Chapter 11
Recent Advances in Understanding of Plant Responses to Excess Metals: Exposure, Accumulation, and Tolerance

Marjana Regvar(✉) and Katarina Vogel-Mikuš

Abstract Toxicity has been the primary driver of research on excess metals; but in recent decades, the biology of metal accumulation/hyperaccumulation became the main focus of research. The main aim is to develop phytoremedial techniques for diminishing the environmental impact of metal pollution. From the toxicological point of view, only the bioavailable soil metal fractions can affect morphology and/or physiology; therefore methods for quantification of metal stress in plants are continuously evolving. Phenotypic plasticity encoded by fixed genetic components in different species determines a plant's responses to excess metals. Metal exclusion, accumulation, and hyperaccumulation are major plant strategies when facing excess metals, with metal tolerance mechanisms as a prerequisite for coping with the metal(s) in question. Considerable research efforts are being directed into the field of biotechnology of metal hyperaccumulation, but the complexity of plant responses to excess metals makes this task extremely difficult.

1 Introduction

Plant functioning and its regulation were first studied using chemical, physiological, and genetic approaches, dramatic progress has, however, been made as a result of molecular biology techniques. Structural genomic approaches yield new insights on genome mappings, sequencing, genome organization and comparative genome data, while functional genomic approaches (transcriptomics, proteomics, and metabolomics) are needed to link the structural data to plant functioning (Hesse and Höfgen 2001). Model plants such as *Arabidopsis thaliana* and *Thlaspi caerulescens* (Cobbett 2003, Assunção et al. 2003) are used in many laboratories to improve the ratio between the knowledge gained and the working load input. Comparative studies on nonmodel organisms are, on the other hand, used to test the universal

---

Marjana Regvar
Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111,
SI-1000 Ljubljana, Slovenia
marjana.regvar@bf.uni-lj.si

N.A. Khan et al. (eds.), *Sulfur Assimilation and Abiotic Stress in Plants.*

applicability of the proposed models. Both, powerful analytical techniques and a multidisciplinary approach by experts from different fields of research have contributed significantly to our knowledge of plant responses to excess metals. This chapter focuses on the latest discoveries in metal bioavailability, accumulation, distribution, and detoxification in plants.

2 Toxic Metal Exposure

Major sources of atmospheric emissions are mining, different types of metal production, and combustion of fossil fuels. The principal sources of toxic metals in soils are the disposal of ash residues from coal combustion, disposal of commercial products on land, and contributions from the atmosphere, whereas emissions into aquatic ecosystems originate mainly from the atmosphere, metal smelters, coal burning, and the dumping of sewage sludge. Soil and water metal contents are particularly relevant for plants and algae, and only a fraction of the total soil metals is available for plant uptake (Clemens 2006). Considerable controversy exists in the literature on the precise definition of *bioavailability*, since it has been linked to metal ion activity in soil solution, the exchangeable soil metal fraction, and more recently, to the concentrations of metals that cause ecotoxicity, but the scientific community universally accepts none of these definitions. From the toxicological point of view, only the *bioavailable soil metal fractions* are the ones that can affect the morphology and/or physiology of an organism. The availability of metal ions in soils depends on the soil (pH, surface charge, organic matter content, clay content, oxide minerals, redox potential, soil solution composition), plant (plant species or cultivar, plant parts, age of the plant, interactions with microorganisms), and environmental factors (climatic conditions, management practices, irrigation practices, topography) (Naidu et al. 2003, Moore 2003, Tsao 2003). Biological availability, strictly speaking, means available to living organisms. Thus, although chemical extractions involving dilute salt solutions have been used to measure the bioavailable pool of metals in soils, the availability of metals for plants can only be assessed using in situ techniques that involve growing the organisms of interest in contaminated materials and quantifying the uptake of metal into the organism and assessing the toxicological response. Therefore bioavailability as determined by plant studies should be more correctly termed *phytoavailability* (Naidu et al. 2003).

2.1 Bioavailability at the Soil-Plant Interface

Plant roots, soil microbes, and their interactions can increase metal bioavailability through secretion of protons, organic acids, phytosiderophores (PSs), amino acids, and enzymes (see Yang et al. 2005). Secretion of protons by roots acidifies the rhizosphere and increases metal dissolution. Proton extrusion of the roots is operated by plasma membrane H^+-ATPases and other H^+ pumps. In the lupin, P deficiency induces citrate exudation by enhancing the activity of plasma membrane H^+-ATPase and H^+ export (Ligaba et al. 2004). Al-induced exudation of malate, as the basis for the mechanism of Al tolerance in wheat, was found to be accompanied by changes in plasma membrane surface potential and the activation of H^+-ATPase (Ahn et al. 2004). Acetic acid and succinate were found in the rhizosphere of a Cd-accumulating genotype of wheat (Kyle), but not in the nonaccumulating genotype (Arcola) (Cieslinski et al. 1998). Phytosiderophores can be released under Fe deficiency from cereals to increase the mobilization of Fe, Zn, Cu, and Mn in the soil (Römheld 1991). In some dicots root reductases, such as *A. thaliana* NAOH-dependent Fe^{3+}-chelate reductase (NFR) can reduce Fe^{3+} or Cu^{2+} under low Fe and Cu supply to increase plant uptake (Welch et al. 1993, Bagnaresi et al. 1999). No direct evidence for a relationship between root exudation and metal hyperaccumulation in *T. caerulescens* was found (McGrath et al. 1997, 2001, Zhao et al. 2001). Changed soil conditions in the rhizosphere and increased solubility of the retained Zn in the rhizosphere soil of the Zn hyperaccumulator *T. caerulescens* by root-microbe interactions were, however, reported (Whiting et al. 2001). Bacteria have been shown to catalyse redox transformations leading to a decrease in soil metal bioavailability (Yang et al. 2005). For example, a strain of *Xanthomonas maltophyla* was shown to catalyze the reduction and precipitation of highly mobile Cr^{6+} to Cr^{3+}, a significantly less mobile and environmentally less hazardous species, and to induce the transformation of other toxic metal ions including Pb^{2+}, Hg^{2+}, Au^{3+}, Te^{4+}, Ag^+ and oxy-anions such as SeO_4^- (Lasat 2002). Similarly, the bulk of evidence tends to indicate that mycorrhizal fungi inhibit metal uptake by binding metals to components of the mycelium (Joner and Leyval 1997, Joner et al. 2000). Studies of genes of arbuscular mycorrhizal fungi that are putatively expressed under heavy metal stress have recently been reported (González-Guerrero et al. 2005, Hildebrandt et al. 2007).

3 Metal Accumulation and Tolerance in Plants

Besides bioavailability, uptake and translocation efficiencies determine metal accumulation and distribution in plants (Clemens 2006). Roots are the plant organs in closest contact with metal-contaminated soils; therefore they are the most affected by metals. Resistance to excess metals can either be achieved by *avoidance*, when the plant is able to restrict metal uptake into the cells, or *tolerance*, when the plant is able to survive the presence of excess metals. Only a limited number (approximately 420) of plant species have developed the ability to accumulate more than 10,000 $\mu g\ g^{-1}$ of Mn, or Zn; 1,000 $\mu g\ g^{-1}$ Ni, Cu, Pb or Se; and 100 $\mu g\ g^{-1}$ of Cd, far in excess of normal physiological requirements (if any), and far in excess of the levels found in the majority of other species tolerant of metalliferous soils. These so-called *hyperaccumulating plants* possess specific genetic determinants that enable efficient metal uptake and enhanced transport from the roots to the shoots (Baker 1981, 1987, Reeves and Baker 2000, Reeves 2006). Transporters of ligands for metal ions contributing to metal uptake, transport, and detoxification have recently been reviewed (Haydon and Cobbett 2007).

3.1 Metal Transporters

Plant genomes encode large families of metal transporters that vary in their substrate specificities, expression pattern, and cellular localization in governing metal translocation throughout the plant (Colangelo and Guerinot 2006, Haydon and Cobbett 2007). This section focuses on the recently most intensively studied metal transporters in plants.

3.1.1 Metal Uptake Proteins

Metal-uptake transporters are either acting at the plasma membrane (PM) to move metals into the cytoplasm from the apoplast or from intracellular compartments (Fig. 11.1) and involve the yellow stripe-like (YSL) family, the natural resistance associated macrophage protein (NRAMP) family, the zinc-regulated and iron-regulated transporter-like protein (ZIP) families, and the high-affinity Cu uptake proteins (COPT) family (Table 11.1), as extensively reviewed in Yang et al. (2005) and Colangelo and Guerinot (2006).

3.1.1.1 YSL Family

Yellow stripe-like proteins (Table 11.1) are believed to mediate the uptake of metals that are complexed with plant-derived phytosiderophores (PS) or nicotianamine (NA), a non-proteinogenic amino acid found throughout the plant kingdom that serves as a precursor for PS synthesis in grasses (Curie 2001). At the biochemical level, the best-studied member of this plant-specific family is YS1 from maize (ZmYS1), which accumulates in the roots and leaves of Fe-starved plants and functions as a proton-coupled symporter for Fe–PS transport. Analogy with yeast and oocyte transport studies indicates that ZmYS1 might also have other substrates, including Zn, Ni, and Cu. On the basis of sequence similarity to ZmYS1, *A. thaliana* has eight predicted YSL proteins. Considering that nongrasses do not produce or use PS, AtYSLs most probably transport metal–NA complexes (Roberts et al 2004, Schaaf et al. 2004, Colangelo and Guerinot 2006). The gene expression of two family members has recently been studied in detail. *AtYSL2* transcript accumulation increases under conditions of Fe sufficiency or Fe resupply and in response to Cu and Zn. Its mRNA expression pattern and its protein localization in lateral membranes suggest that AtYSL2 might function in the lateral transport of metals in veins (DiDonato et al. 2004, Schaaf et al. 2005, Colangelo and Guerinot 2006). *AtYSL1* is a shoot-specific gene whose transcript levels increase in response to high-Fe conditions. Its expression in young siliquas and the chalazal endosperm, together with data from NA- and Fe-distribution studies, support its role in Fe loading of seeds. *ysl1* shoots contain elevated NA levels, whereas *ysl1* seeds contain two to fourfold less NA (and less Fe) than wild-type plants. The germination of *ysl1* seeds under Fe starvation is slower than that of wild-type plants, and the defect can be overcome by Fe supplementation during germination (Le Jean et al. 2005). Thus, it is apparent that Fe and possibly Zn, Mn, and Cu homeostasis are dependent on YSLs. Further examination of the localization and substrate specificity of various YSLs, and genetic analysis

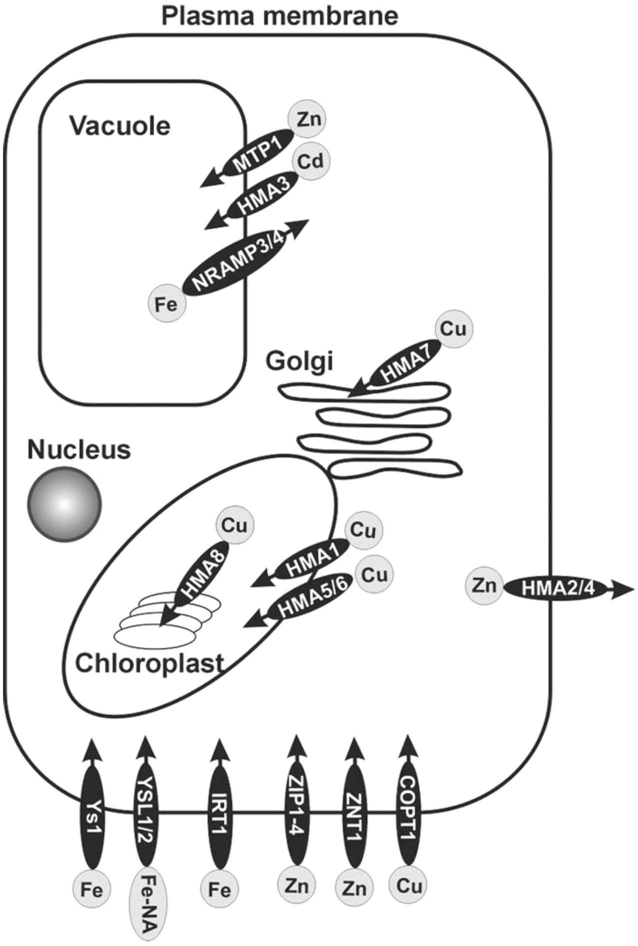

Fig. 11.1 Extra- and intracellular metal transport in plants. Metal delivery to and remobilization from intracellular compartments are important considerations in cellular ion homeostasis and tolerance. The localization of metal transporters with their preferential substrates are summarized in a generic cell, with arrows indicating the direction of transport (adopted after Clemens 2001, Colangelo and Guerinot 2006)

of double (or higher order) mutant plants will help to assign functions to this recently identified plant-specific family of transporters (Colangelo and Guerinot 2006).

3.1.1.2 ZIP Family

Zinc-regulated and iron-regulated transporter-like proteins (Table 11.1) generally contribute to metal-ion homeostasis through the transport of cations into the cytoplasm. More than 100 ZIP proteins have been identified in bacteria, fungi, animals, and plants

Table 11.1 Metal transporters listed by gene family, their tissue expression, cellular localization, function, inducing conditions and proposed/known substrates*

Metal uptake proteins	Tissue expression	Cellular localization and Function	Inducing conditions	Proposed/known substrate	Reference(s)
YSL					
AtYSL1	Siliquae, leaves (xylem parenchyma), flowers, chalazal endosperm of the seeds	Long-distance circulation of Fe-NA, delivery to seeds	+Fe	Fe-NA	Le Jean et al. 2005
AtYSL2	Roots endoderm, pericycle, shoots, reproductive organs, particularly in xylem parenchyma cells	PM, lateral movement of metals in the vasculature, Fe and Zn homeostasis	+Fe, downregulated by –Zn	-	DiDonato et al. 2004
					Schaaf et al. 2005
OsYSL2	Leaves (phloem), roots, seeds	PM, phloem transport of Fe	-Fe in shoots	Fe^{2+}-, Mn^{2+}-NA	Kolke et al. 2004
TcYSL3	Roots, shoots, flowers	-	-	Fe/Ni-NA	Gendre et al. 2007
TcYSL5	Shoots	-	-	Fe/Ni-NA	Gendre et al. 2007
TcYSL7	Flowers	-	-	Fe/Ni-NA	Gendre et al. 2007
ZmYS1	Roots, shoots	Fe^{3+}-PS, Fe^{2+}-NA translocation	-Fe	Fe^{3+}-PS, Fe^{3+}-, Fe^{2+}-, Ni^{2+}-, Cu^{2+}-NA	Schaaf et al. 2004
					Roberts et al. 2004
NRAMP					
AtNRAMP1/ AtNRAMP2	Roots, shoots	Plastid envelope?, Fe distribution in the cell, Fe resistance	-Fe in roots	Fe^{2+}	Curie et al. 2000
AtNRAMP3	Vascular bundles of roots, stems, leaves	VM, Fe-starvation dependent accumulation of Mn-, Zn and Fe acquisition	-	Fe^{2+}	Thomine et al. 2003
AtNRAMP3/ NRAMP4	seeds	VM, Fe remobilization from the vacuole during seed germination	-Fe	Fe^{2+}; Cd^{2+}	Lanquar et al. 2005
OsNRAMP1-2	Roots, leaves	-	-	-	Belouchi et al. 1997
TjNRAMP4				Ni^{2+}	Mizuno et al. 2005

ZIP					
AtIRT1	External cell layers of the roots	PM, Fe^{2+} uptake from soil	-Fe	Fe^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}	Vert et al. 2002
OsIRT1	Roots	PM, Fe^{2+} uptake from soil	-Fe, -Cu	Fe^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}	Bughio et al. 2002
OsZIP4	Leaf vascular bundles and mesophyll cells, root vascular bundles, shoot and root meristems	PM, translocation of Zn^{2+} within the plant	-Zn	Zn^{2+}	Ishimaru et al. 2005
OsZIP1	Roots	-	-Cu	-	Ishimaru et al. 2005
MtZIP1	Root, leaf	-	-	Zn^{2+}	López-Millán et al. 2004
MtZIP3	Root, leaf	-	Downreg. by –Mn and –Fe, slightly upreg. by –Zn	Fe^{2+}	López-Millán et al. 2004
MtZIP4	Roots of – Zn plants, leaves	-	Downreg. by –Mn and –Fe, upreg. by -Zn in leaves,	Mn^{2+}	López-Millán et al. 2004
MtZIP5	Leaves	-	Upreg. by –Zn leaves	Zn^{2+}, Fe^{2+}	López-Millán et al. 2004
MtZIP6	Roots of – Fe plants, leaves	-	-	Zn^{2+}, Fe^{2+}	López-Millán et al. 2004
MtZIP7	Leaves	-	-	Mn^{2+}	López-Millán et al. 2004
TcZNT1	Roots, shoots	PM, high affinity Zn^{2+} and low affinity Cd^{2+} uptake	Downreg. by Zn	Zn^{2+}	Pence et al. 2000
TjZNT1	-	-	-	Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}	Mizuno et al. 2005
TjZNT2	-	-	-	Ni^{2+}, Mn^{2+}, Cd^{2+}	Mizuno et al. 2005

(continued)

Table 11.1 (continued)

Metal uptake proteins	Tissue expression	Cellular localization and Function	Inducing conditions	Proposed/known substrate	Reference(s)
COPT					
AtCOPT1	Root tips, trichomes, stomata, pollen, embryos	Cu acquisition and accumulation	Downreg. by Cu	Cu	Sancenón et al. 2004
Metal efflux proteins					
P_{1B}-ATPases					
Zn/Cd/Pb/Co cluster					
AtHMA1	Green tissues	Chloroplast envelope, Cu^{2+} import and homeostasis	Cu^{2+}	Cu^{2+}	Seigneurin-Berny et al. 2006
AtHMA2	All plant organs, especially vascular tissues	PM, Zn^{2+} efflux, Zn^{2+} phloem loading, Zn^{2+} homeostasis	Zn^{2+}, Cd^{2+}, less by Pb^{2+}, Ni^{2+}, Cu^{2+} and Co^{2+}	Zn^{2+}, Cd^{2+}	Eren and Argüello 2004, Hussain et al. 2004
AtHMA3	Roots, old rosette and cauline leaves	VM, vacuolar uptake of Cd^{2+} and Pb^{2+} and cellular detoxification	Cd^{2+}	Cd^{2+}, Pb^{2+}	Gravot et al. 2004
AhHMA3	Shoots, roots	Detoxification of Zn	Zn^{2+}	Zn^{2+}	Becher et al. 2004
AtHMA4	Tissues surrounding the root vascular tissues	PM, Zn^{2+} xylem loading in roots, root to shoot translocation, increased Zn^{2+}, Cd^{2+}, Co^{2+} tolerance, detoxification by enhanced efflux in root and shoot apoplast	Zn^{2+}, Cd^{2+}, Pb^{2+}	Zn^{2+}, Cd^{2+}	Verret et al. 2004, 2005 Hussain et al. 2004
Cu/Ag cluster					
AtHMA5	Roots (especially pericycle cells), flowers	Plastid membrane, Cu^{2+} compartmentation and detoxification	Cu^{2+} excess	Cu^{2+}	Andrés-Colás et al. 2005
AtHMA7 (RAN1)	-	Post Golgi compartment, transport of Cu^{2+} into the secretory pathway, and delivering it to secreted or membrane-bound proteins	-	Cu^{2+}	Hirayama et al. 1999, Woeste and Kieber, 2000, Williams and Mills 2005

AtHMA6 (PAA1)	Roots, shoots	Plastid envelope, Cu^{2+} transport into stroma	-	Cu^{2+}	Shikanai et al. 2003, Abdel-Ghany et al. 2005
AtHMA8 (PAA2)	Shoots	Chloroplast tylacoid memenbranes	-	Cu^{2+}	Abdel-Ghany et al. 2005
CDF					
AtMTP1	Roots, shoots, flowers	VM, sequestration of Zn^{2+} into vacuoles, to maintain Zn homeostasis	-	Zn	Kobae et al. 2004, Desbrosses-Fonrouge et al. 2005
AhMTP1	Shoots, roots under high Zn^{2+}	VM, transport of Zn^{2+} into the vacuole	Zn excess in roots	Zn	Dräger et al. 2004
TgMTP1	Roots	PM, VM, Zn^{2+} efflux from the cells	-	Zn	Kim et al. 2004
other					
LCT1	-	-	-	Cd^{2+}, Ca^{2+}	Clemens et al. 1998
NtCBP4	-	PM	-	Pb^{2+}	Arazi et al. 1999

VM, vacuolar membrane; PM, plasma membrane, PS, phytosyderophores; NA- nicotianamine. At-*Arabidopsis thaliana*, Os-*Orhyza sativa*, Zm- *Zea mays*, Tc – *Thlaspi caerulescens*, Tg- *Thlaspi goesingense*, Tj- *Thlaspi japonicum*, Mt- *Medicago truncatula*, Ah – *Arabidopsis halleri*, Nt- *Nicotiana tabaccum*

(Colangelo and Guerinot 2006). *A. thaliana* iron regulated transporter1 (AtIRT1), the founding member of the ZIP family, encodes the major Fe transporter expressed in the external cell layers of the roots, specifically in response to iron starvation, whereas the closely related OsIRT1 appears to play a similar role under Fe limiting conditions in rice (Vert et al. 2002, Bughio et al. 2002). Characterization of OsIRT1 and recent identification of OsIRT2 as an Fe^{2+} transporter reveal that grasses utilize two strategies for Fe uptake: the assimilation of Fe^{2+} and the uptake of Fe^{3+}–PS. ZIP proteins contribute to Zn homeostasis in *A. thaliana* and rice. OsZIP4, which shares a greater than 50% identity with AtZIP1 and OsIRT1 and was able to overcome the growth defect of the Zn-uptake-defective *zrt1 zrt2* yeast strain, but no similar functional complementation was observed in the Fe-, Mn- or Cu-uptake mutants, indicating that OsZIP4 is selective for Zn transport. Its transcript accumulates under Zn deficiency and has been detected in the vascular bundles and apical meristems of roots and shoots, suggesting it may be responsible for Zn transport within plants (Ishimaru et al. 2005, 2006, Colangelo and Guerinot 2006). Six cDNAs encoding ZIP family members were recently identified in the model legume *Medicago truncatula* (Table 11.1), and their ability to complement yeast Zn-, Mn-, and/or Fe-uptake mutants was demonstrated, indicating a function in metal transport. In addition, the metal responsiveness of *MtZIP1* steady-state mRNA levels supports its role in metal homeostasis. MtZIP1 is 59.6% identical to AtZIP1 and appears to function as a Zn transporter (Lopez-Millan et al. 2004, Colangelo and Guerinot 2006).

3.1.1.3 COPT Family

The copper transporter family of high-affinity Cu uptake proteins is found throughout the eukaryotes, including five members in *A. thaliana* named COPT1–COPT5. To date, only COPT1 has been characterized in detail. Plants that express antisense *COPT1* exhibit a decrease in Cu uptake and reduced Cu accumulation in leaves. Consistent with reports on *COPT1* expression in pollen and root tips, *COPT1* antisense plants display pollen development defects and increased root length, which can be reversed by Cu supplementation, pointing to a role for COPT1 in plant growth and development (Sancenón et al. 2003, 2004).

3.1.1.4 NRAMP Family

Plant natural resistance associated macrophage protein (NRAMP) family members (Table 11.1) have been implicated in the transport of several divalent cations, including Fe (Colangelo and Guerinot 2006). AtNRAMP1 can functionally complement an Fe uptake mutant of yeast and increase resistance to toxic Fe levels in plants that overexpress *AtNRAMP1* (Curie et al. 2000). *AtNRAMP3* is expressed in the vascular bundles of leaves and in the central cylinder of roots, irrespective of the Fe-nutritional conditions. By analyzing the metal content of *AtNRAMP3* disruption mutants and the *AtNRAMP3* overexpressing plants it was demonstrated that *AtNRAMP3* diminishes Mn and Zn accumulation upon Fe deficiency. Furthermore, strong ectopic expression of *AtNRAMP3* downregulates expression of the primary Fe uptake transporter IRT1 and root ferric chelate reductase (FRO2), indicating complex regulation among these mechanisms. Using transient expression of the GFP (green fluorescence protein)-tagged AtNRAMP3 protein, it was shown that AtNRAMP3 resides on the vacuolar membrane and was proposed to control Fe-acquisition genes, Mn and Zn accumulation upon Fe starvation and Cd sensitivity by mobilizing Fe, Cd, and other metals from the vacuolar compartment (Thomine et al. 2003). The closely related genes *AtNRAMP3* and *AtNRAMP4* share similar tissue-specific expression patterns, transcriptional regulation by Fe, and subcellular localization at the vacuolar membrane. *nramp3 nramp4* mutant seeds store Fe properly, but the metal is retained in the vacuole globoids and is not released during seed germination as in wild-type seeds, indicating the role of the AtNRAMP3 and AtNRAMP4 in retrieval of globoid-associated Fe during seed germination for seedling development (Lanquar et al. 2005). Both transporters were also shown to transport toxic metals such as Cd^{2+} and disruption of *AtNRAMP3* gene leads to slightly enhanced Cd root growth resistance in *Arabidopsis*, while its overexpression results in root growth hypersensitivity and increased accumulation of Fe on Cd^{2+} treatment (Thomine et al. 2000).

3.1.1.5 Transporters Involved in the Uptake of Nonessential Elements

With the exception of the recently described Cd carbonic anhydrase of marine diatoms, no biological function is known to date for the potentially highly toxic metals Cd and Pb (Lane and Morel 2000, Clemens 2006). It is therefore highly likely that these nonessential metals enter cells through cation transporter(s) with broad specificity, which are induced especially under low-iron conditions. The transporters IRT1, ZNT1, and AtNRAMP3 were shown to transport Cd^{2+} (Pence et al. 2000, Thomine et al. 2000). A search among putative cation transporter cDNAs for effects on growth in the presence of Cd^{2+} upon expression in yeast led to the observation that wheat low-affinity cation transporter 1 (LCT1) renders yeast cells more Cd^{2+} sensitive. LCT1 was originally cloned by complementation of the K^+ high-affinity uptake-deficient yeast mutant CY162 and shown to also mediate Na^+ influx. Its expression leads to increased Cd^{2+} and Ca^{2+} uptake activity in *S. cerevisiae* (Clemens et al. 1998). However, no *LCT1* homologues have been found in *Arabidopsis* or other nonplant species genomes (Mäser et al. 2001).

The first example of a plant transporter possibly mediating Pb^{2+} uptake was the calmodulin-binding cyclic nucleotide-gated channel (NtCBP4) localized in the plasma membrane of tobacco. Overexpression of *NtCBP4* resulted in increased sensitivity toward Pb^{2+} and correlated with enhanced Pb^{2+} accumulation (Arazi et al. 1999).

3.1.2 Metal Efflux Proteins

Transporters that are involved in *metal efflux from the cytoplasm* (Fig. 11.1), either by movement across the plasma membrane (PM) or into organelles, include the P_{1B}-ATPase family and the cation diffusion facilitator (CDF) family (Table 11.1) (Yang et al. 2005, Colangelo and Guerinot 2006). They play significant roles in metal detoxification and tolerance by compartmentation of metals or by loading them into the root or shoot apoplast.

3.1.2.1 P-type ATPase Family

The family of P-type ATP-ases uses energy from ATP hydrolysis to translocate cations across biological membranes and can be divided into several subfamilies, including the heavy metal transporting P_{1B}-ATPases. The eight P_{1B}-ATPases in *A. thaliana* are designated heavy metal transporting P-type ATP-ase HMA1 through HMA8. Four family members (HMA1–HMA4) group with the Zn/Cd/Pb/Co divalent cation transporter class of P_{1B}-ATPases, whereas HMA5–HMA8 encode Cu/Ag monovalent cation transporters (Table 11.1) (Williams and Mills 2005, Baxter et al. 2003). Metal transport studies of HMA2 and HMA4 in yeast showed that HMA2 is indeed a Zn^{2+} ATPase, which can also be activated by Cd^{2+} to a similar extent, and to a lesser extent by other divalent cations, while HMA4 transports Zn, Cd, and Pb (Eren and Argüello 2004, Verret et al. 2005). Reverse genetic approaches have been successful in revealing the functions of HMA family members *in planta*. HMA2 and HMA4 play a role in Zn translocation from roots to shoots, since *hma2 hma4* double mutant plants were chlorotic, stunted, and failed to set seeds. They could be resuscitated by watering with high levels of Zn (1 or 3 mM) but not with Co or Cu. Zn levels of *hma2 hma4* plants were two to fourfold lower than those of wild-type plants when grown under 10 mM Zn (+Zn conditions), whereas 35SHMA4 plants (in which HMA4 is overexpressed) accumulated elevated levels of Zn and Cd in leaves. A metal transport assay in yeast showed that HMA2 transports metal substrates out of the cytoplasm, which is in line with the *in planta* localization of HMA2 and HMA4 to the PM, suggesting that these proteins translocate heavy metals out of cells. The vascular expression of HMA2 and HMA4 points to a possible role of these mentioned transport proteins in xylem loading or unloading (Verret et al. 2004, Hussain et al. 2004). A similar root expression pattern was previously observed for the boron transporter BOR1 and for the phosphate transporter PHO1, which have both been implicated in xylem loading. The role of AtHMA2 and AtHMA4 in the translocation of heavy metals from root to shoot tissues makes these transporters potential targets for the generation of transgenic plants that are designed to clean up soils that have been contaminated with toxic metals, such as Cd and Pb (Krämer 2005, Colangelo and Guerinot 2006).

Members of the Cu/Ag-transporting class of P_{1B}-ATPases include HMA7 (responsive to antagonist1 [RAN1]), which is important for Cu delivery to hormone receptors in post-Golgi compartments (Hirayama et al. 1999, Woeste and Kieber

2000). Recently, *HMA5* was characterized as a root-enhanced, Cu-induced gene. The amino-terminal MxCxxC Cu- binding motifs of HMA5 interact with *A. thaliana* ATX1-like Cu chaperones in yeast two-hybrid screens. Considering the Cu hypersensitivity of *hma5* plants, it appears that this HMA family member is involved in Cu detoxification of roots in response to high Cu levels (Andres-Colas et al. 2006). Three P_{1B}-ATPases are involved in Cu transport in the chloroplast. HMA1 and HMA6 (P-type ATPase of *Arabidopsis1* [PAA1]) are localized on the plastid envelope, where they deliver Cu to the stroma. A complete loss of Cu accumulation in chloroplasts might be expected in *hma1 hma6* plants. HMA8 (PAA2) resides at the thylakoid membrane and functions in Cu delivery to the thylakoid lumen. Consistent with its localization *hma8* plants display greatly reduced Cu levels in thylakoids compared to whole chloroplasts, supporting its role in Cu transport to the thylakoid lumen (Abdel-Ghany et al. 2005, Shikanai et al. 2003).

3.1.2.2 CDF Family

The ubiquitous CDF family of metal transporters encodes proton antiporters that efflux heavy metals out of the cytoplasm (Table 11.1) (Colangelo and Guerinot 2006). The first CDF gene characterized in *A. thaliana* was the zinc transporter gene ZAT1, later renamed METAL TOLERANCE PROTEIN1 (MTP1). Its overexpression confers Zn tolerance *in planta* (van der Zaal et al. 1999, Delhaize et al. 2003). Transient expression of AtMTP1::green fluorescent protein (GFP) in *A. thaliana* protoplasts indicates that AtMTP1 is localized in the vacuolar membranes of leaf and root cells, suggesting a role in Zn sequestration in the vacuole. Evidence that *mtp1* knockdown plants accumulate less Zn in various tissues indicates that the proposed defect in vacuolar Zn storage affects Zn uptake or distribution (Kobae et al. 2004, Desbrosses-Fonrouge et al. 2005).

3.2 Long-Distance Transport of Metals

Once taken up by roots, metals are loaded in the xylem sap and translocated to the aerial parts of the plant by the transpiration stream. Only a small fraction of a metal in plants is thought to exist as free ions. A number of small, organic molecules have been implicated in metal ion homeostasis as metal ion ligands facilitating their uptake and transport (Haydon and Cobbett 2007). Metal transporters such as AtHMA2 or AtHMA4 play a role in metal xylem or phloem loading, while AtHMA4, AtNRAMP3, OsZIP4, ZmYSL1, AtISL1, OsISL2 (Table 11.1) may serve for long-distance transport of metals or metal complexes with either phytosiderophores (PS) or nicotianamine (NA) (Hussain et al. 2004, Thomine et al. 2003, Schaaf et al. 2004, Roberts et al. 2004, Koike et al. 2004, Le Jean et al. 2005). In *A. thaliana* expressing *TaPCS1* (phytochelatin synthase from *Triticum aestivum*) gene, significantly decreased Cd^{2+} accumulation in roots and enhanced long-distance Cd^{2+} transport into stems and rosette leaves was found, indicating phytochelatins (PCs) may play an essential role in Cd transport from roots to shoots (Gong et al. 2003). In addition, organic acids, especially citrate, are known as the main metal chelators in the xylem, especially of iron (Briat and Lebrun 1999). Amino acids in the xylem sap are also potential metal chelators of nickel, since increasing free histidine in the xylem sap enhanced translocation of this metal to the shoots, and this could explain nickel hyperaccumulation in some plants (Krämer et al. 1996). Also NA, a methionine derivative involved in sensing Fe status within the plant and translocation of Cu from roots to aerial parts, was shown to bind various metals (Briat and Lebrun 1999, Pianelli et al. 2005).

The mobility of metals from source (leaves) to sink (seeds, roots) tissues via the phloem sap is less understood. It is known that phloem sap contains Fe, Cu, Zn, and Mn arising from their mobilization in source organs. The two principal unknowns regarding phloem metal-loading are the valence state of metals as they enter or transit the phloem system and the identity of the ligands involved in phloem loading. The sole molecule identified as a potential phloem metal transporter is NA, which has been found in a stoichiometry of 1.25 with four metal ions (Fe, Cu, Zn, and Mn) in the phloem sap, leading the authors to speculate on united transport (Stephan et al. 1994). NA could therefore transport metals on long distance, once they have been loaded in the phloem, but this loading would probably require another chelator. Indeed, unidentified compounds of apparent molecular weight much higher than NA that are able to chelate various metals have been found. In *Ricinus communis*, phloem metal chelates of 1,000-l,500 Da for Zn, 1,000-5,000 Da for Mn, and 2,400 Da for iron have been reported. A 4,000 Da Zn-binding peptide has also been identified in *Citrus*. The chemical identity and the role of these metal complexes in the phloem metal-transport processes, if any, remain to be clarified (Briat and Lebrun 1999).

3.3 Metal Uptake and Transport in Metal Hyperaccumulating Plants

Transport mechanisms on the plasma membranes contribute decisively to symplastic metal loading, necessary for further metal translocation to the shoots, a trait that is highly enhanced in metal hyperaccumulating plants (Flowers and Yeo 1992). Molecular studies of metal hyperaccumulating species have revealed that the upregulation of metal transporters from a variety of families is one way in which some hyperaccumulating species achieve increased metal translocation to aerial tissues (Colangelo and Guerinot 2006).

3.3.1 Metal Uptake Proteins

3.3.1.1 YSL and ZIP Family

The role of *YSL* genes (Table 11.1) was demonstrated in metal accumulation by metal hyperaccumulating plant species. In *T. caerulescens YSL* genes are expressed at higher rates and with distinct patterns when compared with their *A. thaliana* homologues. While *TcYSL7* was highly expressed in the flowers, *TcYSL5* was more highly expressed in the shoots, and the expression of *TcYSL3* was equivalent in all the organs tested. In situ hybridizations have shown that *TcYSL7* and *TcYSL5* are expressed around the vasculature of the shoots and in the central cylinder in the roots. Exposure to heavy metals (Zn, Cd, Ni) does not affect the high and constitutive expression of the *TcYSL* genes. By mutant yeast complementation and uptake measurements it was demonstrated that TcYSL3 is an Fe/Ni-NA influx transporter mediating NA-metal, particularly NA-Ni, circulation in metal hyperaccumulating plants (Gendre et al. 2006).

ZIP genes (Table 11.1) were also shown to be an important feature in metal uptake by metal hyperaccumulating plant species. High endogenous *TcZNT1* expression in roots and shoots of the Zn/Cd hyperaccumlator *T. caerulescens* was associated with increased Zn^{2+} uptake in the roots, and the same mechanism likely underlay the enhanced Zn^{2+} uptake into leaf cells. *TcZNT1* encodes a high-affinity Zn transporter that can also mediate low-affinity Cd transport. It would be important to elucidate the regulatory components linking plant Zn status to *ZNT1* gene expression in order to understand how alterations in this pathway contribute to metal hyperaccumulation (Pence et al. 2000, Lasat et al. 2000). The Zn hyperaccumulator *Arabidopsis halleri* has elevated levels (23–24-fold) of the *ZIP6* transcript in roots and shoots, implying its involvement in build-up of this potentially toxic metal (Becher et al. 2004). Two ZIP genes, *TjZNT1* and *TjZNT2*, have been cloned from the Ni hyperaccumulator *Thlaspi japonicum*. Yeast expressing either *TjZNT1* or *TjZNT2* shows increased resistance to Ni^{2+} (Mizuno et al. 2005), highlighting a potential role for these genes in Ni tolerance. Further studies, however, will be needed to determine their functions implicated in Ni hyperaccumulation *in planta* (Colangelo and Guerinot 2006).

3.3.2 Metal Efflux Proteins

3.3.2.1 P-type ATPase and CDF Family

The Zn/Cd hyperaccumulator *A. halleri* safely accumulates 100-fold more Zn than nonaccumulating species. Increased expression of *MTP1* in shoots and *AhHMA3* in shoots and roots exposed to high Zn^{2+} (Table 11.1) were proven to be involved in Zn accumulation and detoxification. *A. halleri* contains three independently segregating and differentially regulated *MTP1* genes, and in a backcross between the hyperaccumulator *A. halleri* and the nonaccumulator *Arabidopsis lyrata*, two *MTP1* loci cosegregate with Zn tolerance. Evidence for AhMTP1-3 mediating Zn transport is provided by functional complementation of the Zn-sensitive phenotype of *zrc1 cot1*, a Zn-hypersensitive strain that lacks the vacuolar CDF family members ZINC RESISTANCE CONFERRING1 (ZRC1) and COBALT TRANSPORTER1 (COT1). Like AtMTP1, AhMTP1-3 localizes on the vacuolar membrane (Dräger et al. 2004, Becher et al. 2004). Expression of *MTP1* or related genes is constitutively high, specifically in the shoots of metal-tolerant metal hyperaccumulating plants, while in roots the transcript levels depend on metal concentrations. Under exposure to low Zn concentrations, root *MTP1* transcript levels in *A. halleri* are comparable to those in *A. thaliana*, whereas at high Zn supply, enhanced Zn sequestration in the root cells of *A. halleri* may participate in protecting the shoot from accumulating excess Zn. The regulation of *MTP1* expression, as observed in *A. halleri*, could thus serve as a model to improve the performance of transgenic plants in the phytoremediation of metal-contaminated soils (Dräger et al. 2004). *Thlaspi goesingense* is an Ni/Zn hyperaccumulator that also relies on enhanced expression of CDF transporters for metal tolerance (Persans et al. 2001). When fused to an epitope of the *Haemophilus influenzae* haemagglutinin protein (TgMTP1b::HA), TgMTP1b localized on vacuolar and plasma membranes. When expressed in *zrc1 cot1* plants, they showed a two-fold reduction in Zn levels, and the level of expression was found to correlate positively with the rate of Zn efflux. These results indicate that TgMTP1b does not confer resistance to high Zn by reversing the defect in vacuolar Zn compartmentalization of *zrc1 cot1*, rather, long- and short-term uptake studies support its role in Zn efflux. Together with its localization at the PM in *A. thaliana* protoplasts, evidence supports a role for MTP1 in Zn efflux at the PM in *T. goesingense*, possibly leading to reduced Zn vacuolar compartmentation in roots and enhanced xylem loading (Kim et al. 2004).

4 Metal Partitioning and Detoxification as Tolerance Strategies in Plants

4.1 Partitioning of Metals at Organ and Tissue Levels

Metal *exclusion from the roots* is achieved by several mechanisms. Enhanced lysis of epidermal and root cap cells with enhanced mucilage secretion were demonstrated to contribute to plant metal tolerance (Llugany et al. 2003, Delisle et al. 2001). Rhizodeposition includes the passive exudation of low molecular weight molecules (sugars, amino acids, organic acids, and hormones), active secretion of high molecular weight molecules (complex carbohydrates, enzymes), lysates, gases, and mucigel (Marschner 1995). Among these, organic acids (malate and citrate) were demonstrated to confer metal tolerance by chelation, although they do not seem to be universally applicable (Ernst et al. 1992, Salt 2001). Nevertheless, in the case of aluminum tolerance, exposure to Al^{3+} triggers the exudation of organic acids, thus preventing its toxicity, which seems to be the result of the constitutive expression of all the necessary metabolic machinery involved. In addition, Cu tolerance in *A. thaliana* was connected to a rapid release of citrate and Pb tolerance in rice to the stimulation of oxalate efflux (Ryan et al. 2001). Symbiosis with arbuscular mycorrhizal fungi was demonstrated to contribute to metal tolerance in plants (Joner and Leyval 1997, Vogel-Mikuš et al. 2006), presumably by root exclusion due to the enhanced adsorption and immobilization of metals on the fungal cell walls and/or compartmentization with complexation mainly in the vacuoles (Joner and Leyval 1997, Joner et al. 2000, Leyval and Joner 2001), thus contributing to the stabilization and succession of plant communities (Van der Heijden et al. 1998, Regvar et al. 2006). Its importance for phytoremediation of metal-polluted sites was recently reviewed elsewhere (Vogel-Mikuš and Regvar 2006).

4.1.1 Distribution of Metals in Roots

Shoot exclusion, on the other hand, indicates that metals have accumulated in the roots but are excluded from further transport. Low metal accumulation with *bioaccumulation factors* (BAF = C_{shoot}/C_{soil}) <1 and minute *translocation factors* (TF = C_{root}/C_{shoot})<<1, indicate *metal exclusion* from the shoots. The majority of plants use this so-called excluder strategy (Salt 2001, Baker 1981, Dahmani-Muller et al. 2000) to cope with excess metals. Thus, when roots absorb metals, they accumulate primarily in the rhizodermis and cortex, and a notable amount of metals are found in the root hairs (Seregin and Ivanov 2001). Metals that reach root cortical cells via apoplastic transport are retained on the walls bound to polygalacturonic acids, with the affinity for metal ions decreasing in the order Pb>Cr>Cu>Ca>Zn, or sequestered in the root vacuoles (Ernst et al. 1992, Clemens 2006). At sublethal concentrations, metals are not found in the stelar parenchyma, while at levels approaching lethal concentrations, when the endodermal barrier is broken, Cd and Pb pass over through the cortex and endodermis and are found in considerable amounts in the cells of the vascular cylinder (Seregin and Ivanov 2001).

4.1.2 Distribution and Partitioning of Metals in Shoots

In nonaccumulators, only low concentrations are found in the stem and leaf tissues, depending primarily on the activity of metal-sequestering pathways in root cells, that probably play a key role in determining the rate of translocation to the aerial parts. A second factor then would be the degree of accessibility and mobilization of the sequestered metal. Thirdly, the efficiency of radial symplastic passage through the root and across the endodermis is important, and finally, xylem loading, i.e., the efflux from xylem parenchyma cells into the xylem (Seregin and Ivanov 2001, Clemens et al. 2002). Metal distribution in plant foliage is species-specific, and its visualization is needed to understand the patterns of accumulation and tolerance of metal-treated plants (Cosio et al. 2005). In young sprouts of soybean, Cd was located in the stems and leaf veins together with leaf age-driven gradients (Cunningham et al. 1975). Uneven Cd accumulation with correspondence between the Cd spots observed on autoradiographs and the necrotic dots observed on the margins of the leaves was found on the leaf surface of *Salix viminalis* and *T. caerulescens* ecotype Prayon (Cosio et al. 2005). At the leaf tissue level the highest Ni concentrations were observed in epidermal cells of the Ni hyperaccumulator *Stackhousia tryonii*, double that recorded in palisade cells (Bhatia et al. 2004).

Similarly, in the Zn/Cd hyperaccumulator *T. caerulescens* preferential localization of metals was observed in the lower and upper epidermis (Vazquez et al. 1994, Küpper et al 1999, Cosio et al. 2005). In addition, Zn was preferentially stored in large vacoularized epidermal cells, while absent from the cells of the stomatal complex (Frey et al. 2000). However, Salt et al. (1995) found an even distribution of points of higher concentration of Cd at the leaf surface of Indian mustard and clearly showed the preferential accumulation of Cd in trichomes. Sequestration of metals in trichomes has been reported in a number of annual plants (Ager et al. 2002, Küupper et al. 1999). They are ideal for allocation of heavy metals because of their localization at the leaf periphery (Cosio et al. 2005), whereas in plants lacking trichomes, metals were found to be located in vessels and at the sites of the transpiration flow pathway, with the epidermis playing an important role in sequestration and metal detoxification (Seregin and Ivanov 2001).

4.1.3 Distribution and Partitioning of Metals in Seeds

The seed coat presents the first barrier to metal absorption in germinating seeds. Cd, Pb, Zn, and Ni were found mainly in the cell walls of seed coats and did not enter the embryos, even at lethal concentrations (Seregin and Ivanov 2001). However, in Zn/Cd hyperaccumulating *Thlaspi praecox* a significant amount of Cd (up to $1350\,\mu g^{-1}$) was accumulated in seeds. Elemental localization within seeds using micro-PIXE showed Cd was mainly accumulated in embryonic tissues, indicating high Cd mobility within the plant tissues. In addition, Zn also preferentially accumulated in embryonic tissues compared to seed coats, although in much lower concentrations (up to $200\,\mu g^{-1}$), while the highest Pb concentrations were observed in the seed coats (Vogel-Mikuš et al. 2007). The rate of metal accumulation in seeds seems to be species-specific, depending mainly on the metal in question; the ability of the plant to sequester metal(s) in roots and leaves, thus lowering free symplastic metal concentrations; and on the transport mechanisms involved in seed metal loading.

4.2 Metal Detoxification at Cellular and Subcellular Levels

Binding metals to the cell walls represents an important detoxification mechanism in both nonaccumulating and metal hyperaccumulating plants, especially at low metal concentrations and short time of exposure. Most studies have shown that in roots of metal hyperaccumulating *T. caerulescens* the majority of Cd was bound to the apoplast, but at higher concentrations Cd was also detected in the root symplast (Wojcik et al. 2005, Vazquez et al. 1992). In metal hyperaccumulating plants, accumulation of large amounts of metals requires the presence of intracellular high-capacity detoxification mechanisms, such as binding to organic acids, amino acids, phytochelatins and metallothioneins, and sequestration in the vacuoles acting as a central storage for ions (Clemens et al. 2002, Briat and Lebrun 1999). Increased expression of *NAS* (nicotianamine syntase) genes was found in *A. halleri* roots when compared to *A. thaliana*, corresponding to 3.5 times higher levels of NA. The higher rate of NA synthesis found for *A. halleri* roots at the transcript, protein, and metabolite levels suggests NA plays an important role in Zn homeostasis in dicots and might well represent one of the molecular factors conferring Zn tolerance and hyperaccumulation on *A. halleri* (Weber et al. 2004). *A. thaliana* plants overexpressing the *TcNAS1* gene overaccumulated NA, and increased NA levels were quantitatively correlated with increased Ni tolerance, indicating the great importance of NA in metal homeostasis of hyperaccumulating plants. These results indicate that it may now be possible to consider transferring NA synthase genes to fast growing plants of high biomass for use in phytoremediation (Pianelli et al. 2005). The function of transport of NA-metal chelates, required for the loading and unloading of vessels, has been assigned to the YSL-like family of proteins. Rapid induction of synthesis and accumulation of phytochelatins in response to Cd, Ag, Pb, Cu, Hg, Zn, Sn, Au, and As was demonstrated (Grill et al. 1987, Schat et al. 2002), with subsequent storage of the metals into vacuoles, providing efficient metal tolerance (Salt and Krämer 2000). The tendency of metals to induce phytochelatins in cell suspension cultures of *Rauvolfia sepentina* decreased in the order Hg >> Cd, As, Fe > Cu, Ni > Sb, Au > Sn, Se, Bi > Pb, Zn (Grill et al. 1987). The latest results, however, indicate that phytochelatin synthesis and metal complexation may contribute to Cd, Zn, and Cu tolerance in metal nontolerant plant populations, whereas they are not responsible for tolerance in metal hyperaccumulating plants, possibly due to the high energy demands required for phytochelatin synthesis, which may be too costly (Schat et al. 2002). Instead of phytochelatins, Cys and other low molecular weight thiols were proposed to be involved in Cd tolerance in *T. caerulescens* (Hernández-Allica et al. 2006). X-ray absorption spectroscopy showed that in mature and senescent leaves of *T. caerulescens* Cd and Zn were mainly complexed to oxygen-ligands, while in young leaves, Cd was mainly bound to S- ligands, and Zn was mainly bound to histidine (Küpper et al. 2004), which also plays an important role in the transport and detoxification of Ni in the Ni hyperaccumulator *T. goesingense* (Krämer et al. 2000).

5 Conclusion

Recent data from structural and functional genomics on uptake and sequestration of metals at plant organ, tissue, and cell levels shed new light on the understanding of the frequently observed exclusion, accumulation, or even hyperaccumulaton of metals in plants. General mechanisms for detoxification of metals in plants at the cellular level are the binding of metals to the apoplast (cell walls and trichomes) and chelation of the metals by ligands, followed by sequestration of the metal-ligand complex into the vacuole (Yang et al. 2005). Intense metal accumulation in the above-ground parts provides a promising approach for both cleaning up anthropogenically contaminated soils (phytoremediation) and for commercial extraction (phytomining) of metals from naturally metal-rich (serpentine) soils (McGrath et al. 1993), or stabilization of highly polluted and eroded sites (Regvar et al. 2006). Still, a deeper understanding of the metal uptake, transport, and detoxification mechanisms of plants is needed before these phytoremedial biotechnologies can brought effectively from theory into practice.

References

Abdel-Ghany SE, Muller-Moule P, Niyogi KK, Pilon M, Shikanai T (2005) Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. Plant Cell 17:1233-51

Ahn SJ, Rengel Z, Matsumoto H (2004) Aluminum-induced plasma membrane surface potential and H^+-ATPase activity in near-isogenic wheat lines differing in tolerance to aluminum. New Phytol 162:71-9

Ager FJ, Ynsa MD, Dominguez-Solis JR, Gotor C, Respaldiza MA, Romero LC (2002) Cadmium localization and quantification in the plant *Arabidopsis thaliana* using micro-PIXE. Nuclear Instrum Meth B 189:494-8

Andres-Colas N, Sancenon V, Rodriguez-Navarro S, Mayo S, Thiele DJ, Ecker JR, 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 45:225-36

Arazi T, Sunkar R, Kaplan B, Fromm H (1999) A tobacco plasma membrane calmodulin-binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. Plant J 20:171-82

Assunção AGL, Schat H, Arts MGM (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351-60

Bagnaresi P, Thoiron S, Mansion M, Rosignol M, Pupillo P, Briat JF (1999) Cloning and characterization of a maize cytochrome-b(5) reductase with Fe^{3+}- chelate reduction capability. Biochem J 338:499-505

Baker AJM (1981) Accumulators and excluders – strategies in the response of plants to heavy metals. J Plant Nutr 3:643-54

Baker, AJM (1987) Metal tolerance. New Phytol 106:93-111

Baxter I, Tchieu J, Sussman MR, Boutry M, Palmgren MG, Gribskov M, Harper JF, Axelsen KB (2003) Genomic comparison of P-type ATPase ion pumps in *Arabidopsis* and rice. Plant Physiol 132:618-28

Becher M, Talke IN, 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 37:251-68

Bhatia NP, Orlic I, Siegele R, Ashwath N, Baker AJM, Walsh KB (2004) Quantitative cellular localisation of nickel in leaves and stems of the hyperaccumulator plant *Stackhousia tryonii* Bailey using nuclear-microprobe (Micro-PIXE) and energy dispersive X-ray microanalysis (EDXMA) techniques. Funct Plant Biol 31:1-14

Briat JF, Lebrun M (1999) Plant responses to metal toxicity. CR Acad Sci Paris 322:43-54

Bughio N, Yamaguchi H, Nishizawa NK, Nakanishi H, Mori S (2002) Cloning an iron-regulated metal transporter from rice. J Exp Bot 53:1677-82

Cieslinski G, Van Rees KC, Szmigielska AM, Huang PM (1998) Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant Soil 203:109-17

Clemens S, Antosiewicz DM, Ward JM, Schachtman DP, Schroeder JI (1998) The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. Proc Natl Acad Sci USA 95:12043-8

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309-15

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochemie 88:1707-19

Cobbett C (2003) Heavy metals and plants – model systems and hyperaccumulators. New Phytol 159:289-93

Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport through plants. Curr Opin Plant Biol 9:322-30

Cosio C, DeSantis L, Frey B, Diallo S, Keller C (2005) Distribution of cadmium in leaves of *Thlaspi caerulescens*. J Exp Bot 56: 765-75

Cunningham LM, Collings FW, Hutchinson TC (1975) Physiological and biochemical aspects of cadmium toxicity in soybean. I. Toxicity symptoms and autoradiographic distribution of Cd in roots, stems and leaves. In: International conference on heavy metals in the environment, Symposium Proceedings, Institute for Environmental Studies, Universityof Toronto, Ontario, Canada 2: 97-120

Curie C, Alonso JM, Le Jean M, Ecker JR, Briat JF (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. Biochem J 347:749-55

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409:346-9

Dahmani-Muller HF, van Oort B, Gelie M, Balabane (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Poll 109:231-8

Delhaize E, Kataoka T, Hebb DM, White RG, Ryan PR (2003) Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. Plant Cell 15: 1131-42

Delisle G, Champoux M, Houde M, (2001) Characterization of oxidase and cell death in Al-sensitive and tolerant wheat roots. Plant Cell Physiol 42:324-33

Desbrosses-Fonrouge AG, Voigt K, Schroder A, Arrivault S, Thomine S, Kramer U (2005) *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. FEBS Lett 579:4165-74

DiDonato RJ Jr, Roberts LA, Sanderson T, Eisley RB, Walker EL (2004) *Arabidopsis* YELLOW STRIPE-LIKE2 (YSL2): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. Plant J 39:403-14

Dräger DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Kramer U (2004) Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins cosegregate with zinc tolerance and account for high MTP1 transcript levels. Plant J 39:425-39

Eren E, Argüello JM (2004) Arabidopsis HMA2, a divalent heavy metaltransporting P(IB)-type ATPase, is involved in cytoplasmic Zn^{2+} homeostasis. Plant Physiol 136:3712-23

Ernst WHO, Verkleij JAC, Schat H (1992) Metal tolerance in plants. Acta Bot Neerl 41:229-48

Flowers TJ, Yeo AR (1992) Solute transport in plants. Chapman & Hall, Edmundsbury

Frey B, Keller C, Zierold K, Schulin R (2000) Distribution of Zn in functionally different leaf epidermal cells of the hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 23:675-87

Gendre D, Czernic P, Conéjéro G, Pianelli K, Briat J- F, Lebrun M, Mari S (2006) TcYSL3, a member of the YSL gene family from the hyperaccumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. Plant J 49:1-15

Gong JM, Lee DA, Schroeder JI (2003) Long-distance root-to-shoot transport of phytochelatins and cadmium in *Arabidopsis*. Proc Natl Acad Sci USA 100:10118-23

González-Guerrero M, Azcón-Aguillar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol, N (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130-40

Gravot A, Lieutaud A, Verret F, Auroy P, Vavasseur A, Richaud P (2004) AtHMA3, a plant P_{1B}-ATPase, functions as a Cd/Pb transporter in yeast. FEBS Lett 561:22-8

Grill E, Winnacker E-L, Zenk MH 1987. Phytochelatins, a class of heavy-metal-binding peptides from plants are functionally analogous to metallothioneins. Proc Natl Acad Sci USA 84: 439-43

Haydon MJ, Cobbett CS (2007) Transporters of ligands for essential metal ions in plants. New Phytol 174:499-506

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity and productivity. Nature 396:69-72

Hernández-Allica J, Garbisu C, Becerril JM, Barrutia O, Garcia-Plazola JI, Zhao FJ, McGrath SP (2006) Synthesis of low molecular weight thiols in response to Cd exposure in *Thlaspi caerulescens*. Plant Cell Environ 29:1422-9

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139-46

Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, Nourizadeh S, Alonso JM, Dailey WP, Dancis A, Ecker JR (1999) Responsive-to-antagonist1, a Menkes/Wilson diseaserelated copper transporter, is required for ethylene signaling in *Arabidopsis*. Cell 97:383-93

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobbett CS (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. Plant Cell 16:1327-39

Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J Exp Bot 56:3207-14

Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M, Kobayashi T, Wada Y, Watanabe S, Matsuhashi S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2006) Rice plants take up iron as an Fe^{3+}- phytosiderophore and as Fe^{2+}. Plant J 45:335-46

Joner EJ, Leycal C (1997) Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol 135:353-60

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227-34

Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun DJ, Salt DE (2004) The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. Plant J 39:237-51

Kobae Y, Uemura T, Sato MH, Ohnishi M, Mimura T, Nakagawa T, Maeshima M (2004) Zinc transporter of Arabidopsis thaliana AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. Plant Cell Physiol 45:1749-58

Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J 39:415-24

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith AC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature (London) 379:635-8

Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. Plant Physiol 122:1343-53

Krämer U (2005) Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotech 16:133-41

Küpper H, Zhao FJ, McGrath SP (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305-11

Küpper H, Mijovilovich A, Klaucke-Mayer W, Kroneck PHM (2004) Tissue and age-dependent differences in the complexation of cadmium and zinc in the cadmium/zinc hyperaccumulator *Thlaspi caerulescens* Ganges ecotype revealed by X-ray absorption spectroscopy. Plant Physiol 134:748-57

Lane T, Morel F (2000) A biological function for cadmium in marine diatoms. P Natl Acad Sci USA 97:4627-31

Lanquar V, Lelievre F, Bolte S, Hames C, Alcon C, Neumann D, Vansuyt G, Curie C, Schroder A, Kramer U (2005) Mobilization of vacuolar iron by AtNramp3 and AtNramp4 is essential for seed germination on low iron. EMBO J 24:4041-51

Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 51:71-9

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109-20

Llugany M, Lombini A, Poschenrieder C, Barcelo J (2003) Different mechanisms account for enhanced copper resistance in *Silene armeria* from mine spoil and serpentine sites. Plant Soil 251:55-63

Leyval C, Joner EJ (2001) Bioavailability of heavy metals in the mycorrhizosphere. In: Gobran RG, Wenzel WW, Lombi E (eds) Trace metals in the rhizosphere, CRC Press, Florida, pp 165-85

Le Jean M, Schikora A, Mari S, Briat JF, Curie C (2005) A loss-of function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. Plant J 44:769-82

Ligaba A, Yamaguchi M, Shen H, Sasaki T, Yamamoto Y, Matsumoto H (2004) Phosphorus deficiency enhances plasma membrane H^+-ATPase activity and citrate exudation in greater purple lupin (*Lupinus pilosus*). Funct Plant Biol 31:1075-83

Lopez-Millan AF, Ellis DR, Grusak MA (2004) Identification and characterization of several new members of the ZIP family of metal ion transporters in *Medicago truncatula*. Plant Mol Biol 54:583-96

Marschner H, 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press, London

Mäser P, Thomine S, Schroeder I, Ward JM, Hirschi K, Sze H, Talke IN, Amtmann A, Maathuis FJM, Sanders D, Harper JF, Tchieu J, Gribskov M, Persans MW, Salt DE, Kim SA, Guerinot ML (2001) Phylogenetic Relationships within cation transporter families of *Arabidopsis*. Plant Physiol 126:1646-67

McGrath SP, Sidoli CMD, Baker AJM and Reeves RD (1993) The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: Eijsackers HJP, Hamers T (eds) Integrated soil and sediment research: a basis for proper protection, Kluwer Academic Publishers, Dordrecht, pp 673-6

McGrath SP, Shen ZG, Zhao FJ (1997) Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. Plant Soil 180:153-9

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207-14

Mizuno T, Usui K, Horie K, Nosaka S, Mizuno N, Obata H (2005) Cloning of three ZIP/Nramp transporter genes from a Ni hyperaccumulator plant *Thlaspi japonicum* and their Ni^{2+}-transport abilities. Plant Physiol Biochem 43:793-801

Moore MR (2003) Risk assessment in environmental contamination and environmental health. In: Naidu R, Gupta VVSR, Rogers S, Kookana RS, Bolan NS, Adriano DC (eds) Bioavailability, toxicity and risk relationships in ecosystems, Science Publishers, Inc., Enfield, pp. 3-19

Naidu R, Rogers S, Gupta VVSR, Kookana RS, Bolan NS, Adriano DC (2003) Bioavailability of metals in the soil plant environment and its potential role in risk assessment. In: Naidu R, Gupta VVSR, Rogers S, Kookana RS, Bolan NS, Adriano DC (eds) Bioavailability, toxicity and risk relationships in ecosystems, Science Publishers, Inc., Enfield, pp. 21-57

Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci USA 97:4956-60

Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. Proc Natl Acad Sci USA 98: 9995-10000

Pianelli K, Mari S, Marque's L, Vacchina V, Lobinski R, Lebrun M, Czernic P (2005) Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. Trans Res 14:739-48

Reeves RD, Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals. Using plants to clean up the environment, John Whiley & Sons, Inc., NY, pp. 193-229

Reeves RD (2006) Hyperaccumulation of trace elements by plants. In Morel JL, Echevarria G, Goncharova N (eds) Phytoremediation of metal contaminates soils (Nato Science Series: IV: Earth and Environmental Sciences), Springer-Verlag, pp 25-52

Regvar M, Vogel-Mikuš K, Kugonič N, Turk B, Batič F (2006) Vegetational and mycorrhizal successions at a metal polluted site: Indications for the direction of phytostabilisation? Environ Pollut 144:976-84

Roberts LA, Pierson AJ, Panaviene Z, Walker EL (2004) Yellow stripe1, Expanded roles for the maize iron-phytosiderophore transporter. Plant Physiol 135:112-20

Römheld V (1991) The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil 130:127-34

Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Ann Rev Plant Physiol Plant Mol Biol 52:527-60

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian Mustard. Plant Physiol 109:1427-33

Salt DE, Krämer U (2000) Mechanisms of metal hyperaccumulation in plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. John Wiley & Sons Inc, New York, pp. 193-229

Salt D (2001) Responses and adaptations of plants to metal stress. In: Hawkesford MJ, Buchner P (eds) Molecular analysis of plant adaptation to the environment. Kluwer Academic Publishers, Dodrecht, pp. 159-79

Sancenón V, Puig S, Mira H, Thiele DJ, Penarrubia L (2003) Identification of a copper transporter family in *Arabidopsis thaliana*. Plant Mol Biol 51:577-87

Sancenón V, Puig S, Mateu-Andres I, Dorcey E, Thiele DJ, Penarrubia L (2004) The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. J Biol Chem 279:15348-55

Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wiren N (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J Biol Chem 279:9091-6

Schaaf G, Schikora A, Haberle J, Vert G, Ludewig U, Briat JF, Curie C, von Wiren N (2005) A putative function for the *Arabidopsis* Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. Plant Cell Physiol 46:762-74

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker P (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53:2381-92

Seigneurin-Berny D, Gravot A, Auroy P, Mazard C, Kraut A, Finazzi G, Grunwald D, Rappaport F, Vavasseur A, Joyard J et al (2006) HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. J Biol Chem 281:2882-92

Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 48:523-44

Shikanai T, Muller-Moule P, Munekage Y, Niyogi KK, Pilon M (2003) PAA1, a P-type ATPase of *Arabidopsis*, functions in copper transport in chloroplasts. Plant Cell 15:1333-46

Stephan UW, Schmidke I, Pich A (1994) Phloem translocation of Fe, Cu, Mn, and Zn in *Ricinus* seedlings in relation to the concentrations of nicotianamine, an endogenous chelator of divalent metal ions, in different seedling parts. Plant Soil 165:181-8

Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI (2000) Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp genes. Proc Natl Acad Sci USA 97:4991-6

Thomine S, Lelievre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H (2003) AtNramp3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J 34:685-95

Tsao DT (2003) Phytoremediation. Adv Biochem Eng Biot 78. Springer-Verlag Berlin

Vázquez MD, Barceló J, Poschenreider C, Mádico J, Hatton P, Baker AJM, Cope GH (1992) Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. J Plant Physiol 140:350-55

Vázquez MD, Poschenreider C, Barceló J, Baker AJM, Hatton P, Cope GH (1994) Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J & C Presl. Bot Acta 107:243-50

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 576:306-12

Verret F, Gravot A, Auroy P, Preveral 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 His11 stretch. FEBS Lett 579:1515-22

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat JF, Curie C (2002) IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14:1223-33

Vogel-Mikuš K, Regvar M (2006) Arbuscular mycorrhiza as a tolerance strategy in metal contaminated soils-prospects in phytoremediation. Nova Science Publishers, pp. 37-56

Vogel-Mikuš K, Pongrac P, Kump P, Nečemer M, Regvar M (2006) Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. Environ Poll 139:362-71

Vogel-Mikuš K, Pongrac P, Kump P, Nečemer M, Simčič J, Pelicon J, Budnar M, Povh B, Regvar M (2007) Localisation and quantification of elements within seeds of Cd/Zn hyperaccumulator *Thlaspi praecox* by micro-PIXE. Environ Poll 147:50-9

Weber M, Harada E, Vess C, 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 37:269-81

Welch RM, Norwell WA, Schafer SC, Shaff Je, Kochian LV (1993) Introduction of iron (III) and copper (II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status: does the root-cell plasmalema Fe (III)-chelate reductase perform a general role in regulating cation uptake? Planta 190:555-61

Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Eviron Sci Technol 15:3144-50

Williams LE, Mills RF (2005) P(1B)-ATPases-an ancient family of transition metal pumps with diverse functions in plants. Trends Plant Sci 10:491-502

Woeste KE, Kieber JJ (2000) A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype. Plant Cell 12:443-55

Wójcik M, Vangronsveld J, Haen JD, Tukiendorf A (2005) Cadmium tolerance in *Thlaspi caerulescens* II. Localization of cadmium in *Thlaspi caerulescens*. Environ Exp Bot 53:163-71

Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339-53

van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JA, Hooykaas PJ (1999) Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol 119:1047-55

Zhao FJ, Hamon RE, McLaughlin MJ (2001) Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. New Phytol 151:613-20