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Abstract

As environmental issues become more and more stringent, the biotechnological approaches to maintain clean environments are receiving increasing attention. Heavy metal pollution is of great concern as it ultimately forces heavy metals into the food chain leading to serious ecological and health problems.

Removal of excess heavy metals from contaminated sites could be achieved by means of organisms that bioaccumulate heavy metals without developing toxicity symptoms, features that are characteristic to hyperaccumulating plants. This review focuses on the applicability of hyperaccumulation phenomenon to heavy metal bioremediation as well as on the possibility to extend the hyperaccumulation concept to organisms other than plants.

Chapter 13

Hyperaccumulation: A Key to Heavy Metal Bioremediation

Ileana Cornelia Farcasanu, Mihaela Matache, Virgil Iordache,
and Aurora Neagoe

13.1 Introduction

Industrialization, along with numerous benefits, brought up important issues such as environment awareness and environment protection. Strict regulations compel industries to find ways to limit the discharge of pollutants into the environment or to use eco-friendly approaches to clean up contaminated sites. Heavy metals are challenging pollutants, as they are natural components of the earth's crust, they are persistent in the environment and are nondegradable. Regarding the interaction with the living organisms, they have a dualistic behavior. On the one hand, many of the heavy metals are essential in minute amounts for the normal metabolism, binding to and stabilizing biomolecules, or acting as cofactors for various enzymatic processes. On the other hand, heavy metals can be toxic in high concentrations, mainly by nonspecific binding to biomolecules or by interference to other metals' metabolism. The sources of heavy metal pollution can be industrial effluents, automobile exhaustion, mining industry, leaching of metal ions from the soil into lakes, rivers and aquifers by acid rain, etc. Heavy metal contamination of soils and waters is alarming due to potential penetration through the food chain leading to serious health problems at all levels (Duruibe et al. 2007; Fraústo da Silva and Williams 2001; Sánchez 2008). The term "heavy metal" is ill defined and has raised controversy (Duffus 2002), but it is still commonly used when referring to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. The term is usually applied not only to common transition metals, such as Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn, but also to metalloids such as As or Se. Among these, Co, Cu, Mn, Ni, Zn, and Se are elements that are essential for life in

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29 very low amounts (essential trace elements). These are taken up by cells via intricate
30 active transport systems and are maintained at relatively constant levels by means of
31 strictly regulated homeostasis mechanisms. Other heavy elements such as Cd, Hg,
32 Pb, As are not essential for life, but they can compete with the essential trace
33 elements either for the transport systems or in binding to various biomolecules.
34 Essential or not, when present in high concentrations, the heavy metals become
35 toxic, causing serious damages to organisms.

36 Although avoidance of pollution should be a pre-requisite for any anthropomor-
37 phic activity, this is not always achieved. Cleaning polluted or contaminated sites is
38 never an easy task, especially when classical physicochemical approaches are
39 expensive or inefficient, produce secondary pollution, or simply fail. As pollution-
40 related issues become more stringent, cost-effective, sustainable, and environmen-
41 tally friendly methods for removal of hazardous substances are more and more
42 needed. Bioremediation by microorganisms, algae or plants is often considered as
43 inexpensive, safe and efficient way of cleaning up wastes, sediments, and soils.
44 Heavy metal bioremediation may be regarded as the removal of the excess hazard-
45 ous substance following the interaction between the pollutant and (1) one or more
46 living species; (2) dead or inactivated biomass. Bioremediation can be achieved
47 through a plethora of mechanisms, but the most actions against heavy metals
48 involve biosorption and bioaccumulation, actions that can take place in situ or in
49 bioreactors designed both for the hazardous substance and for the bioremediator.
50 Biosorption implies binding of the heavy metal ions to the cell surface, usually by
51 noncovalent, electrostatic forces and is considered a cost-effective biotechnology
52 for the treatment of high-volume-and-low-concentration complex wastewater.
53 Most times, biosorption refers to a property of certain types of inactive, dead,
54 microbial biomass to bind and concentrate heavy metals from dilute aqueous
55 solutions. The biomass exhibits this property, acting as an ion exchanger of
56 biological origin, and it is particularly the cell wall structure of certain algae,
57 fungi and bacteria, which was found responsible for this phenomenon.
58 Bioaccumulation is another attractive possibility to remove heavy metals from
59 the environment. In contrast to biosorption, bioaccumulation can be achieved
60 only by living cells, requiring metabolically driven continuous uptake that needs
61 to occur with a rate greater than the excretion rate. The retention of a chemical
62 within the living cells is a key step for bioremediation and obtaining resistant strains
63 is vital for such a process.

64 **13.2 Hyperaccumulation as a Primary Tool for Heavy** 65 **Metal Bioremediation**

66 Bioaccumulation has bioremediation significance only when correlated with
67 increased (gained) tolerance to the pollutant and easy separation of the
68 bioremediator from the site to be decontaminated. Thus, an ideal heavy

metal bioremediator would have the following characteristics: tolerance to nonphysiological metal concentrations, abundant growth on/in the contaminated site, hyperaccumulating capacity, and facile separation from the bioremediated site. Nevertheless, heavy metals inhibit very often the biological remediation processes due to metal sensitivity of most organisms. Under such circumstances, strategies for efficient operation have to be considered and heavy metal hyperaccumulating plants seem to be the best models to follow when designing or developing a suitable heavy metal bioremediator. Hyperaccumulation was a term first used by Brooks et al. (1977) for plants that are endemic to metalliferous soils and are able to tolerate and accumulate metals in their above-ground tissues. Metal hyperaccumulator plants are naturally capable of accumulating trace elements, in their above-ground tissues, without developing any toxicity symptoms (Baker 2002; Baker and Brooks 1989). The concentrations of these elements in dry leaf biomass are usually up to 100-fold higher than the concentrations in the soil (McGrath and Zhao 2003). Natural hyperaccumulator species are often an indication of elevated soil heavy metal concentrations, and hence they can function as bioindicators of contamination; their potential role in phytoremediation, phytoextraction, and phytomining has been extensively studied (for review see Boyd 2010; Chaney et al. 2007; Cheng 2003; Krämer 2005; Lone et al. 2008). Although metal hyperaccumulator plants seem very promising, most produce little biomass and are therefore used mainly as model organisms for research purposes (Krämer 2005). In this paper, studies concerning the use of hyperaccumulating plants for understanding the hyperaccumulation and tolerance mechanisms, the use of transgenic approaches to obtain new hyperaccumulators, as well as the possibility to extend the plant hyperaccumulation concept to other organisms are reviewed.

13.3 Metals Commonly Hyperaccumulated by Plants

Most plants, when exposed to potentially toxic metals in their growing medium, take up the metal into the root, but restrict its further translocation to the shoot. A very rare class of plants, named hyperaccumulators, translocate substantial amounts to their shoots, so that shoot:root ratios exceed unity (Baker 1981; Macnair 2003). Most hyperaccumulators that have been identified so far hyperaccumulate nickel, but hyperaccumulators of cadmium, arsenic and zinc are also well characterized (Macnair 2003). To be considered a genuine hyperaccumulator, plants need to accumulate high concentrations of metals in any part that grows above the ground (Baker and Brooks 1989; Baker et al. 2000) in concentrations 10–500 times higher than the same plant species from nonpolluted environments (Yanqun et al. 2005). In addition, the shoot-to-root concentrations ratios must be higher than one (McGrath and Zhao 2003; Yanqun et al. 2005), meaning higher concentrations in the plant than in the soil. Most metal hyperaccumulators are endemic to soils with high concentrations of metal (Baker and Brooks 1989; Pollard et al. 2002). Table 13.1 summarizes the most studied heavy metal plant hyperaccumulators. In particular,

Table 13.1 Some heavy metal plant accumulators

	Plant name	Metal accumulated	Possible tolerance mechanism	Comments	References (selected)
t1.1	<i>Alyssum</i> sp.	Ni	Chelated by free L-histidine Ni ²⁺ /H ⁺ antiport V-ATPase at the tonoplast that can drive vacuolar accumulation of Ni through a secondary active transport mechanism	<i>Alyssum</i> accounts for 48 of the 318 known Ni hyperaccumulator species known	Baker et al. (1994, 2000), Brooks et al. (1977), Corem et al. (2009), Ingle et al. (2005b, 2008), Krämer et al. (1996), Reeves and Baker (2000)
t1.3	<i>Arabidopsis halleri</i>	Cd, Ni, Zn	Slow vacuolar (SV) channel activity Metal chelation Metal sequestration <i>AHMA4</i> increased activity	Proteomic analysis in <i>A. lesbiacum</i> One of the closest wild relatives of <i>A. thaliana</i> Transcriptomic studies Proteomic analysis of shoots in response to Cd, Zn and rhizosphere microorganisms Field and soil studies Fern common in metal-contaminated areas in Asia	Becher et al. (2004), Farinati et al. (2009), Hanikenne et al. (2008), Küpper et al. (2000), Marquès et al. (2004), McGrath et al. (2006), Talke et al. (2006), Vera-Estrella et al. (2009), Weber et al. (2004), Zhao et al. (2006)
t1.11	<i>Athyrium yokoscense</i>	As, Cd	High activities of antioxidative enzymes	Crop plant	Morishita and Boratynski (1992), Van et al. (2006)
t1.12	<i>Avena strigosa</i>	Cd	High amounts of total soluble phenolics	Crop plant	Uraguchi et al. (2006)
t1.13	<i>Crotalaria juncea</i>	Cd	High activities of antioxidative enzymes	Crop plant	Uraguchi et al. (2006)
t1.14	<i>Fagopyrum esculentum</i>	Pb	High amounts of total soluble phenolics	One of the species co-accumulates Ni	Tamura et al. (2005)
t1.15	<i>Gossia</i> sp.	Mn/foliar	Organic acid chelation		Fernando et al. (2007, 2009)

t1.20	<i>Maytenus founieri</i> ssp.	Mn	Sequestration primarily in dermal tissues	Tree species, difficult to follow on short periods	Fernando et al. (2007)
t1.21	<i>Phytolacca acinosa</i>	Mn	Sequestration of Mn in leaf epidermis	Herbaceous Mn hyperaccumulator, with high biomass and fast growth	Xue et al. (2004, 2005), Xu et al. (2006)
t1.23	<i>Pteris vitata</i>	As	Suppression of endogenous arsenate reduction in roots may serve to enhance root-to-shoot translocation of As	The plant transports 95% of the absorbed arsenic from roots to shoots	Bondada et al. (2004), Caille et al. (2004), Dhankher et al. (2002), Ma et al. (2001), Tu and Ma (2002), Wei et al. (2006, 2007), Zhao et al. (2003)
t1.24	<i>Sedum alfredii</i>	Cd, Pb, Zn	Chelation by phytochelatins Enhanced root-to-shoot translocation Glutathione, rather than phytochelatins involved in Zn and Pb transport	Applicability in the field Transcriptomic analysis under Zn induction	Chao et al. (2010), Long et al. (2009), Lu et al. (2008), Ni and Wei (2003), Sun et al. (2005, 2007), Yang et al. (2004)
t1.25	<i>Thlaspi caerulescens</i>	Cd, Ni, Zn	Metal chelation with organic ligands	One of the most studied model organisms for hyperaccumulation mechanisms	Baker et al. (2000), Basic et al. (2006), Hammond et al. (2006), Küpper et al. (2004), McGrath et al. (2006), Milner and Kochian (2008), van de Mortel et al. (2006), Papoyan et al. (2007), Rigola et al. (2006), Ueno et al. (2005), Yanai et al. (2006)
t1.28			Increased expression of <i>ZNT1</i> , <i>MTP1</i> , and <i>HMA4</i> genes	Stimulated shoot metal accumulation Transcriptome studies	
t1.29			Enhanced ability to compartmentalize Ni in shoot vacuoles	Variations between populations and ecotypes	
t1.30				Field and soil studies	
t1.31					
t1.32					
t1.33	<i>Thlaspi goesingense</i>	Ni	Enhanced ability to compartmentalize Ni in shoot vacuoles	Vacuolar metal ion transport proteins, termed metal tolerance proteins (TgMTPs) described	Krämer et al. (2000), Persans et al. (2001)

110 *Thlaspi caerulescens* and *Arabidopsis halleri*, have been studied extensively for
111 their ability to hyperaccumulate several heavy metals, mainly Zn, Cd, and Ni, as
112 well as the species from *Alisum* genus, known for their ability to hyperaccumulate
113 Ni. To be considered a hyperaccumulator, the plants need to store more than
114 1,000 mg kg⁻¹ (dry weight biomass) of metal for Ni, Cu, Co, Cr, or Pb, or
115 10,000 mg kg⁻¹ for Zn or Mn (Baker and Brooks 1989). Some of the heavy metals
116 more commonly stored by hyperaccumulator plants are presented below.

117 13.3.1 Cadmium

118 Cd is nonessential but a major pollutant that is extremely toxic to organisms. Soils
119 can be contaminated with Cd as a result of fertilization with phosphates as well as
120 from mining and smelting industries (McGrath et al. 2001; Sanità di Toppi and
121 Gabbrielli 1999). Daily consumption of Cd-contaminated foods poses a risk to
122 human health (Watanabe et al. 2000). The principal plant used for Cd
123 phytoextraction is *Thlaspi caerulescens*, a Zn/Cd hyperaccumulator (Baker et al.
124 2000) owing its potential to the exceeding bioconcentration factor of Cd and Zn in
125 the shoots that enables a remarkable yield of both metals from contaminated soil
126 (McGrath and Zhao 2003; McGrath et al. 2001). Usually, Cd overlaps the Zn
127 tolerance and hyperaccumulation (Uraguchi et al. 2006; Yang et al. 2004). Apart
128 from *T. caerulescens*, *Arabidopsis halleri* (Küpper et al. 2000), *Sedum alfredii*
129 (Yang et al. 2004; Zhao et al. 2006), and *Athyrium yokoscense*, (Morishita and
130 Boratynski 1992) have been reported as evident Cd-hyperaccumulator plants.
131 These are all wild metal-accumulators, growing slowly in the field. Uraguchi
132 et al. (2006) studied the behavior of crop species and found two Cd hyperaccu-
133 mulators, *Avena strigosa* and *Crotalaria juncea*. In this case, the Cd tolerance
134 seems to be the result of enhanced activities of antioxidative enzymes and of
135 augmentation in total soluble phenolics, both well-known antioxidant defense
136 strategies. In plant cells, Cd tends to be stored in the apoplast and in vacuoles. In
137 addition to the metal compartmentation mechanism, antioxidative ability might
138 play an important role in the tolerance. Cd is a redox-inactive metal that is
139 incapable of producing reactive oxygen species (ROS) directly, but can indirectly
140 promote oxidative stress by disrupting physiological processes. Also,
141 phytochelatin (PCs) and other thiol (SH)-containing compounds have been pro-
142 posed to play an important role in the detoxification and tolerance of some heavy
143 metals. For instance, glutathione may be responsible for Cd and tolerance in mine
144 population of *Sedum alfredii* (Sun et al. 2007).

145 13.3.2 Copper

146 Cu is an essential element, and Cu poisoning is rarely an issue of environmental risk.
147 Nevertheless, sources of Cu contamination are the electroplating industry, smelting

and refining, mining, biosolids (Liu et al. 2005). There are not many Cu hyperaccumulators described, and most of them are not Cu-specific, like some varieties of *Thlaspi caerulescens*, or *Athyrium yokoscense* (Cheng 2003; Honjo et al. 1984).

13.3.3 Manganese

Manganese, an essential trace element that is found in varying amounts in all tissues, is one of the most widely used metals in industry. Exposure to excess manganese results in manganese toxicity, including Parkinson-like symptoms (Chan et al. 2000; Erikson and Aschner 2003; Gerber et al. 2002), and abnormalities of the immune system (Vartanian et al. 1999). Manganese hyperaccumulation has been arbitrarily defined by a threshold foliar concentration of 10,000 mg kg⁻¹ dry weight (Baker and Brooks 1989). Plants that hyperaccumulate Mn are predominantly woody and hence unsuited to short-term controlled study (Fernando et al. 2008). The number of species has varied with taxonomic changes, and currently nine are recognized worldwide (Bidwell et al. 2002; Reeves and Baker 2000; Xue et al. 2004). The heterogeneity of the Mn-hyperaccumulative trait was studied in *Phytolacca acinosa* a herbaceous species, under controlled conditions (Xue et al. 2005) and in natural populations of the tree *Gossia bidwillii* (Fernando et al. 2007). Recently, Fernando et al. (2009) demonstrated up to seven new Mn hyperaccumulators, mostly tropical rainforest species from the *Gossia* genus, one of them exhibiting also elevated foliar Ni concentrations. Among the Mn hyperaccumulating plants, *P. acinosa* is a good candidate for phytoremediation of Mn polluted soil for its high biomass and fast growth. Xu et al. (2006) determined Mn distribution within the hyperaccumulating plant and found that the highest Mn content was in the vascular tissues of root, stem, petiole and midrib and that, Mn content in leaf epidermis was higher than that in mesophyll, which suggested that the sequestration of Mn in leaf epidermis might be one of the detoxification mechanisms of *P. acinosa*.

13.3.4 Nickel

Approximately 450 species of metal-hyperaccumulating plants are currently known, of which more than 330 are Ni-hyperaccumulators (Reeves 2003; Reeves and Adigüzel 2004; Reeves and Baker 2000). Ni is ubiquitously distributed in nature and constitutes a trace element in most living cells. In high concentrations, it is toxic to most cells and is also listed as a possible human carcinogen (group 2B) and associated with reproductive problems and birth defects. Ni-hyperaccumulators are able to take up more than 1,000 mg kg⁻¹ dry weight (Baker et al. 1994) without significant detrimental effect on plant survival or health. In terms of remediation using classical chemical approaches, Ni is one of the most recalcitrant pollutants; therefore, Ni hyperaccumulating plants have received special attention. Ni-hyperaccumulators belong to a group of taxonomically diverse plants that can

186 accumulate Ni to concentrations in excess of 0.1% shoot dry biomass (Baker and
187 Brooks 1989; Reeves and Baker 2000). The genus *Alyssum* accounts for 48 of the
188 known Ni hyperaccumulator species, and includes *Alyssum lesbiacum* which is
189 capable of accumulating Ni to over 3% shoot dry biomass (Baker et al. 2000;
190 Reeves and Baker 2000). Ni accumulates predominantly in the aerial tissues of
191 hyperaccumulators, and the shoot epidermal cells are a major site of Ni deposition
192 in hyperaccumulator plants, including several *Alyssum* species, *Cleome heratensis*,
193 *Hybanthus floribundus* and *Senecio coronatus* (Asemaneh et al. 2006; Bidwell et al.
194 2004; de la Fuente et al. 2007; Küpper et al. 2001; Marquès et al. 2004; Mesjasz-
195 Przybyłowicz et al. 1994).

196 13.3.5 Lead

197 Pb is a nonessential heavy metal discharged in the environment from mining and
198 smelting of metalliferous ores, burning of leaded gasoline, municipal sewage,
199 industrial wastes enriched in Pb, paints (Gispert et al. 2003; Seward and Richardson
200 1990) There are certain cultivars of Indian mustard (*Brassica juncea*) (Kumar et al.
201 1995), the fern *Athyrium yokoscense* (Honjo et al. 1984), or the leguminous shrub
202 *Sesbania drummondii* (Sahi et al. 2002) that have been reported as Pb hyperaccu-
203 mulators. Tamura et al. (2005) found that common buckwheat (*Fagopyrum*
204 *esculentum* Moench), known as an aluminum (Al) accumulator (Ma and Hiradate
205 2000; Ma et al. 1997; Shen et al. 2002), can accumulate a high concentration of lead
206 (Pb) in the shoot and especially in the leaf. Since buckwheat can grow with
207 relatively high biomass productivity this plant may prove to be a useful
208 phytoremediator of Pb-contaminated soils around the world.

209 13.3.6 Zinc

210 Zn is another essential trace element that has deleterious effect on organism when
211 present in excess. Zn pollution sources can be electroplating industry, smelting and
212 refining, mining, biosolids (Liu et al. 2005). Around 14 species have been described
213 as Zn hyperaccumulators defined as containing more than 10 g kg⁻¹ shoot dry
214 weight (Baker et al. 2000). *Thlaspi caerulescens* has been widely studied for its
215 remarkable properties to tolerate toxic levels of Zn as well as Cd, and sometimes Ni
216 (reviewed by Milner and Kochian 2008). Along with *Arabidopsis halleri*,
217 *T. caerulescens* has been in the primelight for studies concerning the hyperaccu-
218 mulation process. Because *T. caerulescens* is a slow-growing plant species that
219 does not generate significant shoot biomass, it has been used primarily as a
220 model system for the investigation and identification of the molecular and physio-
221 logical mechanisms of hyperaccumulation, with the ultimate goal of transferring
222 these mechanisms to higher biomass plant species. *A. halleri* has been
223 described as constitutively zinc (Zn)-tolerant as well as Zn-hyperaccumulating

(Bert et al. 2000, 2002, 2003; Macnair 2002; Pauwels et al. 2006). The Zn- 224
 accumulating capacity of this plant is quite remarkable (Kashem et al. 2010). 225
 Another plant reported to accumulate Zn is *Sedum alfredii* Hance. When grown 226
 on an old lead/zinc mining site, Long et al. (2009) found this plant to significantly 227
 hyperaccumulate zinc and cadmium under field conditions. 228

13.3.7 Arsenic 229

Arsenic (As) has been categorized as a toxic and carcinogenic element and contrib- 230
 ute to environmental and human health problems worldwide; the highest number of 231
 cases has been reported in South-East Asian countries (Mandal and Suzuki 2002). 232
 Drinking water from wells located in areas with As-rich underground sediments has 233
 been shown to be the source of arsenocosis (Patel et al. 2005; Yang et al. 2002). Ma 234
 et al. (2001) discovered the first known vascular plant, *Pteris vittata* L. commonly 235
 known as Chinese brake fern to hyperaccumulate arsenic. The brake fern takes up 236
 high concentrations of arsenic (as high as 2.3%) from soil and allocated most of it to 237
 the above-ground parts for final storage (Tu and Ma 2002). Moreover, the hyperaccu- 238
 mulation of arsenic is accompanied by an increased biomass of the above-ground 239
 plant parts, an important characteristic, which is indispensable for phytoremediation 240
 (Ma et al. 2001; Tu and Ma 2002). Other desirable characters permitting brake fern 241
 as an ideal plant for phytoremediation include its perennial growth habit, disease 242
 and pest resistance, fast vigorous growth, and its preference for soils with high pH 243
 where arsenic exists in high abundance (Bondada and Ma 2003; Bondada et al. 244
 2004). Studies on arsenic hyperaccumulation by *P. vittata* were diverse (Caille et al. 245
 2004; Wei et al. 2006, 2007) and other species of ferns, including *Pteris cretica* and 246
Pityrogramma calomelanos, have also been determined to be As-hyperaccu- 247
 mulators and show great potential in phytoremediation (Francesconi et al. 2002; 248
 Visoottiviseth et al. 2002; Wei et al. 2006). The root systems of the As hyperaccu- 249
 mulating fern *P. vittata* possess a higher affinity for arsenate uptake than those of a 250
 related nonaccumulator fern species, and a suppression of endogenous arsenate 251
 reduction in roots may serve to enhance root-to-shoot translocation of As (Dhankher 252
 et al. 2002). Phytochelatins, metal-chelating molecules synthesized by the ubiqui- 253
 tous plant enzyme phytochelatin synthase are known to contribute to As detoxifica- 254
 tion in As hyperaccumulator plants (Zhao et al. 2003). 255

13.4 What is Responsible for the Heavy Metal Tolerance 256
of the Hyperaccumulating Plants? 257

At least three processes appear to make a major contribution to the ability of certain 258
 species to hyperaccumulate metals: enhanced uptake, root-to-shoot translocation, 259
 and detoxification via chelation and sequestration (Clemens et al. 2002; Pollard 260

261 et al. 2002). In addition, hyperaccumulator plants appear to have highly effective
262 antioxidant systems to protect against the potentially damaging effects of metal-
263 induced ROS (Freeman et al. 2004).

264 13.4.1 Metal Chelation

265 Regarding the deleterious effects of heavy metals on the living organisms, it is
266 considered that mainly the osmotically free forms of the metal ions are genuinely
267 toxic, as they are prone to bind nonspecifically to biomolecules or to interfere with
268 the essential metals' metabolism. To avoid the growth impairments caused by the
269 potentially toxic heavy metals, hyperaccumulating plants must possess biochem-
270 ical defense mechanisms. Plants developed a number of strategies to resist this
271 toxicity, including active efflux, sequestration, and binding of heavy metals inside
272 the cells by strong ligands. The primary antidote against the osmotically free ions
273 may be the presence of chelating agents that form easily nontoxic complexes. Thus,
274 an essential component of tolerance is the buffering of free metal ions in the
275 cytoplasm via chelation with high-affinity ligands. The response of plants is
276 complex with considerable variation between species. Several detoxification
277 strategies are known to occur and different effects are observed with different
278 metals and metal concentrations. Still and Williams (1980) first proposed that Ni
279 hyperaccumulation might involve a ligand containing two nitrogen donor centers
280 and one oxygen donor center because this would exhibit a sufficiently high affinity
281 for Ni and could account for the observed preference for Ni over Co in these plants.
282 This was demonstrated by Krämer et al. (1996), who observed a linear relationship
283 between the concentrations of Ni and free histidine appearing in the xylem of
284 hyperaccumulating species of *Alyssum* exposed to a range of Ni concentrations.
285 Thus, it was found that the concentration of free histidine in the roots of the
286 hyperaccumulator species *A. lesbiacum* could be several fold higher than in the
287 nonaccumulator *Brassica juncea*, even in the absence of Ni (Kerkeb and Krämer
288 2003). Histidine forms soluble complex compounds with Ni, which are completely
289 nontoxic. To determine the molecular basis of the histidine response and its
290 contribution to Ni tolerance, Ingle et al. (2005a, b) analyzed the transcripts of the
291 enzymes involved in histidine biosynthesis and found that the transcript levels were
292 constitutively higher in the hyperaccumulating *A. lesbiacum* compared to the
293 congeneric nonaccumulator *A. montanum*. This was noted especially for the first
294 enzyme in the biosynthetic pathway, ATP-phosphoribosyltransferase (ATP-PRT);
295 comparison with the weak hyperaccumulator *A. serpyllifolium* revealed a close
296 correlation between Ni tolerance, root histidine concentration, and ATP-PRT
297 transcript abundance. Thus, it seems that *ATP-PRT* expression plays a major role
298 in regulating the pool of free histidine and contributes to the exceptional Ni
299 tolerance of hyperaccumulator *Alyssum* species. Free histidine seems to be involved
300 in Ni tolerance in other hyperaccumulators as well. Thus, histidine concentration in
301 the roots is 17-fold higher in the Ni hyperaccumulator *Thlaspi goesingense* than in

the nonaccumulator *T. arvense* (Persans et al. 1999). Nevertheless, it seems that the Ni hyperaccumulating phenotype in *T. goesingense* is not determined by the overproduction of His in response to Ni (Persans et al. 1999). Coordination with histidine is not a universal determinant for heavy metal hyperaccumulation, not even for Ni. Phytochelatins are other plant chelators that might be worth considering when metal ion buffering is desired. Complexation with phytochelatin peptides synthesized from glutathione has been identified as an important mechanism for detoxifying metals such as Cd, Pb, and Zn in nonhyperaccumulator plants and plant cell cultures (Cobbett 2000; Zenk 1996), but phytochelatins do not appear to be responsible for hyperaccumulation or metal tolerance in hyperaccumulator species (Ebbs et al. 2002; Krämer et al. 1996, 2000; Shen et al. 2000). Sun et al. (2005) investigated whether phytochelatins were differentially produced in mine populations of *Sedum alfredii* compared with a nonmine control of the same species and found that phytochelatins and cysteine were not responsible for Zn and Pb tolerance in the mine population; instead, Zn and Pb treatments resulted in the increase of glutathione, suggesting that glutathione, rather than phytochelatins, may be involved in Zn and Pb transport, hyperaccumulation/accumulation and tolerance in mine population of *S. alfredii*. There are many indications that organic acids are involved in heavy metal tolerance, transport and storage in plants, including for Cd, Ni, and Zn (Godbold et al. 1984; Krotz et al. 1989; Nigam et al. 2001; Yang et al. 1997). In hyperaccumulators, the levels of citric, malic, malonic, and oxalic acids have been correlated with elevated concentrations of Ni or Zn in the biomass Li et al. 2010; Tolrá et al. 1996). Determining the distribution of Cd and Ni in hairy roots of the Cd hyperaccumulator, *Thlaspi caerulescens* and the Ni hyperaccumulator, *Alyssum bertolonii* that contained high constitutive levels of citric, malic and malonic acids, Boominathan and Doran (2003) found that about 13% of the total Cd in *T. caerulescens* roots and 28% of the total Ni in *A. bertolonii* were associated with organic acids, while the hairy roots remained healthy and grew well. Nicotianamine (NA), a nonproteinogenous amino acid synthesized in all plants through the activity of the enzyme nicotianamine synthase (NAS) is another chelator that can be involved in heavy metal tolerance (Higuchi et al. 1994). Based on yeast screens, Mari et al. (2006) demonstrated root-to-shoot long-distance circulation of nicotianamine and nicotianamine–nickel chelates in the metal hyperaccumulator *Thlaspi caerulescens*, while NA–Ni chelates are absent in the nontolerant nonhyperaccumulator-related species *T. arvense*. Furthermore, *Arabidopsis thaliana* lines overexpressing TcNAS cDNA produce a large amount of NA, correlated with a better resistance to the toxicity of this metal (Pianelli et al. 2005). An intriguing fact is that the Cd/Zn hyperaccumulator *Thlaspi caerulescens* is sensitive toward Cu, which may be a problem for phytoremediation of soils with mixed contamination, demonstrating that hyperaccumulation and metal resistance are highly metal specific. A few individuals of *T. caerulescens* that were more Cu resistant revealed that a large proportion of Cu in *T. caerulescens* leaves is bound by sulfur ligands, in contrast to the known binding environment of cadmium and zinc in the same species, which is dominated by oxygen ligands (Mijovilovich et al. 2009). This observation suggests that hyperaccumulators detoxify hyperaccumulated metals

347 differently compared with nonaccumulated metals. Thus, it seems that hyperaccu-
348 mulators have different strategies of detoxification for metals that are
349 hyperaccumulated compared with nonhyperaccumulated metals. For the
350 hyperaccumulated metals, detoxification is mainly based on active sequestration
351 into the vacuoles of the epidermis, where they are stored only loosely associated with
352 organic acids that are abundant in this organelle (Küpper et al. 2004). Strong ligands
353 such as the phytochelatins and metallothioneins that detoxify heavy metals in
354 nonaccumulator plants do not play a major role in the detoxification of
355 hyperaccumulated metals in hyperaccumulator plants (Küpper et al. 2009;
356 Mijovilovich et al. 2009). At the same time, Ni and Zn are possibly transported by
357 similar ligands and thus competition for binding sites is likely to be a factor in the
358 relationship between uptake of Zn and Ni when levels of both are high in the soil
359 (Assunção et al. 2003).

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360 13.4.2 Metal Transport, Distribution, Compartmentalization

361 Plants developed different strategies to grow on soils rich in heavy metals. Many of
362 them, the excluders, are able to restrict root uptake, and in particular, root-to-shoot
363 translocation of heavy metals (Baker 1981; Küpper et al. 2000, 2001). On the
364 opposite side, hyperaccumulators store high amounts of heavy metals in the
365 above-ground parts. Understanding how metal transport and accumulation pro-
366 cesses differ between normal and hyperaccumulator plants is important. “Normal”
367 nonhyperaccumulator plants tend to store the absorbed heavy metals in the roots,
368 whereas hyperaccumulator plants are capable of transporting most of the
369 accumulated heavy metals to the shoots (Lasat et al. 1998, 2000). Hyperaccumulator
370 plants exhibit stronger influx of heavy metals into the roots than the nonaccumulator
371 species do (Lasat et al. 1996). Studies examining the competitive effects between Zn
372 and Cd transport in *Thlaspi caerulescens* suggested that, at least in the leaf, the
373 heavy metal Cd is transported via cellular Zn transporters (Cosio et al. 2004). While
374 Zn and Cd are transported via the same transporter in the case of the Prayon ecotype,
375 Cd transport in the Ganges ecotype (which hyperaccumulates Cd to a higher degree)
376 takes place through a separate transporter (Lombi et al. 2001). In contrast, in
377 nonaccumulator plants, Zn and Cd uptake and accumulation are negatively
378 correlated (Hart et al. 2002; Wu et al. 2003). Papoyan et al. (2007) suggested that
379 xylem loading may be one of the key sites responsible for the hyperaccumulation of
380 Zn and Cd accumulation in *Thlaspi caerulescens*. Interestingly, the expression of
381 one of the metal transporter involved, (HMA4) in *Arabidopsis thaliana* is
382 downregulated upon exposure to heavy metals (Mills et al. 2003), while its expres-
383 sion in *T. caerulescens* is upregulated upon exposure to high concentrations of Cd
384 and Zn (Papoyan and Kochian 2004). In a quantitative study of cell compartmen-
385 tation, Küpper et al. (2000) showed that the major storage site for Zn and Cd
386 hyperaccumulation in *Arabidopsis halleri* are the leaf mesophyll cells. In *Sedum*
387 *alfredii* Hance, altered Zn transport across the tonoplast in the root stimulate Zn

uptake in the leaf cells, being the major mechanisms involved in the strong Zn hyperaccumulation observed (Yang et al. 2006). At the cellular level, a major role has been ascribed to vacuolar compartmentalization of excess cytosolic metals. Likewise, hyperaccumulation is connected to the ability to transport large amounts of metals into leaf vacuoles (Krämer et al. 2000; Persans et al. 2001). Shira et al. (2009) investigated the transport properties of the Slow Vacuolar (SV) channel identified in leaf vacuoles of *Alyssum bertolonii* Desv. Accumulation of Ni in epidermal cells seemed to be a common feature in the leaves of Ni-hyperaccumulators, such as *Thlaspi goesingense*, *Alyssum* species and *Berkheya coddii* (Broadhurst et al. 2004; Kerkeb and Krämer 2003; Krämer et al. 1996; Küpper et al. 2000, 2001; McNear et al. 2005). The sequestration of toxic metals in leaf epidermis is not a universal detoxification mechanism in all hyperaccumulators. Cu in *Elsholtzia splendens* (Shi et al. 2004) and Mn in *Gossia bidwillii* (Fernando et al. 2006) seem to be more abundant in the mesophyll than in the epidermis of leaves. The cellular distribution of Pb in leaves is less studied. The Pb-As co-hyperaccumulator *Viola principis* H. de Boiss has similar Pb and As compartmentalization patterns in the leaves, as both elements accumulate in the bundle sheath and the palisade mesophyll, while the vascular bundle and the epidermis contained lower levels of Pb and As. The palisade enrichment of Pb and As indicates that *V. principis* H. de Boiss. may have a special mechanism on detoxification of toxic metals within the mesophyll cells (Lei et al. 2008). Apart from complexation with organic ligands, cellular compartmentation is likely to be involved in heavy metal tolerance of the hyperaccumulators. Krämer et al. (2000) estimated that approximately 70% of Ni in *Thlaspi goesingense* was associated with the apoplast, while Küpper et al. (2001) reported that the majority of Ni was localized in the vacuoles in leaf tissue of *Alisum lesbiacum* and *Thlaspi goesingense*. This apparent discrepancy may be due in part to the different methods used to study Ni localization (Smart et al. 2007). The vacuole is the largest subcellular compartment, occupying 70–80% of the total volume of mature parenchymatous plant cells, and plays an important role in the storage of inorganic ions (Martinoia et al. 2007). It is rich in carboxylic acids, such as citric and malic acid, which may serve to chelate Ni with moderately high affinity (Saito et al. 2005; Smart et al. 2007). If the vacuole is a site of Ni sequestration in hyperaccumulators, some form of active transport will be required at the tonoplast membrane to move Ni into the vacuole against its electrochemical gradient. Ingle et al. (2008) highlighted a $\text{Ni}^{2+}/\text{H}^{+}$ antiport system at the tonoplast of *Alisum lesbiacum* that can drive vacuolar accumulation of Ni via a secondary active transport mechanism.

13.5 Molecular Studies

Most hyperaccumulators are slow-growing plant species that do not generate significant shoot biomass. This is why they are used mainly as model systems for the investigation and identification of the underlying molecular and physiological

429 mechanisms of hyperaccumulation, with the ultimate goal of transferring these
430 mechanisms to higher biomass plant species. A number of genes involved in the
431 hyperaccumulating phenotype were identified. Among them, a major role for
432 *AhHMA4* in naturally selected Zn hyperaccumulation and associated Cd and Zn
433 hypertolerance in *Arabidopsis halleri* was demonstrated (Hanikenne et al. 2008).
434 The function of identified genes was also checked by complementation analysis in
435 model organisms such as *Arabidopsis thaliana* or *Saccharomyces cerevisiae*
436 (Table 13.2). The molecular basis of the widely used hyperaccumulator *Thlaspi*
437 *caerulescens* was thoroughly reviewed by Milner and Kochian (2008). More
438 widely, systemic studies such as transcriptomic and proteomic studies are expected
439 to shed light on the intricate mechanisms that lead to hyperaccumulation.

440 13.5.1 Transcriptomic Studies

441 A comparative transcriptome analysis between the Zn/Cd hyperaccumulator
442 *Arabidopsis halleri*, and the nonaccumulator *Arabidopsis thaliana* using the gene
443 chip arrays revealed that hyperexpression may be a general property of metal
444 hyperaccumulators, as a large number of genes were found to be more highly
445 expressed in *A. halleri* compared with *A. thaliana* (Becher et al. 2004; Weber
446 et al. 2004). In a separate study, a set of candidate genes for Zn hyperaccumulation,
447 Zn and Cd hypertolerance, and the adjustment of micronutrient homeostasis in
448 *A. halleri* was identified using a combination of genome-wide cross species
449 microarray analysis and real-time PCR (Talke et al. 2006). In this study, 18 putative
450 metal homeostasis genes were newly identified to be more highly expressed in
451 *A. halleri* than in *A. thaliana*, and 11 previously identified candidate genes were
452 confirmed (Talke et al. 2006). Interestingly, the study showed that in the steady
453 state, *A. halleri* roots, but not the shoots, act as physiologically Zn-deficient under
454 conditions of moderate Zn supply. An analysis of the first EST collection from
455 *Thlaspi caerulescens* obtained from Zn-exposed roots and shoots revealed that
456 *T. caerulescens* expresses a relatively large number of genes, which are expressed
457 at a very low level in *A. thaliana*, and 8% of the total set of expressed uni-genes did
458 not have an *Arabidopsis* ortholog (Rigola et al. 2006). Interestingly, some of the
459 genes shown to be more highly expressed in *A. halleri* also exhibited elevated
460 expression in *T. caerulescens*, suggesting this set of genes might be important for
461 hyperaccumulation in both plant species. Also, cDNA-AFLP analysis of inducible
462 gene expression in the Zn hyperaccumulator *Sedum alfredii* Hance under zinc
463 induction was reported (Chao et al. 2010).

Table 13.2 Transgenic organisms expressing genes isolated from hyperaccumulator plants

t2.1	Target organism	Gene/source	Gained phenotype	Function	Comments	References
t2.2	<i>Arabidopsis thaliana</i>	<i>TcNAS1/Thlaspi caerulescens</i>	Ni tolerance	Nicotianamine synthase	Expression in <i>At</i> resulted in a significant increase in both plant Ni tolerance and Ni accumulation in the shoot	Pianelli et al. (2005)
t2.3		<i>ATP-PRT</i> cDNA/ <i>Alissum lesbiacum</i>	Ni tolerance, but no Ni hyperaccumulation	Histidine synthesis pathway	Overexpression increased the pool of free His up to 15-fold	Ingle et al. (2005a)
t2.4		<i>AhHMA4/Arabidopsis halleri</i>	Zn and Cd tolerance	CPx-ATPase	Metal substrates: Zn and Cd	Talke et al. (2006)
t2.5	<i>Saccharomyces cerevisiae</i>	<i>PvPCS1/Pteris vittata</i>	Cd tolerance	Phytochelatin synthase		Dong et al. (2005)
t2.6		<i>TcHMA4/Thlaspi caerulescens</i>	Cd tolerance	CPx-ATPase	Expression in <i>Tc</i> is induced by both Zn-deficiency and high-Zn treatments, as well as in response to high Cd	Bernard et al. (2004), Papoyan and Kochian (2004)
t2.7		<i>TcMT3</i>	(metallothionein)/ <i>Thlaspi caerulescens</i>	Cd and Cu tolerance	Cysteine-rich, low-molecular-weight, metal-binding proteins	Maintains normal Cu homeostasis under high cytoplasmic Cd and Zn
t2.8		Roosens et al. (2004)				
t2.9		<i>TcNAS1</i> cDNA/ <i>Thlaspi caerulescens</i>	Ni tolerance	Synthesis of nicotianamine, a nonproteinaceous amino acid, capable of chelating metal ions	Nicotianamine synthase	Vacchina et al. (2003)
t2.10		<i>TcZNT1/Thlaspi caerulescens</i>	Mediate Zn and Cd uptake	Plasma membrane-localized transporter		Pence et al. (2000)

(continued)

13.5.2 Proteomic Studies

464

Broader molecular characterization of hyperaccumulators has been performed 465
mainly by transcriptomics. In proteomics, even though large and hydrophobic 466
transporters and low abundant or small polypeptides may remain undetected in 467
2-DE-based proteomics, many other proteins, such as regulatory proteins and those 468
contributing to stress protection that appear to have importance in the hyperaccu- 469
mulation phenotype might be detected (Farinati et al. 2009; van de Mortel et al. 470
2006; Verbruggen et al. 2009). In addition, posttranscriptional regulation could be 471
very important, and there is often no proportionality between the transcript and 472
protein abundance. Different profiling techniques are thus clearly complementary, 473
and the proteomics approach is of increasing interest in exploring the hyperaccu- 474
mulation phenomenon. A proteomic analysis of the Ni hyperaccumulator plant 475
Alyssum lesbiacum was carried out to identify proteins that may play a role in the 476
Ni tolerance and accumulation characteristic. As very few polypeptides were found 477
to change in abundance in root or shoot tissue after plants were exposed to 478
conditions representing the optimum for growth and hyperaccumulation of Ni in 479
the shoot, it was concluded that constitutively expressed genes may be sufficient to 480
allow for effective chelation and sequestration of Ni without the need for additional 481
protein synthesis (Ingle et al. 2005b). Tuomainen et al. (2006) identified differences 482
in protein intensities among three *Thlaspi caerulescens* accessions with pronounced 483
differences in tolerance, uptake and root-to-shoot translocation of Zn and Cd, 484
noticing clearest differences mainly among the *Thlaspi* accessions, while the effects 485
of metal exposures were less pronounced. When looking at protein profiles of 486
Thlaspi caerulescens, two accessions, and lines derived from the two accession 487
proteins that showed co-segregation with high or low Zn accumulation were 488
manganese superoxide dismutase, glutathione S-transferase, S-formyl glutathione 489
hydrolase (Tuomainen et al. 2010). 490

13.6 The Hyperaccumulation Concept Applied to Organisms 491 Other than Plants 492

Searching through literature uncovers the fact that the heavy metal hyperaccu- 493
mulation seems to be restricted to plants, as seen from the number of studies or from 494
the unequivocal definition of a hyperaccumulator. Hyperaccumulation is a phenom- 495
enon that generated the idea of phytoremediation and phytoextraction, immediately 496
related to eco-friendly bioremediation processes. Even if the large-scale application 497
of hyperaccumulating plants in bioremediation is in its infancy, it is undeniable that 498
this is somehow restricted to soils. Phycoremediation, or the use of algae in 499
bioremediation is another increasing possibility that would enlarge the applicability 500
to contaminated waters (Olguin 2003). Marine organisms are of increasing interest 501
and exceptionally high levels of trace metals have been reported in specific tissues 502

503 of certain *Polychaetes* (Gibbs et al. 1981; Ishii et al. 1994; Fattorini et al. 2005,
504 2010; Sandrini et al. 2006). In a remarkable review, Gifford et al. 2007 introduced
505 the concept of zooremediation and defined an animal heavy metal
506 hyperaccumulator by similitude with plants as animal species known to accumulate
507 $>100 \text{ mg kg}^{-1}$ Cd, Cr, Co or Pb; or $>1,000 \text{ mg kg}^{-1}$ Ni, Cu, Se, As or Al; or
508 $>10,000 \text{ mg kg}^{-1}$ Zn or Mn. Even though this field would probably be limited to
509 invertebrates for ethical reasons, emerging data are tempting and self-financing zoo
510 remediation models such as pearl oysters, sponges are proposed as models (Gifford
511 et al. 2007). Work concerning the use of zooremediation is less intense than in
512 plants, one system is already in use for the recovery of Cd in waste scallop tissue: in
513 scallops (Seki and Suzuki 1997; Shiraishi et al. 2003, [http://www.unirex-jp.com/
514 engcadmium/engcadmium.htm](http://www.unirex-jp.com/engcadmium/engcadmium.htm)). Although microorganisms are the most common
515 group of organisms used for bioremediation, biosorption is the main governing
516 process used to remove heavy metals from contaminated sites. Manipulating heavy
517 metal resistance of bacteria by overexpressing genes from hyperaccumulating
518 plants has been reported (Freeman et al. 2005), but the term hyperaccumulation
519 may be awkward to apply in this case. Molecular mechanisms concerning heavy
520 metal metabolism are widely studied in bacteria, and expressing various bacterial
521 genes into plants to obtain hyperaccumulating transgenics is a promising approach
522 (Dhankher et al. 2002, 2003; Rugh et al. 1996). The genus *Saccharomyces* looks
523 like the group to which the term is worth extrapolating, mainly because of the
524 general tendency to extend plant molecular processes to model organisms such as
525 *Saccharomyces cerevisiae* (Table 13.2). The ease of growth and the exceptional
526 elegance of genetic manipulation make the yeast cells a versatile tool for biotech-
527 nology. *S. cerevisiae* is not a heavy metal accumulator, and attempts to obtain
528 tolerant hyperaccumulating in *S. cerevisiae* yeast strains failed, noticing a
529 biunivocal relationship tolerance-exclusion, hyperaccumulation-sensitivity. Never-
530 theless, there are studies indicating that heavy metal sensitive but accumulating
531 *kamikaze* strains could be used for heavy metal bioremediation through
532 bioaccumulation (Ruta et al. 2010). Moreover, the cell surface of yeast cells can
533 be engineered for heavy metal increases absorption capacity using the molecular
534 display (arming) technology in which heterologous proteins that are expected to
535 offer novel functionality to cell wall can be expressed on the surface of the cell
536 (Georgiou et al. 1997; Murai et al. 1997; Shibasaki et al. 2009). Using such
537 technology, cells with improved heavy metal biosorption abilities were obtained
538 (Kambe-Honjoh et al. 2000; Nakajima et al. 2001; Kotrba and Rumi 2010).
539 Combining improved biosorbents with bioaccumulative induction may be the
540 basis of obtaining hyperaccumulating yeast strains.

541 13.7 Conclusions

542 As heavy metal pollution poses serious problems and needs to be overcome in a
543 friendly, noninvasive way, the extraordinary phenotype of hyperaccumulating
544 plants seems to be the appropriate tool to design bioremediation systems capable

of removing the contaminating heavy metals from various sites. In recent years, 545
 major scientific progress has been made in understanding the physiological and 546
 molecular mechanisms of metal uptake and transport in these plants. General metal 547
 hyperaccumulators, however, may be inadequate for bioremediation technology 548
 because of their small size and slow growth rates. At present, therefore, the focus is 549
 on searching for new metal hyperaccumulators or on trying to improve metal uptake 550
 in plants and other organisms using metal chelators and on producing transgenic 551
 organisms that show metal hyperaccumulation. 552

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References

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Asemaneh T, Ghaderian SM, Crawford SA, Marshall AT, Baker AJM (2006) Cellular and 558
 subcellular compartmentation of Ni in the Eurasian serpentine plants *Alyssum bracteatum*, 559
Alyssum murale (*Brassicaceae*) and *Cleome heratensis* (*Capparaceae*). *Planta* 225:193–202 560
 Assunção AGL, Schat H, Aarts MGM (2003) *Thlaspi caerulescens*, an attractive model species to 561
 study heavy metal hyperaccumulation in plants. *New Phytol* 159:351–360 562
 Baker AJM (1981) Accumulation and excluders – strategies in the response of plants to heavy 563
 metals. *J Plant Nutr* 3:643–654 564
 Baker AJM (2002) The use of tolerant plants and hyperaccumulators. In: Wong MH, Bradshaw 565
 AD (eds) *Restoration and management of derelict land: modern approaches*, [derived from an 566
Advanced Study Institute], Kowloon, China, Nov 2000, pp 138–148 567
 Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements – 568
 a review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81–126 569
 Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal 570
 decontamination of polluted soils using crops of metal-accumulating plants. *Resour Conservat* 571
Recycl 11:41–49 572
 Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: 573
 a review of the ecology and physiology of a biological resource for phytoremediation of 574
 metal-polluted soils. In: Terry N, Baelos G (eds) *Phytoremediation of contaminated soil and* 575
water. Lewis, Boca Raton, FL, pp 85–107 576
 Basic N, Salamin N, Keller C, Galland N, Besnard G (2006) Cadmium hyperaccumulation and 577
 genetic differentiation of *Thlaspi caerulescens* populations. *Biochem Syst Ecol* 34:667–677 578
 Becher M, Talke IN, Krall L, Krämer U (2004) Cross-species microarray transcript profiling 579
 reveals high constitutive expression of metalhomeostasis genes in shoots of the zinc 580
 hyperaccumulator *Arabidopsis halleri*. *Plant J* 37:251–268 581
 Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N (2004) A novel CPx-ATPase from the 582
 cadmium hyperaccumulator *Thlaspi caerulescens*. *FEBS Lett* 569:140–148 583
 Bert V, Macnair MR, De Lague rie P, Saumitou-Laprade P, Petit D (2000) Zinc tolerance and 584
 accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* 585
 (*Brassicaceae*). *New Phytol* 146:225–233 586
 Bert V, Bonnin I, Saumitou-Laprade P, de Laguerie P, Petit D (2002) Do *Arabidopsis halleri* from 587
 nonmetallicolous populations accumulate zinc and cadmium more effectively than those from 588
 metallicolous populations? *New Phytol* 155:47–57 589

- 590 Bert V, Meerts P, Saumitou-Laprade P, Salis P, Gruber W, Verbruggen N (2003) Genetic basis of
591 Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. Plant Soil 249:9–18
- 592 Bidwell SD, Crawford SA, Woodrow IE, Sommer-Knudsen J, Marshall AT (2004) Sub-cellular
593 localization of Ni in the hyperaccumulator, *Hybanthus floribundus* (Lindley) F. Muell. Plant
594 Cell Environ 27:705–716
- 595 Bondada BR, Ma LQ (2003) Tolerance of heavy metals in vascular plants: arsenic hyperaccu-
596 mulation by Chinese brake fern (*Pteris vittata* L.). In: Chandra S, Srivastava M (eds) Pteridol-
597 ogy in new millenium. Kluwer, The Netherlands, pp 397–420
- 598 Bondada BR, Tu S, Ma LQ (2004) Absorption of foliar-applied arsenic by the arsenic hyperaccu-
599 mulating fern (*Pteris vittata* L.). Sci Total Environ 332:61–70
- 600 Boominathan R, Doran PM (2003) Organic acid complexation, heavy metal distribution and the
601 effect of ATPase inhibition in hairy roots of hyperaccumulator plant species. J Biotechnol
602 101:131–146
- 603 Boyd RS (2010) Heavy metal pollutants and chemical ecology: exploring new frontiers. J Chem
604 Ecol 36:46–58
- 605 Broadhurst CL, Chaney RL, Angle JS, Mangel TK, Erbe EF, Murphy CA (2004) Simultaneous
606 hyperaccumulation of nickel, manganese, and calcium in *Alyssum Leaf* Trichomes. Environ Sci
607 Technol 38:5797–5802
- 608 Brooks RR, Lee J, Reeves R, Jaffre T (1977) Detection of nickeliferous rocks by analysis of
609 herbarium specimens of indicator plants. J Geochem Explor 7:49–58
- 610 Caille N, Swanwick S, Zhao FJ, McGrath SP (2004) Arsenic hyperaccumulation by *Pteris vittata*
611 arsenic contaminated soils and the effect of liming and phosphate fertilization. Environ Pollut
612 132:113–120
- 613 Chan DW, Son SC, Block W, Ye R, Douglas P, Pelley J, Goodarzi AA, Khanna KK, Wold MS,
614 Taya Y, Lavin MF, Lees-Miller SP (2000) Purification and characterization of ATM from
615 human placenta, a manganese-dependent, wortmanninsensitive serine/threonine protein
616 kinase. J Biol Chem 275:7803–7810
- 617 Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved
618 understanding of hyperaccumulation yields commercial phytoextraction and phytomining
619 technologies. J Environ Qual 36:1429–1443
- 620 Chao Y, Zhang M, Feng Y, Yang X, Islam E (2010) cDNA-AFLP analysis of inducible gene
621 expression in zinc hyperaccumulator *Sedum alfredii* Hance under zinc induction. Environ Exp
622 Bot 68:107–112
- 623 Cheng S (2003) Heavy metals in plants and phytoremediation: a state-of-the-art report with special
624 reference to literature published in Chinese journals. Environ Sci Pollut Res Int 10:335–340
- 625 Clemens S, Palmgren M, Krämer U (2002) A long way ahead: understanding and engineering
626 plant metal accumulation. Trends Plant Sci 7:309–315
- 627 Cobbett CS (2000) Phytochelatin biosynthesis and function in heavy-metal detoxification. Curr
628 Opin Plant Biol 3:211–216
- 629 Corem S, Carpaneto A, Soliani P, Cornara L, Gambale F, Scholz-Starke J (2009) Response to
630 cytosolic nickel of Slow Vacuolar channels in the hyperaccumulator plant *Alyssum bertolonii*.
631 Eur Biophys J 38:495–501
- 632 Cosio C, Martinoia E, Keller C (2004) Hyperaccumulation of cadmium and zinc in *Thlaspi*
633 *caerulescens* and *Arabidopsis halleri* at the leaf cellular level. Plant Physiol 134:716–725
- 634 de la Fuente V, Rodriguez N, Diez-Garretas B, Rufo L, Asensi A, Amils R (2007) Nickel
635 distribution in the hyperaccumulator *Alyssum serpyllifolium* Desf. spp. from the Iberian
636 Peninsula. Plant Biosyst 141:170–180
- 637 Dhankher OP, Li Y, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB (2002)
638 Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate
639 reductase and g-glutamylcysteine synthetase expression. Nat Biotechnol 20:1140–1145
- 640 Dhankher OP, Shasti NA, Rosen BP, Fuhrmann M, Meagher RB (2003) Increased cadmium
641 tolerance and accumulation by plants expressing bacterial arsenate reductase. New Phytol
642 159:431–441

- Dong R, Formentin E, Losseso C, Carimi F, Benedetti P, Terzi M, Lo Schiavo F (2005) Molecular cloning and characterization of a phytochelatin synthase gene, *PvPCS1*, from *Pteris vittata* L. *J Ind Microbiol Biotechnol* 32:527–533 643–645
- Duffus JH (2002) “Heavy metals” a meaningless term? (IUPAC Technical Report). *Pure Appl Chem* 74:793–807 646–647
- Duruibe JO, Ogwoegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human biotoxic effects. *Int J Phys Sci* 2:112–118 648–649
- Ebbs S, Lau I, Ahner B, Kochian L (2002) Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (J. & C. Presl). *Planta* 214:635–640 650–652
- Erikson KM, Aschner M (2003) Manganese neurotoxicity and glutamate-GABA interaction. *Neurochem Int* 43:475–480 653–654
- Farinati S, DalCorso G, Bona E, Corbella M, Lampis S, Cecconi D, Polati R, Berta G, Vallini G, Furini A (2009) Proteomic analysis of *Arabidopsis halleri* shoots in response to the heavy metals cadmium and zinc and rhizosphere microorganisms. *Proteomics* 9:4837–4850 656–657
- Fattorini D, Notti A, Halt MN, Gambi MC, Regoli F (2005) Levels and chemical speciation of arsenic in polychaetes: a review. *Mar Ecol* 26:255–264 658–659
- Fattorini D, Notti A, Nigro M, Regoli F (2010) Hyperaccumulation of vanadium in the Antarctic polychaete *Perkinsiana littoralis* as a natural chemical defense against predation. *Environ Sci Pollut Res* 17:220–228 660–662
- Fernando DR, Batianoff GN, Baker AJM, Woodrow IE (2006) In vivo localization of manganese in the hyperaccumulator *Gossia bidwillii* (Benth.) N. Snow & Guymier (Myrtaceae) by cryo-SEM/EDAX. *Plant Cell Environ* 29:1012–1020 663–665
- Fernando DR, Baker AJM, Woodrow IE, Batianoff GN, Bakkaus EJ, Collins RN (2007) Variability of Mn hyperaccumulation in the Australian rainforest tree *Gossia bidwillii* (Myrtaceae). *Plant Soil* 293:145–152 666–668
- Fernando DR, Woodrow IE, Jaffré T, Dumontet V, Marshall AT, Baker AJM (2008) Foliar manganese accumulation by *Maytenus founieri* (Celastraceae) in its native New Caledonian habitats: populational variation and localization by X-ray microanalysis. *New Phytol* 177:178–185 669–672
- Fernando DR, Guymier G, Reeves RD, Woodrow IE, Baker AJ, Batianoff GN (2009) Foliar Mn accumulation in eastern Australian herbarium specimens: prospecting for ‘new’ Mn hyperaccumulators and potential applications in taxonomy. *Ann Bot* 103:93–939 673–675
- Francesconi K, Visoottiviset P, Sridokchan W, Goessler W (2002) Arsenic species in an As hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of As-contaminated soils. *Sci Total Environ* 284:27–35 676–678
- Fraústo da Silva JJR, Williams RJP (2001) The biological chemistry of the elements: the inorganic chemistry of life, 2nd edn. Oxford University Press, Oxford 679–680
- Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. *Plant Cell* 16:2176–2191 681–683
- Freeman JL, Persans MW, Nieman K, Salt DE (2005) Nickel and cobalt resistance engineered in *Escherichia coli* by overexpression of serine acetyltransferase from the nickel hyperaccumulator plant *Thlaspi goesingense*. *Appl Environ Microb* 71:8627–8633 684–686
- Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, Curtiss R 3rd (1997) Display of heterologous proteins on the surface of microorganisms: from the screening of combinatorial libraries to live recombinant vaccines. *Nat Biotechnol* 15:29–34 687–689
- Gerber GB, Leonard A, Hantson P (2002) Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Crit Rev Oncol Hematol* 42:25–34 690–691
- Gibbs PE, Bryan GW, Ryan KP (1981) Copper accumulation by the polychaete *Melinna palmata*: an antipredation mechanism? *J Mar Biol Ass UK* 61:707–722 692–693

- 694 Gifford S, Dunstan RH, O'Connor W, Koller CE, MacFarlane GR (2007) Aquatic zooremediation:
695 deploying animals to remediate contaminated aquatic environments. *Trends Biotechnol*
696 25:60–65
- 697 Gispert C, Ros R, de Haro A, Walker DJ, Pilar Bernal M, Serrano R, Avino JN (2003) A plant
698 genetically modified that accumulates Pb is especially promising for phytoremediation.
699 *Biochem Biophys Res Commun* 303:440–445
- 700 Godbold DL, Horst WJ, Collins JC, Thurman DA, Marschner H (1984) Accumulation of zinc and
701 organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*.
702 *J Plant Physiol* 116:59–69
- 703 Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJ, Whiting SN, May ST, Broadley
704 MR (2006) A comparison of the *Thlaspi caerulescens* and *Thlaspi arvense* shoot
705 transcriptomes. *New Phytol* 170:239–260
- 706 Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U
707 (2008) Evolution of metal hyperaccumulation required cis-regulatory changes and triplication
708 of HMA4. *Nature* 453:391–395
- 709 Hart JJ, Welch RM, Norvell WA, Kochian LV (2002) Transport interactions between cadmium
710 and zinc in roots of bread and durum wheat seedlings. *Physiol Plant* 116:73–78
- 711 Higuchi K, Kanazawa K, Nishizawa NK, Chino M, Mori S (1994) Purification and characteriza-
712 tion of nicotianamine synthase from Fe-deficient barley roots. *Plant Soil* 165:173–179
- 713 Honjo T, Hatta A, Taniguchi K (1984) Characterization of heavy metals in indicator plants –
714 studies on the accumulation of lead and tolerance of gregarious fern, *Athyrium yokoscense*, in
715 the polluted areas from the lead tile of the ruins of Kanazawa Castle, now the campus of
716 Kanazawa University. *J Phytogeogr Taxon* 32:68–80
- 717 Ingle RA, Mugford ST, Rees JD, Campbell MM, Smith JAC (2005a) Constitutively high expres-
718 sion of histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator
719 plants. *Plant Cell* 17:2089–2106
- 720 Ingle RA, Smith JAC, Sweetlove LJ (2005b) Responses to nickel in the proteome of the
721 hyperaccumulator *Alyssum lesbiacum*. *Biometals* 18:627–641
- 722 Ingle RA, Fricker MD, Smith JAC (2008) Evidence for nickel/proton antiport activity at the
723 tonoplast of the hyperaccumulator plant *Alyssum lesbiacum*. *Plant Biol* 10:746–753
- 724 Ishii T, Otake T, Okoshi K, Nakahara M, Nakamura R (1994) Intracellular localization of
725 vanadium in the fan worm *Pseudopotamilla ocellata*. *Mar Biol* 121:143–151
- 726 Kambe-Honjoh H, Ohsumi K, Shimoi H, Nakajima H, Kitamoto K (2000) Molecular breeding of
727 yeast with higher metal-adsorption capacity by expression of histidine-repeat insertion in the
728 protein anchored to the cell wall. *J Gen Appl Microbiol* 46:113–117
- 729 Kashem MA, Singh BR, Kubota H, Sugawara R, Kitajima N, Kondo T, Kawai S (2010) Zinc
730 tolerance and uptake by *Arabidopsis halleri* ssp. *gemma* grown in nutrient solution. *Environ*
731 *Sci Pollut Res* 17:1174–1176
- 732 Kerkeb L, Krämer U (2003) The role of free histidine in xylem loading of nickel in *Alyssum*
733 *lesbiacum* and *Brassica juncea*. *Plant Physiol* 131:716–724
- 734 Kotrba P, Rumi T (2010) Surface display of metal fixation motifs of bacterial P1-type ATPase
735 specifically promotes biosorption of Pb(2+) by *Saccharomyces cerevisiae*. *Appl Environ*
736 *Microbiol* 76:2615–2622
- 737 Krämer U (2005) Phytoremediation: novel approaches to cleaning up polluted soils. *Curr Opin*
738 *Biotechnol* 16:133–141
- 739 Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a
740 metal chelator in plants that accumulate nickel. *Nature* 379:635–638
- 741 Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and
742 speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. *Plant Physiol*
743 122:1343–1353
- 744 Krotz RM, Evangelou BP, Wagner GJ (1989) Relationships between cadmium, zinc, Cd-peptide,
745 and organic acid in tobacco suspension cells. *Plant Physiol* 91:780–787

- Kumar NPBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. *Environ Sci Technol* 29:1232–1238 746
747
- Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* 212:75–84 748
749
750
- Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP (2001) Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goeingense*. *J Exp Bot* 52:2291–2300 751
752
753
- Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) Tissue- and age-dependent differences in the complexation of cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by x-ray absorption spectroscopy. *Plant Physiol* 134:748–757 754
755
757
- Küpper H, Götz B, Mijovilovich A, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper in higher plants. I. Characterization of copper accumulation, speciation, and toxicity in *Crassula helmsii* as a new copper accumulator. *Plant Physiol* 151:702–714 758
759
760
- Lasat MM, Baker A, Kochian L (1996) Physiological characterization of root Zn²⁺ absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiol* 112:1715–1722 761
762
763
- Lasat MM, Baker A, Kochian L (1998) Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens*. *Plant Physiol* 118:875–883 764
765
766
- 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* 51:71–79 767
768
- Lei M, Chen TB, Huang ZC, Wang YD, Huang YY (2008) Simultaneous compartmentalization of lead and arsenic in co-hyperaccumulator *Viola principis* H. de Boiss.: an application of SRXRF microprobe. *Chemosphere* 72:1491–1496 769
770
771
- Li WC, Ye ZH, Wong MH (2010) Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii*. *Plant Soil* 326:453–467 772
773
774
- Liu XM, Wu QT, Banks MK (2005) Effect of simultaneous establishment of *Sedum alfredii* and *Zea mays* on heavy metal accumulation in plants. *Int J Phytoremediation* 7:43–53 775
776
- Lombi E, Zhao F, McGrath S, Young S, Sacchi G (2001) Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytol* 149:53–60 777
778
779
- Lone MI, He Z, Stoffella PJ, Yang X (2008) Phytoremediation of heavy metal polluted soils and water: progress and perspectives. *J Zhejiang Univ Sci B* 9:210–220 780
781
- Long XX, Zhang YG, Dai J, Zhou Q (2009) Zinc, cadmium and lead accumulation and characteristics of rhizosphere microbial population associated with hyperaccumulator *Sedum alfredii* Hance under natural conditions. *Bull Environ Contam Toxicol* 82:460–467 782
783
784
- Lu L, Tian S, Yang X, Wang X, Brown P, Li T, He Z (2008) Enhanced root-to-shoot translocation of cadmium in the hyperaccumulating ecotype of *Sedum alfredii*. *J Exp Bot* 59:3203–3213 785
786
- Ma JF, Hiradate S (2000) Form of aluminium for uptake and translocation in buckwheat (*Fagopyrum esculentum* Moench). *Planta* 211:355–360 787
788
- Ma JF, Zheng SJ, Matsumoto H, Hiradate S (1997) Detoxifying aluminium with buckwheat. *Nature* 390:569–570 789
790
- Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic. *Nature* 409:579 791
792
- Macnair MR (2002) Within and between population genetic variation for zinc accumulation in *Arabidopsis halleri*. *New Phytol* 155:59–66 793
794
- Macnair MR (2003) The hyperaccumulation of metals by plants. *Adv Bot Res* 40:63–106 795
- Mandal BK, Suzuki KT (2002) Arsenic round the world: a review. *Talanta* 58:201–235 796

- 797 Mari S, Gendre D, Pianelli K, Ouerdane L, Lobinski R, Briat JF, Lebrun M, Czernic P (2006)
798 Root-to-shoot long-distance circulation of nicotianamine and nicotianamine-nickel chelates in
799 the metal hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 57:4111–4122
- 800 Marquès L, Cossegal M, Bodin S, Czernic P, Lebrun M (2004) Heavy metal specificity of cellular
801 tolerance in two hyperaccumulating plants, *Arabidopsis halleri* and *Thlaspi caerulescens*. New
802 Phytol 164:289–295
- 803 Martinoia E, Maeshima M, Neuhaus HE (2007) Vacuolar transporters and their essential role in
804 plant metabolism. J Experim Bot 58:83–102
- 805 McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils.
806 Curr Opin Biotechnol 14:277–282
- 807 McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in
808 phytoremediation of metal-contaminated soils. Plant Soil 232:207–214
- 809 McGrath SP, Lombi E, Gray CW, Caille N, Dunham SJ, Zhao FJ (2006) Field evaluation of Cd and
810 Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis*
811 *halleri*. Environ Pollut 141:115–125
- 812 McNear DH, Peltier E, Everhart J, Chaney RL, Sutton S, Newville M, Rivers M, Sparks DL (2005)
813 Application of quantitative fluorescence and absorption-edge computed microtomography to
814 image metal compartmentalization in *Alyssum murale*. Environ Sci Technol 39:2210–2218
- 815 Mesjasz-Przybyłowicz J, Balkwill K, Przybyłowicz WJ, Annegarn HJ (1994) Proton microprobe
816 and X-ray fluorescence investigations of nickel distribution in serpentine flora from South
817 Africa. Nucl Instrum Meth Phys Res B 89:208–212
- 818 Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Goöt B, Küpper H (2009)
819 Complexation and toxicity of copper in higher plants. II. Different mechanisms for copper
820 versus cadmium detoxification in the copper-sensitive cadmium/zinc hyperaccumulator
821 *Thlaspi caerulescens* (Ganges ecotype). Plant Physiol 151:715–731
- 822 Mills R, Krijger G, Baccarini P, Hall J, Williams L (2003) Functional expression of AtHMA4,
823 a P1B-type ATPase of the Zn/Co/Cd/Pb subclass. Plant J 35:164–176
- 824 Milner MJ, Kochian LV (2008) Investigating heavy-metal hyperaccumulation using *Thlaspi*
825 *caerulescens* as a model system. Ann Bot Lond 102:3–13
- 826 Morishita T, Boratynski K (1992) Accumulation of Cd and other metals in organs of plants
827 growing around metal smelters in Japan. Soil Sci Pl Nutr 38:781–785
- 828 Murai T, Ueda M, Yamamura M, Atomi H, Shibasaki Y, Kamasawa N, Osumi M, Amachi T,
829 Tanaka A (1997) Construction of a starch-utilizing yeast by cell surface engineering. Appl
830 Environ Microbiol 63:1362–1366
- 831 Nakajima H, Iwasaki T, Kitamoto K (2001) Metalloidsorption by *Saccharomyces cerevisiae* cells
832 expressing invertase-metallothionein (Suc2-Cup1) fusion protein localized to the cell surface.
833 J Gen Appl Microbiol 47:47–51
- 834 Ni TH, Wei YZ (2003) Subcellular distribution of cadmium in mining ecotype *Sedum alfredii*.
835 Acta Bot Sin 45:925–928
- 836 Nigam R, Srivastava S, Prakash S, Srivastava MM (2001) Cadmium mobilisation and plant
837 availability: the impact of organic acids commonly exuded from roots. Plant Soil 230:107–113
- 838 Olguin EJ (2003) Phytoremediation: key issues for cost-effective nutrient removal processes.
839 Biotechnol Adv 22:81–91
- 840 Papoyan A, Kochian LV (2004) Identification of *Thlaspi caerulescens* genes that may be involved
841 in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal
842 transporting ATPase. Plant Physiol 136:3814–3823
- 843 Papoyan A, Pineros M, Kochian LV (2007) Plant Cd²⁺ and Zn²⁺ status effects on root and shoot
844 heavy metal accumulation in *Thlaspi caerulescens*. New Phytol 175:51–58
- 845 Patel KS, Shrivastava K, Brandt RN, Jakubowski WC, Hoffmann P (2005) Arsenic contamination in
846 water, soil, sediment and rice of central India. Environ Geochem Health 27:131–145
- 847 Pauwels M, Frérot H, Bonnin I, Saumitou-Laprade P (2006) A broadscale study of population
848 differentiation for Zn-tolerance in an emerging model species for tolerance study: *Arabidopsis*
849 *halleri* (Brassicaceae). J Evol Biol 19:1838–1850

Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular basis for heavy metal hyperaccumulation in <i>Thlaspi caerulescens</i> . Proc Natl Acad Sci USA 97:4956–4960	850 851 852
Persans MW, Yan X, Patnoe J-MML, Krämer U, Salt DE (1999) Molecular dissection of the role of histidine in nickel hyperaccumulation in <i>Thlaspi goesingense</i> (Hálácsy). Plant Physiol 121:1117–1126	853 854 855
Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in <i>Thlaspi goesingense</i> . Proc Natl Acad Sci USA 98:9995–10000	856 857 858
Pianelli K, Mari S, Marques L, Lebrun M, Czerniec P (2005) Nicotianamine over-accumulation confers resistance to nickel in <i>Arabidopsis thaliana</i> . Transgenic Res 14:739–748	859 860
Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. Crit Rev Plant Sci 21:539–566	861 862
Reeves RD (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. Plant Soil 249:57–65	863 864
Reeves RD, Adigüzel NN (2004) Rare plants and nickel accumulators from Turkish serpentine soils, with special reference to <i>Centaurea</i> species. Turk J Bot 28:147–153	865 866
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. Wiley, New York, NY, pp 193–221	867 868 869
Rigola D, Fiers M, Vurro E, Aarts MGM (2006) The heavy metal hyperaccumulator <i>Thlaspi caerulescens</i> expresses many species-specific genes, as identified by comparative expressed sequence tag analysis. New Phytol 170:753–766	870 871 872
Roosens N, Bernard C, Lepäe R, Verbruggen N (2004) Adaptive evolution of metallothionein 3 in the Cd/Zn hyperaccumulator <i>Thlaspi caerulescens</i> . Z Naturforsch 60:224–228	873 874
Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic <i>Arabidopsis thaliana</i> plants expressing a modified bacterial <i>merA</i> gene. Proc Natl Acad Sci USA 93:3182–3187	875 876 877
Ruta LL, Paraschivescu CC, Matache M, Avramescu S, Farcasanu IC (2010) Removing heavy metals from synthetic effluents using “kamikaze” <i>Saccharomyces cerevisiae</i> cells. Appl Microbiol Biotechnol 85:763–771	878 879 880
Sahi SV, Bryant NL, Sharma NC, Singh SR (2002) Characterization of a lead hyperaccumulator shrub, <i>Sesbania drummondii</i> . Environ Sci Technol 36:4676–4680	881 882
Saito A, Higuchi K, Hirai M, Nakane R, Yoshida M, Tadano T (2005) Selection and characterization of a nickel-tolerant cell line from tobacco (<i>Nicotiana tabacum</i> cv. bright yellow-2) suspension culture. Physiol Plant 125:441–453	883 884 885
Sánchez ML (ed) (2008) Causes and effects of heavy metal pollution. Nova Science, Hauppauge	886
Sandrini JZ, Regoli F, Fattorini D, Notti A, Inacio AF, Linde-Arias AR, Laurino J, Bainy AC, Marins LF, Monserrat JM (2006) Short-term responses to cadmium exposure in the estuarine polychaete <i>Laeonereis acuta</i> (polychaeta, Nereididae): subcellular distribution and oxidative stress generation. Environ Toxicol Chem 25:1337–1344	887 888 889 890
Sanità di Toppi LS, Gabbriellini R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130	891 892
Scarano G, Morelli E (2002) Characterization of cadmium-and lead phytochelatin complexes formed in a marine microalga in response to metal exposure. Biometals 15:145–151	893 894
Seki H, Suzuki A (1997) A new method for the removal of toxic metal ions from acid-sensitive biomaterial. J Colloid Interface Sci 190:206–211	895 896
Seward MRD, Richardson DHS (1990) Atmospheric sources of metal pollution and effects on vegetation. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC, Florida, pp 75–92	897 898 899
Shen ZG, Li XD, Chen HM (2000) Comparison of elemental composition and solubility in the zinc hyperaccumulator <i>Thlaspi caerulescens</i> with the non-hyperaccumulator <i>Thlaspi ochroleucum</i> . Bull Environ Contam Toxicol 65:343–350	900 901 902

- 903 Shen R, Ma JF, Kyo M, Iwashita T (2002) Compartmentation of aluminium in leaves of an Al-
904 accumulator, *Fagopyrum esculentum* Moench. *Planta* 215:394–398
- 905 Shi JY, Chen YX, Huang YY, He W (2004) SRXRF microprobe as a technique for studying
906 elements distribution in *Elsholtzia splendens*. *Micron* 35:557–564
- 907 Shibasaki S, Maeda H, Ueda M (2009) Molecular display technology using yeast-arming technol-
908 ogy. *Anal Sci* 25:41–49
- 909 Shira C, Carpaneto A, Soliani P, Cornara L, Gambale F, Scholz-Starke J (2009) Response to
910 cytosolic nickel of Slow Vacuolar channels in the hyperaccumulator plant *Alyssum bertolonii*.
911 *Eur Biophys J* 38:495–501
- 912 Shiraishi T, Tamada M, Saito K, Sugo T (2003) Recovery of cadmium from waste of scallop
913 processing with amidoxime adsorbent synthesized by graftpolymerization. *Radiat Phys Chem*
914 66:43–47
- 915 Smart KE, Kilburn MR, Salter CJ, Smith JAC, Grovenor CRM (2007) NanoSIMS and EPMA
916 analysis of nickel localisation in leaves of the hyperaccumulator plant *Alyssum lesbiacum*. *Int*
917 *J Mass Spectrom* 260:107–114
- 918 Still ER, Williams RJP (1980) Potential methods for selective accumulation of nickel(II) ions by
919 plants. *J Inorg Biochem* 13:35–40
- 920 Sun Q, Ye ZH, Wang XR, Wong MH (2005) Increase of glutathione in mine population of *Sedum*
921 *alfredii*: a Zn hyperaccumulator and pb accumulator. *Phytochem* 66:2549–2556
- 922 Sun Q, Ye ZH, Wang XR, Wong MH (2007) Cadmium hyperaccumulation leads to an increase of
923 glutathione rather than phytochelatins in the cadmium hyperaccumulator *Sedum alfredii*.
924 *J Plant Physiol* 164:1489–1498
- 925 Talke I, Hanikenne M, Krämer U (2006) Zinc-dependent global transcriptional control, transcrip-
926 tional deregulation, and higher gene copy number for genes in metal homeostasis of the
927 hyperaccumulator *Arabidopsis halleri*. *Plant Physiol* 142:148–167
- 928 Tamura H, Honda M, Sato T, Kamachi H (2005) Pb hyperaccumulation and tolerance in common
929 buckwheat (*Fagopyrum esculentum* Moench). *J Plant Res* 118:355–359
- 930 Tolrá RP, Poschenrieder C, Barceló J (1996) Zinc hyperaccumulation in *Thlaspi caerulescens*. II.
931 Influence on organic acids. *J Plant Nutr* 19:1541–1550
- 932 Tsuji N, Hirayanagi N, Okada M, Miyasaka H, Hirata K, Zenk MH, Miyamoto K (2002)
933 Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by
934 Zn-induced phytochelatin synthesis. *Biochem Biophys Res Commun* 293:653–659
- 935 Tu C, Ma LQ (2002) Effects of arsenic concentrations and forms on arsenic uptake by the
936 hyperaccumulator Ladder Brake. *J Environ Qual* 31:641–647
- 937 Tuomainen MH, Nunan N, Lehesranta SJ, Tervahauta AI, Hassinen VH, Schat H, Koistinen KM,
938 Auriola S, McNicol J, Kärenlampi SO (2006) Multivariate analysis of protein profiles of metal
939 hyperaccumulator *Thlaspi caerulescens* accessions. *Proteomics* 6:3696–3706
- 940 Tuomainen M, Tervahauta A, Hassinen V, Schat H, Koistinen KM, Lehesranta S, Rantalainen K,
941 Häyrynen J, Auriola S, Anttonen M, Kärenlampi S (2010) Proteomics of *Thlaspi caerulescens*
942 accessions and an interaccession cross segregating for zinc accumulation. *J Exper Bot*
943 61:1075–1087
- 944 Ueno D, Ma JF, Iwashita T, Zhao FJ, McGrath SP (2005) Identification of the form of Cd in the
945 leaves of a superior Cd-accumulating ecotype of *Thlaspi caerulescens* using ^{113}Cd -NMR.
946 *Planta* 221:928–936
- 947 Uraguchi S, Watanabe I, Yoshitomi A, Kiyono M, Kuno K (2006) Characteristics of cadmium
948 accumulation and tolerance in novel Cd-accumulating crops, *Avena strigosa* and *Crotalaria*
949 *juncea*. *J Exp Bot* 57:2955–2965
- 950 Vacchina V, Mari S, Czernic P, Marques L, Pianelli K, Schaumloeffel D, Lebrun M, Lobinski R (2003)
951 Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-
952 inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using
953 yeast complementation. *Anal Chem* 75:2740–2745
- 954 Van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, Moerland PD,
955 Loren V, van Themaat E, Koornneef M, Aarts MGM (2006) Large expression differences in

genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of <i>Arabidopsis thaliana</i> and the related metal hyperaccumulator <i>Thlaspi caerulescens</i> . <i>Plant Physiol</i> 142:1127–1147	956 957 958
Van TK, Kang Y, Fukui T, Sakurai K, Iwasaki K, Aikawa Y, Phuong NM (2006) Arsenic and heavy metal accumulation by <i>Athyrium yokoscense</i> from contaminated soils. <i>Soil Sci Plant Nutr</i> 52:701–710	959 960 961
Vartanian JP, Sala M, Henry M, Hobson SW, Meyerhans A (1999) Manganese cations increase the mutation rate of human immune deficiency virus type 1 ex vivo. <i>J Gen Virol</i> 80:1983–1986	962 963
Vera-Estrella R, Miranda-Vergara MC, Barkla BJ (2009) Zinc tolerance and accumulation in stable cell suspension cultures and in vitro regenerated plants of the emerging model plant <i>Arabidopsis halleri</i> (<i>Brassicaceae</i>). <i>Planta</i> 229:977–986	964 965 966
Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. <i>New Phytol</i> 181:759–776	967 968
Visoottiviset P, Francesconi K, Sridokchan W (2002) The potential of Thai indigenous plant species for the phytoremediation of As contaminated land. <i>Environ Pollut</i> 118:453–461	969 970
Watanabe T, Moon CS, Zhang ZW, Shimbo S, Nakatsuka H, Matsuda-Inoguchi N, Higashikawa K, Ikeda M (2000) Cadmium exposure of women in general populations in Japan during 1991–1997 compared with 1977–1991. <i>Int Arch Occup Environ Health</i> 73:26–34	971 972 973
Weber M, Harada E, Vess C, von Roepenack-Lahaye E, Clemens S (2004) Comparative microarray analysis of <i>Arabidopsis thaliana</i> and <i>Arabidopsis halleri</i> roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. <i>Plant J</i> 37:269–281	974 975 976 977
Wei CY, Wang C, Sun X, Wang WY (2006) Factors influencing arsenic accumulation by <i>Pteris vittata</i> : a comparative field study at two sites. <i>Environ Pollut</i> 141:488–493	978 979
Wei CY, Wang C, Sun X, Wang WY (2007) Arsenic accumulation by ferns: a field survey in southern China. <i>Environ Geochem Health</i> 29:169–177	980 981
Wu F, Zhang G, Yu J (2003) Interaction of cadmium and four microelements for uptake and translocation in different barley genotypes. <i>Commun Soil Sci Plant Anal</i> 34:2003–2020	982 983
Xu XH, Shi JY, Chen YX, Xue SG, Wu B, Huang YY (2006) An investigation of cellular distribution of manganese in hyperaccumulator plant <i>Phytolacca acinosa</i> Roxb. Using SRXRF analysis. <i>J Environ Sci (China)</i> 18:746–751	984 985 986
Xue SG, Chen YX, Reeves RD, Baker AJM, Lin Q, Fernando DR (2004) Manganese uptake and accumulation by the hyperaccumulator plant <i>Phytolacca acinosa</i> Roxb. (<i>Phytolaccaceae</i>). <i>Environ Pollut</i> 131:393–399	987 988 989
Xue SG, Chen YX, Baker AJM (2005) Manganese uptake and accumulation by two populations of <i>Phytolacca acinosa</i> Roxb. (<i>Phytolaccaceae</i>). <i>Water Air Soil Pollut</i> 160:3–14	990 991
Yanai J, Zhao FJ, McGrath SP, Kosaki T (2006) Effect of soil characteristics on Cd uptake by the hyperaccumulator <i>Thlaspi caerulescens</i> . <i>Environ Pollut</i> 139:167–175	992 993
Yang XE, Baligar VC, Foster JC, Martens DC (1997) Accumulation and transport of nickel in relation to organic acids in ryegrass and maize grown with different nickel levels. <i>Plant Soil</i> 196:271–276	994 995 996
Yang LS, Peterson PJ, Williams WP, Wang WY, Hou SF, Tan JA (2002) The relationship between exposure to arsenic concentrations in drinking water and the development of skin lesions in farmers from Inner Mongolia, China. <i>Environ Geochem Health</i> 24:293–303	997 998 999
Yang XE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ (2004) Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (<i>Sedum alfredii</i> Hance). <i>Plant Soil</i> 259:181–189	1000 1001 1002
Yang X, Li T, Yang J, He Z, Lu L, Meng F (2006) Zinc compartmentation in root, transport into xylem, and absorption into leaf cells in the hyperaccumulating species of <i>Sedum alfredii</i> Hance. <i>Planta</i> 224:185–195	1003 1004 1005
Yanqun Z, Yuan L, Jianjun C, Haiyan C, Li Q, Schwartz C (2005) Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. <i>Environ Int</i> 31:755–762	1006 1007 1008

- 1009 Zenk MH (1996) Heavy metal detoxification in higher plants: a review. *Gene* 179:21–30
- 1010 Zhao FJ, Wang JR, Barker JHA, Schat H, Bleeker PM, McGrath SP (2003) The role of
1011 phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytol*
1012 159:403–410
- 1013 Zhao FJ, Jiang RF, Dunham SJ, McGrath SP (2006) Cadmium uptake, translocation and tolerance
1014 in the hyperaccumulator *Arabidopsis halleri*. *New Phytol* 172:646–654

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