Acad. Sci. USA* 93: 13383-13388. [This manuscript describes the cloning of three Arabidopsis thaliana cDNA's that are shown to encode APS reductases suggesting that plants may not use PAPS reductases.]

Shaw W.H. and Anderson J.W. (1972) Purification, properties and substrate specificity of adenosine triphosphate sulphurylase from spinach leaf tissue. *Biochem. J.*,127: 237-247. [This early work describes the purification and characterization of an ATP sulfurylase from spinach, including determining co-

factors, substrates (such as selenate), and K_m's.]

Singleton C. and Le Brun N.E. (2007) Atx1-like chaperones and their cognate P-type ATPases: copper-binding and transfer. *Biometals* 20: 275–289. [The review explains the role and function of Cu-chaperones in transferring Cu (I) to transporters.]

Skorupa J.P. (1998) Selenium poisoning of fish and wildlife in nature: lessons from twelve real-world examples. In *Environmental Chemistry of Selenium*; Marcel Dekker: New York. [Included in this piece is a description of the Kesterson Reservoir, CA disaster caused by high selenium concentrations in improperly disposed agricultural drainage water.]

Song W.Y., Sohn E.J., Martinoia E., Lee Y.J., Yang Y.Y., Jasinski M, Forestier C., Hwang I., Lee Y. (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nature Biotechnology* 21:914 – 919. [This article shows that the overexpression of the Saccharomyces cerevisiae ABC transporter, YCF1, which transports heavy metal-glutathione complexes to the vacuole, can increase both the accumulation and the tolerance to Pb (II) and Cd (II) in plants. These results are discussed in the perspective of phytoremediation.]

Sors T.G., Ellis D.R., Na G.N., Lahner B., Lee S., Leustek T., Pickering I.J., Salt D.E. (2005) Analysis of sulfur and selenium assimilation in Astragalus plants with varying capacities to accumulate selenium. *Plant J.* 42: 785-797. [This paper examines how the activities of enzymes of the sulfur assimilation pathway (ATP sulfurylase, APS reductase, and serine acetyltransferase) and selenocysteine methyltransferase vary with selenium accumulation ability across species of the Astragalus genus.]

Spallholz, J.E. (1997) Free radical generation by selenium compounds and their prooxidant toxicity. *Biomed Environ Sci*. 10:260 –270. [This review covers the possibility that selenium toxicity is related to its ready conversion to free radicals.]

Strohm M., Jouanin L., Kunert K.J., Pruvost C., Polle A., Foyer C.H., Rennenberg H. (1995) Regulation of glutathione synthesis in leaves of transgenic poplar (*Populus tremula x P. alba*) overexpressing glutathione synthetase. *Plant J.* 7: 141-145. [By studying transgenic poplar, the authors determined that glutathione synthesis is controlled by the availability of both cysteine and γ-glutamylcysteine and is related to the expression of γ-glutamylcysteine synthetase and glutathione synthetase.]

Suh M.C., Choi D., Liu J.R. (1998) Cadmium resistance in transgenic tobacco plants expressing the *Nicotia glutinosa* L. metallothionein like gene. *Mol Cells* 31: 678-684. [This paper shows the potential of metallothionein-like genes in the genetic engineering of Cd tolerance.]

Summers A.O. and Sugarman L.I. (1974) Cell-free mercury(II)-reducing activity in a plasmid-bearing strain of *Escherichia coli*. *J. Bacteriol*. 119:242-249. [This early manuscript isolated a fraction containing mercury(II)-reducing activity and characterized substances that destroyed or enhanced the activity and identified Au(III) as an additional substrate but not organic mercury.]

Taji T., Seki M, Satou M., Sakurai T., Kobayashi M., Ishiyama K., Narusaka Y, Narusaka M., Zhu J.K., Shinozaki K. (2004) Comparative genomics in salt tolerance between Arabidopsis and Arabidopsis-Related halophyte salt cress using Arabidopsis microarray. *Plant Physiology* 135: 1697–1709 [This article provides evidence that extreme traits as halophytism evolve through the higher expression of genes involved in basal salt tolerance].

Taghavi S., Barac T., Greenberg B., Borremans B., Vangronsveld J., van der Lelie D. (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Appl Environ Microbiol* 71: 8500-8505. [This article is a landmark in the potential use of horizontal gene transfer to endogenous endophytic bacteria to improve phytoremediation of pollutants.]

Talke I., Hanikenne M., Krämer U. (2006) Zinc dependent global transcriptional control, transcriptional de-regulation and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri. Plant Physiol*, 142: 148–167. [In this article, the use of Arabidopsis thaliana gene chips to identify genes overexpressed in the closely related hyperaccumulator species Arabidopsis halleri is presented. This study shows that under normal growth conditions, roots of the Zn hyperaccumulator behave as Zn deficient, when considering expression of several genes involved in Zn transport. It also further supports the role of HMA4 in Zn translocation.]

Terry N., Zayed A.M., de Souza M.P., Tarun A.S. (2000) Selenium in higher plants. Ann. Rev. Plant Physiology Plant Mol. Biol. 51: 401-432. [This paper reviews selenium toxicity, the parallels and

differences between sulfur and selenium assimilation in plants, and how this knowledge could be applied to phytoremediation.]

Terry N., Banuelos G. (2000) *Phytoremediation of contaminated soil and water*. Lewis Publishers. [This book covers work in phytoremediation from bench to field demonstration projects with analysis of the potential for this field of study and future applications.]

Thomas J.C., Davies E.C., Malick F.K., Endreszl C., Williams C.R., Abbas M., Petrella S., Swisher K., Perron M., Edwards R., Osenkowski P., Urbanczyk N., Wiesend W.N., Murray K.S. (2003) Yeast metallothionein in transgenic tobacco promotes copper uptake from contaminated soils. *Biotechnol Prog*. 19: 273-80. [The yeast CUP1 gene encoding a metallothionein was expressed in tobacco plants. Upon copper stress, those plants were able to accumulate more copper in their leaves.]

University Of Georgia (2003, September 11). UGA Researchers Use Transgenic Trees To Help Clean Up Toxic Waste Site. ScienceDaily. Retrieved March 2, 2008, from http://www.sciencedaily.com/releases/2003/09/030911072408.htm. [This news article reports on a field study with merA cottonwood.]

van de Mortel J., Villanueva L.A., Schat H., Kwekkeboom J., Coughlan S., Moerland P.D., Ver Loren van Themaat E., Koornneef M., Aarts M.G.M. (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens. Plant Physiol* 142: 1127–1147. [This is a global transcriptional analysis of Thlaspi caerulescens using gene chips of Arabidopsis thaliana. It highlights the up-regulation of genes involved in metal homeostasis but also genes involved in lignin biosynthesis and genes of unknown function. The article also discusses the higher deposition of lignin in root endoderm in the metal hyperaccumulator.]

van der Lelie D., Barac T., Taghavi S., Vangronsveld J. (2005) New uses of endophytic bacteria to improve phytoremediation. *Trends Biotechnology* 23: 8-9. [This review explains the hopes and the obstacles of using genetically engineered endophytic bacteria to improve phytoremediation.]

Van der Zaal B.J., Neuteboom L.W., Pinas J.E., Chardonnens A.N., Schat H., Verkleij J.A.C., Hooykaas P.J.J. (1999) Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiology* 119: 1047–1055. [This is a first report about the genetic manipulation of a member of the Arabidopsis CDF transporters, AtZAT (recently renamed MTP1), to increase Zn tolerance and accumulation.]

Van Huysen T., Terry N., Pilon-Smits E.A.H. (2004) Exploring the selenium phytoremediation potential of transgenic *Brassica juncea* overexpressing ATP sulfurylase or cystathionine-γ-synthase. *Int. J. Phytorem*. 6: 111-118. [This study anticipated the later field trial of the APS transgenic plants by evaluating their ability to accumulate selenium on selenium-rich soil in greenhouse studies.]

Van Mantgem P.J., Wu L., Bañuelos G.S. (1996) Bioextraction of selenium by forage and selected field legume species in selenium-laden soils under minimal field management conditions. *Ecotoxicol. Environ. Saf*. 34: 228-238. [This study demonstrates how the amount of selenium removed by soil depends heavily on the plant species, i.e. the concentration that can be tolerated and the biomass achieved, and on the availability (chemical form) of selenium present in the soil.]

Vatamaniuk O., Mari S., Lu Y., Rea P. (1999) AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and in vitro reconstitution. *Proc. Natl. Acad. Sci. USA* 96:7110–7115. [This is a study of phytochelatin synthase and its activation by Cd^{++}.]

Verret F., Gravot A., Auroy P., Leonhardt N., David P., Nussaume L., Vavasseur A., Richaud P. (2004) Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Lett* 576: 306–312. [This report clearly demonstrates in Arabidopsis thaliana the role of HMA4 in zinc and cadmium root-to-shoot translocation and tolerance.]

Verret F., Gravot A., Auroy P., Preval S., Forestier C., Vavasseur A., Richaud P. (2005) Heavy metal transport by AtHMA4 involves the N-terminal degenerated metal binding domain and the C-terminal His(11) stretch. *FEBS Lett* 579: 1515–1522. [This is a study of functional domains of At HMA4.]

Wangeline A.L., Burkhead J.L., Hale K.L., Lindblom S.D., Terry N., Pilon M., Pilon-Smits E.A.H. (2004) Overexpression of ATP sulfurylase in Indian mustard: effects on tolerance and accumulation of

twelve metals. *J. Environ. Qual.* 33:54-60. [This comprehensive study evaluated the ability of the APS Indian mustard to tolerate and accumulate 12 forms of metals in hydroponics experiments.]

Weber M., Harada E., Vess C., von Roepenack-Lahaye E., Clemens S. (2004) Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. *Plant J* 37: 269–281. [Changes in the transcriptome of Arabidopsis thaliana roots and the related hyperaccumulator species Arabidopsis halleri upon Cd^{++} and Cu^{++} exposure are presented and discussed.]

Weber M., Trampczynska A., Clemens S. (2006) Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri*. *Plant, Cell and Environment* 29: 950–963. [This study presents the use of Arabidopsis GeneChips to study the transcriptome of Arabidopsis halleri and supports the role of nicotianamine in Zn hyperaccumulation.]

Wilber C.G. (1980) Toxicology of selenium: a review. *Clin. Toxicol.* 17: 171-230. [This review explains one of the prevailing theories regarding selenium's toxicity, i.e. that the misincorporation of selenoamino acids for sulfur amino acids (cysteine and methionine) is responsible.]

Willems G., Godé C., Verbruggen N., Saumitou-Laprade P. (2007) Quantitative Trait Loci mapping of zinc tolerance in the metallophyte *Arabidopsis halleri* ssp. *Halleri. Genetics* 176: 659-674. [The article presents the identification of 3 QTL for Zn tolerance and the co-localization with known genes involved in metal homeostasis and tolerance.]

Wilson L.G. and Bandurski R.S. (1958) Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. *J. Biol. Chem.* 233: 975-981. [Relevant to the current discussion, this paper shows that ATP sulfurylase activity can use sulfate, selenate, and molybdate as substrates.]

Wycisk K., Kimb E.J., Schroeder J.I., Krämer U. (2004) Enhancing the first enzymatic step in the histidine biosynthesis pathway increases the free histidine pool and nickel tolerance in *Arabidopsis thaliana*. *FEBS Letters* 578: 128–134. [Results support that enhancing histidine biosynthesis in plants is sufficient to improve Ni tolerance.]

Yeargan R., Maiti I.B., Nielsen M.T., Hunt A.G., Wagner G.J. (1992) Tissue partitioning of cadmium in transgenic tobacco seedlings and field grown plants expressing the mouse metallothionein I gene. *Transgenic Research* 1: 261-267. [This is a report on genetic engineering of metallothioneins to decrease Cd accumulation in the leaves of crops by enhancing its retention in the roots.]

Zayed A.M., Lytle C.M., and Terry N. (1998) Accumulation and volatilization of different chemical species of selenium by plants. *Planta* 206: 284-292. [Using X-ray and atomic absorption spectroscopy, the authors demonstrate that the form of selenium used to challenge plants determines the form accumulated and the rate of selenium volatilisation, suggesting the presence of rate-limiting steps in the selenate assimilation pathway.]

Zenk M.H. (1996) Heavy metal detoxification in higher plants: a review. *Gene* 179: 21-30. [This paper constitutes a review of the important role played by phytochelatins in metal detoxification in plants, including the different chemical forms of phytochelatins, a description of phytochelatin synthase and characterization of the cadmium-sensitive cad1 Arabidopsis thaliana mutant.]

Zhigang A., Cuijie L., Yuangang Z., Yejie D., Wachter A., Gromes R., Rausch T. (2006) Expression of BjMT2, a metallothionein 2 from *Brassica juncea*, increases copper and cadmium tolerance in *Escherichia coli* and *Arabidopsis thaliana,* but inhibits root elongation in *Arabidopsis thaliana* seedlings. *J. Exp. Bot.* 57: 3575-3582. [The article is a report on the use of metallothioneins to increase metal tolerance.]

Zhu Y.L., Pilon-Smits E.A.H., Tarun A.S., Weber S.U., Jouanin L., Terry N. (1999) Cadmium tolerance and accumulation in Indian Mustard is enhanced by overexpressing γ-glutamylcysteine synthetase. *Plant Physiology* 121:1169–1177. [The article shows that increasing gluthathione biosynthesis by the overexpression of γEC, has a small, but significant, impact on cadmium tolerance and accumulation.]

Zhu Y.L., Pilon-Smits E.A.H., Jouanin L., Terry N. (1999) Overexpression of glutathione synthetase in Indian Mustard enhances cadmium accumulation and tolerance. *Plant Physiology* 119: 73–79. [The article shows that increasing glutathione biosynthesis by the overexpression of glutathione synthase has a

little but significant impact on cadmium tolerance and accumulation.]

Biographical Sketches

Dr Nathalie Verbruggen did her Ph.D. on proline accumulation in plants after salt stress at the Ghent University (Belgium) with Professor Marc van Montagu. Following this, she conducted research as group leader on molecular responses of plants to osmotic stress. In 1999, she became a full-time professor in the University of Brussels and started a new laboratory to study mechanisms underlying metal homeostasis and detoxification in plants. Projects include cadmium hyperaccumulation, copper hypertolerance and magnesium deficiency. N. Verbruggen is also active in explaining the science behind plant genetic engineering and GMO's.

Dr. Danika LeDuc has been an Assistant Professor of Chemistry & Biochemistry at California State University, East Bay since 2006. She conducts research at the interface of biochemistry and analytical and environmental chemistry, in which she has co-authored over 20 peer reviewed articles and book chapters. Her published work ranges from analysis of selenium and arsenic metabolites to the first field trial using genetically engineered plants for phytoremediation. Her current projects include work on elucidating the molecular mechanism of metal hyperaccumulation in selenium, nickel, and lead accumulating plants using synchrotron X-ray absorption techniques and proteome and transcriptome analyses.