

Chapter 2

Plants in Heavy Metal Soils

Hermann Bothe

2.1 Heavy Metal Soils

Heavy metals have a molecular mass $>5.0 \text{ g cm}^{-3}$ which is distinctly higher than the average particle density of soils (2.65 g cm^{-3}). Several heavy metals such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), or molybdenum (Mo) are essential for the growth of organisms. Others have a single function and only in some organisms such as vanadium (V) in some peroxidases and in V-nitrogenases or nickel (Ni) in hydrogenases. The remainder of the heavy metals is always toxic to organisms: cadmium (Cd), lead (Pb), uranium (U), thallium (Tl), chromium (Cr), silver (Ag), and mercury (Hg). Arsenic (As) and selenium (Se) are nonheavy metals. However, since they partly share toxicity features with heavy metals, they are often referred to as “metalloids” in publications.

All soils contain heavy metals. In nonheavy metal soils, the concentrations of Zn, Cu, Pb, Ni, Cd and Cr range between 0.0001 and 0.065%, whereas Mn and Fe can reach 0.002% and 10.0%, respectively (Ernst 1974). With the exception of iron, all heavy metals above a concentration of 0.1% in the soil become toxic to plants and therefore change the community structure of plants in a polluted habitat. However, each plant species has a specific threshold value for each heavy metal where it exerts toxicity (Ernst 1982). Plants specifically adapted to life on heavy metal-rich soils (“heavy metal soils”) are termed metallophytes.

Zinc-rich soils (0.1–10.0% Zn) often contain a high content of Pb, but virtually no Cd, whereas soils with a high copper content (0.1–3.2% Cu) have higher concentrations of other heavy metals such as Zn, Pb, Co, Ni including Cd (Ernst 1974).

Soils that carry metallophytes can be classified by the content of their main heavy metal: serpentine soils are rich in Ni, seleniferous soils carry Se, calamine

H. Bothe (✉)

Botanical Institute, The University of Cologne, Zùlpicher Str. 47b, 50923 Cologne, Germany
e-mail: Hermann.Bothe@uni-koeln.de

soils have Zn as major pollutant and soils of the African copper belt are rich in Cu, Co, Cr, Ni, and Zn (Alford et al. 2010).

Special cases appear to be the serpentine soils (ultramafic soils) that are rock-forming hydrous magnesium phyllosilicate $(\text{Mg,Fe})_3\text{Si}_2\text{O}_5(\text{OH})_4$ minerals worldwide. They are also characterized by low levels of potassium and phosphorus and a low ratio of calcium/magnesium (Kinzel and Weber 1982). Because they carry high concentrations of Ni, Cr and Co, plants growing there have to cope with similar adverse conditions as those on heavy metal soils.

Most heavy metal soils worldwide have been exploited by human activities from early times. However, due to the insufficient technologies of medieval time and earlier periods, the remnants (heavy metal heaps) are still rich in heavy metals and carry a typical metallophyte vegetation. The concentration of heavy metals can be particularly high around smelters. When close to rivers, the heavy metals are gradually washed off leading to the diminution of the heavy metal vegetation with special concern about the risk of losing endangered plants (Becker and Dierschke 2008; Lucassen et al. 2010).

A special case appears to be soils contaminated by mercury (Hg). Plants growing on Hg-polluted soils show a reduced vitality, as observed at sites in Slovenia (Idrija) by the author. Specific Hg-adapted metallophytes do not seem to exist. However, 13 plant species accumulated Hg out of 87 examined from a highly contaminated waste area originating from a chemical plant in Northern Italy (Massa et al. 2010). In this study, *Polygonum aviculare* was the best accumulator and contained higher levels of Hg in roots than in shoots. The low productivity of this plant prevents its use for phytoextraction of Hg.

2.2 The Heavy Metal Plants (Metallophytes)

A botanist can immediately identify a habitat as heavy metal soil polluted by high concentrations of Zn, Pb or other heavy metals by the composition of the plants. In Central Europe, most heavy metal soils carry four to six typical metallophytes. Highest Zn-concentrations are endured by spring sandwort (*Minuartia (Alsine) verna*, Fig. 2.1h) of the Caryophyllaceae which otherwise occurs only, although abundantly, on chalk meadows and rocks in alpine regions of Europe above the timberline. The thrifts or sea pinks (*Armeria maritima*) of the Plumbaginaceae form a somewhat difficult plant complex. The subspecies *A. maritima* ssp. *halleri* (Fig. 2.1f) grows only on heavy metal heaps in European plains, and can be differentiated morphologically only with difficulty from the subspecies *maritima* of the coastal salt marshes or the alpine ssp. *alpina*. The genus *Armeria* does not appear to be finally resolved taxonomically which is also true for its occurrence in different plant associations (Becker and Dierschke 2008). The same applies to pennycresses of the Brassicaceae (*Thlaspi alpestre* agg.) on heavy metal soils (Koch et al. 1998). *Thlaspi caerulescens* (Fig. 2.1k) and *T. praecox* form ecotypes on heavy metal heaps. The plants on some heavy metal locations are often termed

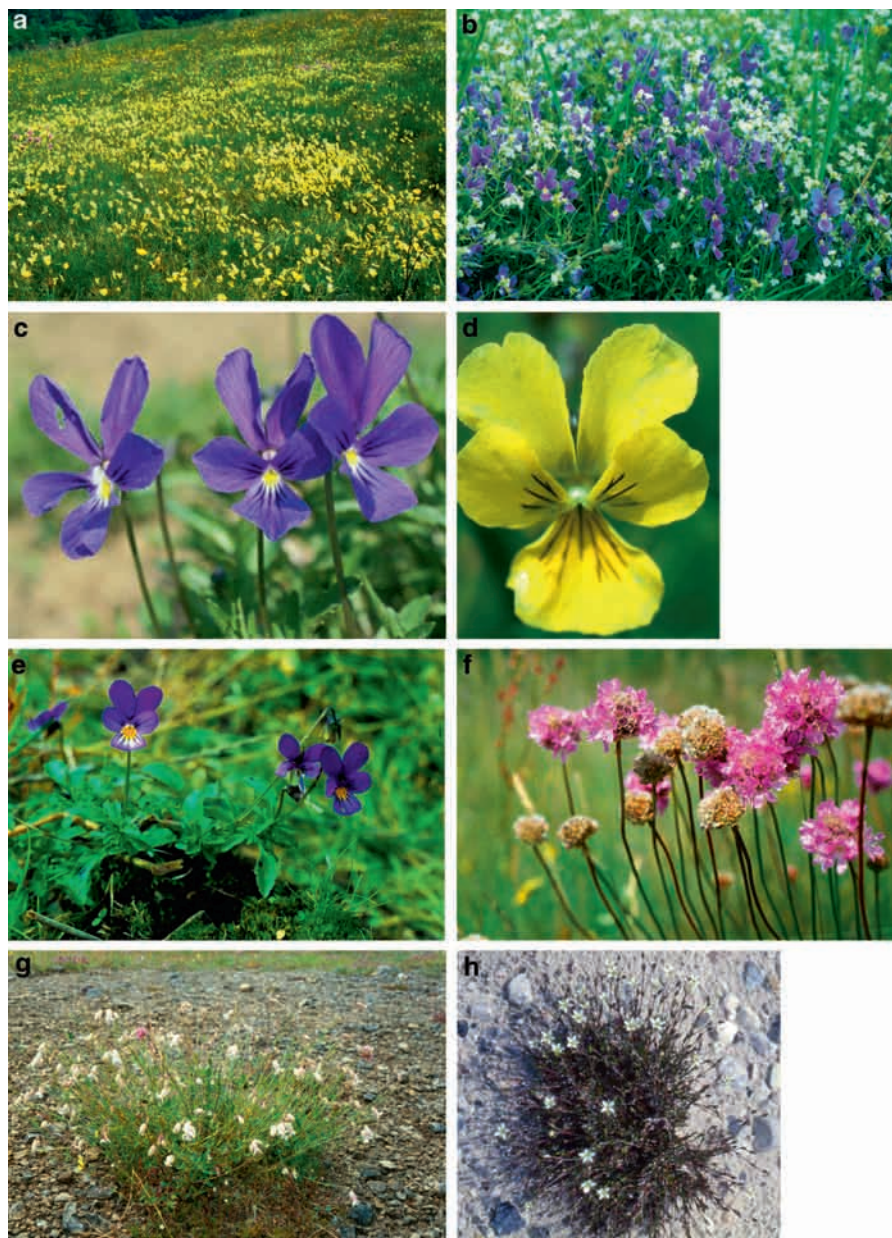


Fig. 2.1 (Continued)

T. caerulea ssp. *calaminaria*, but the differentiation to *T. montanum* and the “genuine” *T. alpestre* is not always obvious. A revision has recently been made on the genus *Thlaspi*; *T. caerulea* is now supposed to be *Noccaea caerulea*



Fig. 2.1 A selection of European heavy metal plants. (a) A meadow on a Zn-rich heavy metal heap at Schlangenberg/Stolberg close to Aachen, Germany. The yellow zinc violet (*Viola lutea* ssp. *calaminaria*) dominates and also *Armeria maritima* ssp. *halleri* is detectable as red flowering spots. Photo from June 1998. (b) The blue-zinc violet (*Viola lutea* ssp. *westfalica*) occurs only in the Pb-ditch and its surrounding heaps at Blankenrode close to Paderborn, Germany. The photo also shows *Cardaminopsis* (*Arabidopsis*) *halleri* (white flowers) at this location. May 1987. (c) The blue-zinc violet (*Viola lutea* ssp. *westfalica*) also grows happily on nonpolluted soils. Photo from the author's own allotment at D-Erftstadt. June 2009. (d) The alpine *Viola lutea* is the ancestor of the zinc violets (Hildebrandt et al. 2006). Zweisimmen/Bern, Switzerland. July 1997. (e) *Viola tricolor* forms specific ecotypes on heavy metal heaps. Siebertal, Harz mountains, Germany. June 1992. (f) *Armeria maritima* ssp. *halleri* from Schlangenberg/Stolberg close to Aachen. June 2002. (g) *Silene vulgaris* var. *humilis* which shows the bent shoots typical of the heavy metal ecotype of this plant species. Schlangenberg/Stolberg close to Aachen. June 2002. (h) *Minuartia* (*Alsine*) *verna* on heavy metal polluted gravel in the Gailitz river bed close to Arnoldstein, Southern Austria. (i) *Thlaspi cepaeifolium* on a heap close to a Zn-smelter at Cave del Predil (Raibl) in Northern Italy, close to the Slovenian border. This is an almost extinct species, but it is not related to *Thlaspi caerulescens*. May 2001. (j) *Alyssum wulfenianum*, an extremely rare heavy metal plant in the Gailitz river close to Arnoldstein, Southern Austria. May 2001. (k) *Thlaspi caerulescens* ssp. *calaminare* (= *Thlaspi calaminare*). Schlangenberg/Stolberg close to Aachen. April 2002. (l) *Thlaspi goesingense* from a serpentine soil at Pernegg/Mur in Steiermark, Austria. June 2002

(Meyer 2006). However, the taxonomy of the genus *Thlaspi* remains controversial (Koch et al. 1998) and it presumably is polyphyletic (Broadley et al. 2007). A separate tribe is the Ni-hyperaccumulator *Thlaspi goesingense* (Fig. 2.11) which thrives on serpentine soils in South-Eastern European countries. This latter is fairly productive and could be a good candidate for phytoremediation purposes on heavy metal heaps.

The bladder campion (*Silene vulgaris*, Fig. 2.1g) of the Caryophyllaceae forms a characteristic ecotype on heavy metal heaps. Whereas the shoots of this plant are straight on nonpolluted soils, they are bent or curved in specimens found on heavy metal heaps (forma *humilis*). I express from my own experience of diverse heavy metal heaps in Germany, Poland and alpine areas in Austria, Slovenia and Italy that this is an indicative feature of the heavy metal ecotype. Any taxonomic relationship of the *humilis* ecotype to the alpine *S. vulgaris* ssp. *prostrata* does not appear to be resolved at present. *S. vulgaris* has frequently been used for physiological and biochemical studies on heavy metal tolerance (e.g. Schat and Tenbookum 1992; Schat and Vooijs 1997; Kovacik et al. 2010).

Haller's rockcress (*Cardaminopsis halleri*, Fig. 2.1b) is even more in the center of interest of experimental studies nowadays. It is closely related to the model plant of modern molecular biology, *Arabidopsis thaliana*, and therefore termed *Arabidopsis halleri* in recent publications (Koch and Matchinger 2007; Macnair et al. 1999; Meyer et al. 2009, 2010). The gene compositions of both *Arabidopsis thaliana* and *Cardaminopsis halleri* are similar, and molecular techniques to identify and manipulate the genes can be applied to both species (Pauwels et al. 2005; Willems et al. 2007; Roosens et al. 2008). In the field, *Cardaminopsis halleri* occurs on heavy metal heaps, but not when the content of heavy metals, particularly Cu, is high in soils (Becker and Dierschke 2008). In contrast to other metallophytes already mentioned, it escapes from heavy metal heaps to neighboring nonpolluted sites and is therefore sometimes called "pseudometallophyte" (e.g. Pauwels et al. 2005, 2006) and its abundance is positively affected by soil depth and moisture (Becker and Dierschke 2008).

My personal comment on the nomenclature *Arabidopsis*–*Cardaminopsis* may be added here. The former genus *Cardaminopsis* is now abandoned to *Arabidopsis* (Koch and Matchinger 2007). All members of the former *Cardaminopsis* possess eight chromosomes in the haploid state whereas *A. thaliana* has only five. Molecular information is based only on ITS and cpDNA sequencing but not on nuclear DNA properties. The evolutionary split between the x-5 *A. thaliana* and the x-8 *Cardaminopsis* occurred around 5 Ma (Bechsgaard et al. 2006), a long time ago. However, in all these considerations, morphological criteria are completely ignored. From knowing natural populations of *A. thaliana* and *Cardaminopsis* species (*C. halleri*, *petraea*, *arenosa*), I would immediately believe that *Arabidopsis* and *Cardaminopsis* belong to different genera, although, as is known, no absolute criteria exist to define a species or a genus. On the other hand, the juxtaposition of members of *Cardaminopsis* to the model plant *A. thaliana* places them more into the focus of current interest. The sequence identity between *A. thaliana* and *C. halleri* is around 94% (Becher et al. 2004). For comparison, chimpanzee and human share a

sequence identity of 98.8% (Cyranowski 2001; Mikkelsen et al. 2005), so a common genus for both species might not be accepted by many people. Another relative of *A. thaliana*, the salt cress *Thellungiella halophila*, is 95% identical, at the cDNA level, but both genera are considered to be separate (Verbruggen et al. 2009).

Other plant ecotypes exist in Central Europe that are particularly adapted to a life on heavy metal soils. For example, a subspecies thriving on heavy metal heaps and termed *Festuca aquisgranensis* has been separated from the *Festuca ovina* aggregate (Patzke and Brown 1990). However, the *Festuca ovina* agg. is a fairly difficult taxon which is not yet finally resolved by morphological criteria. Other plants, particularly grasses (Bradshaw 1952; Wu et al. 1975; Humphreys and Nicholls 1984) may also have evolved heavy metal-adapted ecotypes (subspecies) which can, however, only be identified as separate species by current molecular techniques.

The zinc violets are special beauties (Fig. 2.1a) that occur only on heavy metal sites of very restricted distribution in Western Central Europe (Schwickerath 1944). The yellow zinc violet (*V. lutea* ssp. *calaminaria*) lives on Zn-rich soils in the area between Aachen, Germany and Liège, Belgium, and the blue form (*V. lutea* ssp. *westfalica*, Fig. 2.1b, c) thrives in a ditch and the surrounding heaps in an area of some 1 km × 0.5 km at Blankenrode, Eastern Westphalia, Germany. Both taxa belong to the most endangered plants in Central Europe and are probably the only unambiguous endemic taxa in Central Europe outside the alpine regions. Lack of competitiveness forces them to survive on heavy metal heaps. Molecular analyses showed that they are closely related to the alpine *V. lutea* (Fig. 2.1d) which occurs in Vosges or in the Sudeten mountains and more rarely in the Alps (Hildebrandt et al. 2006). The zinc violets might be relicts of the glacial period where they and their parents *V. lutea* seemingly had a wider distribution. Their patchy occurrence in Central Europe and also in Great Britain is an indication for such a history. Isolation on heavy metal heaps of the zinc violets may have resulted in the separation from their parents into the two separate entities.

In more Eastern and Southern European countries the zinc violets seem to be replaced by wild pansy (heartsease), *Viola tricolor* (Fig. 2.1e), which has developed ecotypes that can cope with fairly high concentrations of heavy metals (Slomka et al. 2011). This plant is, however, not restricted to heavy metal soils but occurs on gravel or sandy soils throughout the areas just mentioned.

When leaving Central Europe, the vegetation on heavy metal sites changes. Heavy metal soils in the area between Carinthia, Austria, Friaul in Italy and Northern Slovenia carry two very remarkable and almost extinct metallophytes: *Alyssum wulfenianum* (Fig. 2.1j) and *Thlaspi cepaeifolium* ssp. *cepaefolium* (Fig. 2.1i) which is unrelated to the *Thlaspi alpestre* agg, mentioned above but has close taxonomic affinities to *Thlaspi rotundifolium*. The need to preserve these extremely rare metallophytes is obvious. A detailed account of the metallophytes and their role in plant associations particularly in Northern and Southern Europe is given by Ernst (1974), and a list of heavy metal plants of the world can be found in Brooks (1998) or Prasad and Hagemeyer (1999). It is estimated that approximately 500 angiosperms representing about 0.2% of all higher plants are metallophytes (Baker and Brooks 1989; cited in Krämer 2010).

A differentiation is often made between absolute (strict or eu-) metallophytes and facultative (pseudo-) metallophytes according to their occurrence either only on polluted sites or on both contaminated and noncontaminated habitats (Lambinon and Augier 1964; Willems et al. 2007). However, as just said, all metallophytes of Central Europe occur outside heavy metal sites. On their natural stands in the European plains, they cannot compete with faster growing plants on nonpolluted soils. Their patchy distribution in Europe may be the result of glacial époques and the postglacial warm period. Such a differentiation between strict and pseudo-metallophytes is possibly justified at the subspecies level, for example for *Viola lutea* ssp. *calaminaria* and *V. l.* ssp. *westfalica* or *Armeria maritima* ssp. *halleri* on one side and *V. lutea* ssp. *lutea* or *Armeria maritima* ssp. *maritima* on the other. Such a differentiation merges into the discussion what is a subspecies in botany.

2.3 Accumulating and Hyperaccumulating Metallophytes

Heavy metal plants differ largely in their heavy metal contents as indicated by analysis of the plants that grew on a Zn-rich heavy metal soil close to Aachen, Germany (Table 2.1). Leaves of *Thlaspi caerulescens* (*T. alpestre* ssp. *calaminare*) and *Minuartia verna* contained the highest amount of Zn. The ratio between Cd and Zn is low in *Minuartia verna* and *Silene cucubalus* var. *humilis* in contrast to the situation in *T. caerulescens*. *Armeria maritima* in particular accumulates Pb. Thus

Table 2.1 Heavy metal content in mmol \times kg⁻¹ in leaves of plants living on the Zn-polluted soil at Breinigerberg close to Aachen, Germany

Plant species	Zn	Pb	Cd
<i>Thlaspi alpestre</i> ssp. <i>calaminare</i> (= <i>T. caerulescens</i>)	159.0	8.21	4.83
<i>Minuartia verna</i>	151.3	6.52	0.65
<i>Armeria maritima</i> ssp. <i>calaminaria</i>	112.8	11.60	1.10
<i>Silene cucubalus</i> var. <i>humilis</i>	40.8	0.29	0.02
<i>Plantago lanceolata</i>	39.2	2.35	0.19
<i>Lotus corniculatus</i>	30.4	0.05	0.02
<i>Anthyllis vulneraria</i>	28.8	0.15	0.03
<i>Festuca ovina</i>	28.3	0.97	0.13
<i>Campanula rotundifolia</i>	24.8	4.70	0.98
<i>Thymus serpyllum</i> agg.	22.9	3.96	0.33
<i>Cladonia rangifera</i> (podetium) (lichen)	21.4	8.08	0.40
<i>Rumex acetosa</i>	21.4	2.12	0.16
<i>Agrostis tenuis</i>	17.4	0.88	0.10
<i>Achillea millefolium</i>	14.8	1.38	0.02
<i>Euphrasia stricta</i>	14.3	0.94	0.10
<i>Viola lutea</i> ssp. <i>calaminaria</i>	8.9	0.19	0.02
<i>Pimpinella saxifraga</i>	8.2	0.26	0.03

The data are taken from Ernst (1982). Extreme values are given in bold

there is no tolerance to heavy metal in general, but each metallophyte has evolved a strategy to cope with an individual heavy metal.

The amount of heavy metals taken up by a plant is dependent on the concentration of heavy metals in the polluted soil. In most plants, heavy metals are predominantly accumulated in roots. The shoot/root ratio is generally below unity in most plants but not in metallophytes. In some heavy metal plants, the concentration of heavy metals in shoots and leaves can be particularly high and the partitioning of heavy metals between shoots and roots differs from one metallophyte to the next and with each individual heavy metal (Krämer 2010).

The zinc violet *V. lutea* ssp. *calaminaria* has very low amounts of Zn, Pb, and Cd in its leaves (Table 2.1). Although conflicting data have been published on the levels of heavy metals in zinc violets (Jedrzejczyk et al. 2002; Noret et al. 2007), these violets have distinctly less heavy metals in their organs and thus show a different pattern of adaptation to heavy metal stress in soils than *Thlaspi caerulescens*, *Minuartia verna*, or *Armeria maritima*. The two basic strategies to respond to heavy metal toxicity emerge from the comparison of the data of Table 2.1. Metallophytes can be either excluders of heavy metals or they can be accumulators (Ramirez-Rodriguez et al. 2005). The zinc violet is a good example of an excluder. The other three species mentioned (*Thlaspi*, *Minuartia*, and *Armeria*) are accumulators. Some plants are hyperaccumulators, however, only for a single or few specific heavy metals. *Thlaspi caerulescens*, *Cardaminopsis halleri*, and the Crassulaceae *Sedum alfredii* (Sun et al. 2007) hyperaccumulate Zn and Cd, but not Pb (Broadley et al. 2007). The Katanga species *Haumaniastrum katangense* is a hyperaccumulator for copper (Chipeng et al. 2010). The Ni-hyperaccumulating species *Alyssum murale* (Ernst 2005), *Alyssum bertolonii* (Boominathan et al. 2004), *Berkheya coddii* (Robinson et al. 2003), or several endemic species of the serpentine flora of Zimbabwe (Brooks and Yang 1984) were suggested as potential enrichers of this element for human exploitation (leaching purposes). The fern *Pteris vittata* accumulates Se from seleniferous soils (Liao et al. 2004) and the violet *Viola baoshanensis* is a Cd-hyperaccumulator (Wu et al. 2010). Hyperaccumulators of Pb do not seem to exist (or are rare) due to the fact that Pb is extremely immobile and is not easily accumulated by plants (Bert et al. 2002). An extreme example for hyperaccumulation is *Sebertia acuminata* which can store up to 26% (w/w) Ni in its latex (Jaffré et al. 1976; Verbruggen et al. 2009).

The degree of tolerance of plants to heavy metals was divided into the three categories: hypotolerance, basal tolerance, and hypertolerance in a more recent review (Ernst et al. 2008). Although some plant species are clear hyperaccumulators for Cu, Zn or Cd, no species can store excess of *all* heavy metals. The borderline between basal tolerance and hyperaccumulation is blurred. A hyperaccumulator may be defined by the threshold value which is approximately a 10 times higher concentration of a heavy metal in the aerial parts compared to the content in a nonhyperaccumulator growing on the same polluted habitat (Bert et al. 2002). Approximately 400 taxa worldwide are hyperaccumulators (Bert et al. 2002). To give more precise values a Zn hyperaccumulator is defined as a plant that

contains $>10,000 \mu\text{g g}^{-1}$ dry weight (1%, w/w), and a Cd hyperaccumulator should have $>100 \mu\text{g g}^{-1}$ dry weight (0.01%, w/w) (Baker 1981; Bert et al. 2002).

Halophytes can be divided into salt-resistant and salt-tolerant species. *Salicornia europaea* and *Suaeda maritima* need a high soil concentration of NaCl for germination and growth and are therefore salt-resistant species. All other halophytes grow better in non-NaCl enriched soils (gardens) than in saline habitats and are therefore salt-tolerant (Hildebrandt et al. 2007). All metallophytes of Central Europe are metal-tolerant (Hildebrandt et al. 2007). Lack of competitiveness forces them to live on heavy metal soils where they are not overgrown by more productive glycophytes. This is also true of the blue-zinc violet (*V. lutea* ssp. *westfalica*) where its germination and growth was claimed to require polluted soils with excess of heavy metals (Nauenburg 1986). The blue-zinc violet was shown to grow with no impaired vitality in garden soils at several locations in Germany in the last years. Its germination requires darkness, but is even more effective in nonpolluted garden earth than in heavy metals soils (Slomka et al. 2011). I am not aware of any clear-cut heavy-metal-resistant plant species worldwide that would be strictly dependent on the presence of excess of heavy metals in its growth substratum.

2.4 Strategies Employed by the Metallophytes to Cope with High Concentrations of Heavy Metals at the Whole Plant Level

Heavy metal tolerance has been developed by plants of totally unrelated taxonomic affinities. It is frequent in Brassicaceae, and also seen in Caryophyllaceae, Plumbaginaceae, Violaceae, Asteraceae, Poaceae, and others. Over 34 different plant families have developed heavy metal tolerant species (Verbruggen et al. 2009). Heavy metal tolerance is thus one of the clear-cut examples of convergence in biology. Therefore, it is not surprising that the strategies to cope with the excess of heavy metals differ from one plant taxon to the next. Similarities in the mechanisms exist between metallophytes and halophytes.

Armeria maritima can thrive both in coastal salt marshes with a high NaCl content (ssp. *maritima*) and on heavy metal heaps (ssp. *halleri*). This plant has special glands, developed from stomata, which apparently serve to excrete excess NaCl (Lüttge 1975; Rozema et al. 1981). Similar glands might also be used to transfer the surplus of heavy metals to the outside by the metallophyte *A. maritima* ssp. *halleri*. However, this has not yet been verified to my knowledge. Halophytes of the genus *Atriplex* secrete NaCl into hairs of the leaves and stems and discard them when overloaded (Lüttge 1975). Such a strategy seems also to be employed by metallophytes, as recently reassessed for an ecotype of *Viola tricolor* from the Bukowno heavy metal heap in Southern Poland (Slomka et al. 2011). *Minuartia verna* has the vegetation point in the center of a bundle of small, relatively long

leaves. The vegetation point continuously forms new maiden leaves essentially free of heavy metals. These leaves are used to deposit heavy metals during the vegetation period and then fall off when overloaded with heavy metals. Most *Thlaspi* species such as *T. caerulescens* are fast growing annuals that rapidly die after flower and seed formation with an overload of heavy metals. Their seeds are essentially free of heavy metals which, therefore, cannot affect them during germination next spring.

The zinc violet as a heavy metal excluder utilizes special arbuscular mycorrhizal fungi (AMF) as recently reviewed in a chapter of another book of this series (Bothe et al. 2010) and elsewhere (Hildebrandt et al. 2007). Roots of zinc violet samples collected from the natural sites are yellow due to the deposition of the yellow pigment, termed mycorradicin (Klingner et al. 1995a). This is a visible indication of mycorrhizal colonization in violets and other plants (Klingner et al. 1995b), and this pigment is deposited into the plant cells due to the action of the fungi. Counting of the degree of mycorrhizal colonization indicated that roots of the zinc violets, either the yellow morph from the Aachen-Liège area or the blue form from Blankenrode, are heavily colonized by AMF. A fungus, *Glomus intraradices* Br1, had been isolated that consistently enabled diverse plants to grow on diverse heavy metal soils, provided fertilization was optimized. Biophysical determinations of the concentrations of heavy metals in roots of maize and also of the blue-zinc violet indicated that plants colonized by AMF contained considerably less heavy metals in their roots than nonfungal control plants, in line with the data of Table 2.1. Uptake of the major part of the heavy metals into the living compartments of the two symbiotic partners might be prevented due to the action of the AMF. It is suggested that the majority of the heavy metals are transferred to the cell walls and the vacuoles of the fungi. The heavy metals that reached the inside of the plant cells are deposited mainly into the inner root cortical cells where the fungal structures reside. The techniques employed did not allow us to discriminate whether the heavy metals in the inner cortical cells were deposited into plant or fungal structures.

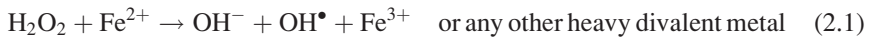
Another example of a metallophyte colonized by AMF is the Asteraceae *Berkheya coddii* of South Africa (Turnau and Mesjasz-Przybyłowicz 2003). The Brassicaceae *Biscutella laevigata* on heavy metal heaps at Olkucz, Southern Poland (Orłowska et al. 2002) or *Thlaspi* sp. (Regvar et al. 2003; Vogel-Mikuš et al. 2006) are occasionally colonized, particularly at the flowering state. However, it is doubtful whether the degree of root colonization by AMF is high enough for a significant contribution to heavy metal tolerance by these plants.

In most cases, heavy metal heaps are not covered by trees. Since heavy metals accumulate gradually in the cells, only some short-living herbs can cope with the adverse soil conditions. In Central Europe some crippled fir (*Pinus sylvestris*) or birch (*Betula pendula*) trees can be found on older heavy metal soils. Such trees may utilize ectomycorrhizal fungi for their survival. Evidence was presented that a copper-tolerant ectomycorrhizal fungus *Suillus luteus* was able to protect pine trees against Cu toxicity (Adriaensen et al. 2005).

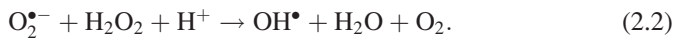
2.5 Toxicity of the Heavy Metals in Cells and Responses of the Plant Cells

Heavy metals can have multiple effects in the plant cytoplasm:

1. They can bind to functionally essential SH-groups in enzymes and thereby inactivate them.
2. They can substitute functional elements in prosthetic groups of enzymes resulting in an inactive catalysis. This is particularly the case for Cd substituting Zn in proteins.
3. They may enhance the generation of ROS (reactive oxygen species) such as $O_2^{\bullet-}$, OH^{\bullet} , H_2O_2 , and 1O_2 . These ROS are generated in cells in either the Fenton reaction:



or the Haber–Weiss reaction:



The reaction velocity of the Haber–Weiss reaction is enhanced by any heavy metal cation which can change the oxidation state (Elstner 1990; Prasad and Hagemeyer 1999).

As indicated in Fig. 2.2, the plant can cope with these adverse affects by multiple responses (Salt 2001; Hall 2002):

- (a) Formation of *Siderophores*: These are large, complex molecules that are excreted from the plant cells into the soil. These chelators bind heavy metals to form a large complex which is not taken up by the cells. Thus the surplus of heavy metals would not reach the cell cytoplasm. Such a role of siderophores is not accepted by all investigators working in the field.
- (b) Synthesis of small molecules such as the *carboxylic acids*: malate, citrate, or oxaloacetate that bind heavy metals with their acid groups. These carboxylic acids may bind heavy metals outside the roots or in the root apoplasm which may prevent uptake of heavy metals. The root symplasm of plants excretes up to 20% of the organic carbon containing such organic acids. Root exudation may change the pH-value in soils and thereby may alter the accessibility and uptake rate of heavy metals into plant roots (Alford et al. 2010). Alternatively, such simple organic acids may be used to selectively deposit heavy metals. In particular, Zn may be bound to malate in the cytoplasm and transferred as a complex across the tonoplast to the vacuole. There Zn-malate may dissociate, then Zn^{2+} may bind to stronger chelators such as citrate and oxaloacetate and finally malate may be retranslocated to the cytoplasm. This long-ago-postulated Zn-malate shuttle hypothesis (Ernst et al. 1992) is still quoted (Broadley et al. 2007) but is not in the focus of current interest.

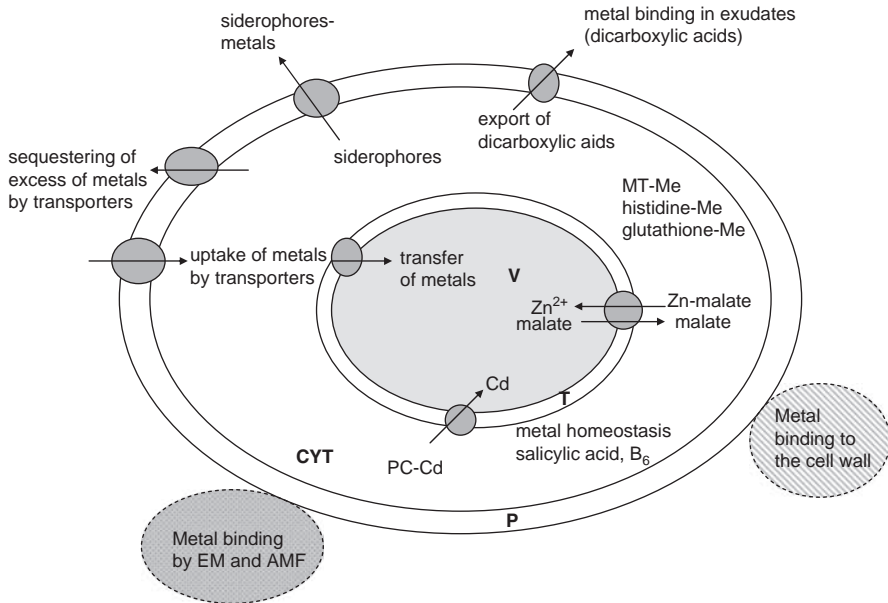


Fig. 2.2 Schematic summary of the factors involved in heavy metal tolerance of plants. Abbreviations: *P* plasma membrane (cytoplasmic membrane); *CYT* cytoplasm, cytosol; *T* tonoplast; *V* vacuole; *Me* metal; *PC* phytochelatin; *MT* metallothionein. Adapted from Hall (2002), however, significantly modified

- (c) Other small molecules that may bind heavy metals at their SH-groups are *free histidines or glutathione*. The role of the free amino acid histidine in detoxifying heavy metals, particularly nickel, has recently been stressed (Ingle et al. 2005; Krämer et al. 1996; Krämer 2010). Excess of heavy metals, particularly Fe, may also be bound by nicotianamine (Krämer 2010). Other candidates for metal binding in the cytoplasm are polyamines (Cicatelli et al. 2010). Small molecules may be used to maintain heavy metal homeostasis in the plant cytoplasm.
- (d) *Metallothioneins*. These are small cysteine-rich proteins. The SH-groups of the cysteines may bind heavy metals in the cytoplasm and may thereby prevent them from exerting toxic effects.
- (e) *Phytochelatin*s. These compounds contain cysteine in a more regular way than metallothioneins. The compositions of the phytochelatin are multiples of glutamate-cysteine ($n = 2-11$) which terminate with one glycine. The SH-groups of the cysteines in the phytochelatin may also serve to bind metals. Phytochelatin are synthesized nontranslationally by phytochelatin synthase with glutathione as substrate (Hall 2002).
- (f) *Heavy metal transporters*. The plasma membrane surrounding the root parenchyma cells acts as a barrier against the uptake of heavy metals from the cytoplasm. This membrane and the tonoplast surrounding the vacuole possess

several classes of transport proteins such as CPx-ATPases for Cu and Cd, ABC transporters for Cd transport into the vacuole, ZIP transporters (ZRT-, IRT-related proteins) for Fe and Zn, *Nramp* transporters with a broad-range affinity for heavy metals and the CDF (cation diffusion facilitator) family (Hall 2002). In general, a plant possesses several genes coding for each class of transporters which may individually be expressed depending on the soil toxicity (heavy metal content), the plant organ and organelle, the plant development state and the annual season. Therefore, the study of the role of an individual transporter amongst the realm of all others is complex. Investigators tend to put their favorite transporter and their encoding gene into the focus. Those heavy metals that are essential for the growth of organisms (Fe, Mn, Cu, Co, Mo) may be taken up in excess by their transporters in metallophytes. Always toxic elements such as Cd, Pb, Cr, U, Ag, Hg, Ni (in most organisms), As and Se might be taken up erroneously; often transporters cannot strictly discriminate between the element wanted and a toxic heavy metal.

- (g) *ROS detoxifying enzymes*. Upon stress caused by high loads of heavy metals or salts, by drought or soil acidity, plants express stress responsive genes. Here glutathione S-transferase is of paramount importance, but, in addition, superoxide dismutases, cytochrome P450, thioredoxin are detected in SSH libraries where the gene expression from stressed and control plants are compared (see Hildebrandt et al. 2007). Such proteins might serve to detoxify ROS generated by excess of heavy metals [(2.1) and (2.2)]. In addition, heat shock proteins (HSPs) are expressed not only under high temperatures but also under a variety of stress conditions, among which is exposure to excess of heavy metals. HSPs not only function as chaperones in protein folding and assembly but may also serve in protecting and repairing proteins from oxidative stress (Hall 2002).
- (h) *Alterations in the root morphology*. The Zn- and Cd-hyperaccumulator *Thlaspi caerulescens* has recently been shown to possess a peri-endodermal layer of cells with irregularly thickened tangential cell walls impregnated by lignin (Broadley et al. 2007; Zelko et al. 2008). The secondary compact cylinder commences close to the root tip and encircles the endodermis and is not seen in the nonmetallophyte *Thlaspi arvense*. Any specific role of this cylinder in the detoxification of heavy metals in *T. caerulescens* is not clear at present. To my knowledge, alterations in the root anatomy of other metallophytes have not been reported but are not unlikely. Alterations in the root structures occur in the halophyte *Aster tripolium* upon NaCl load and mycorrhizal colonization (Scheloske et al. 2004). Increases in root biomasses as well as root hair production and root lengths have been reported for high Zn-containing soil patches compared to Zn-deficient adjacent areas (see Broadley et al. 2007). However, this may not be a general phenomenon for all metallophytes and all heavy metal soils. The different metallophytes have all sorts of roots, from fine hairy root bundles to thick, long primary roots with few, marginally branched side roots at their natural habitats (my own observations).

2.6 Genes and Their Expressions Upon Heavy Metal Stress in Plants

It is often stated that metal tolerances are strictly metal-specific; however, the situation is often not so simple as this (see Schat and Vooijs 1997). Any combined tolerance to different heavy metals in a metallophyte could result from the sum of different metal-specific mechanisms or from less-specific mechanisms that pleiotropically confer tolerances to different metals. Work with *Silene vulgaris* (Schat and Vooijs 1997) showed that tolerance to Cu, Zn and Cd is under nonpleiotropic, independent genetic control. On the other hand, tolerance to Ni and Co seems to be a pleiotropic by-product of Zn tolerance. Hyperaccumulation of Zn and Cd is a constitutive trait in *Cardaminopsis (Arabidopsis) halleri* (Bert et al. 2002). Zn tolerance in *C. halleri* populations from heavy metal soils is, on average, higher than in those from nonpolluted habitats (Pauwels et al. 2006; Meyer et al. 2010). On the other hand, a population from a noncontaminated site accumulated Zn in its roots and shoots more rapidly and more effectively than plants from a polluted habitat (Bert et al. 2000).

Another issue concerns the debate of whether heavy metal tolerance and hyperaccumulation share a common genetic basis or are under control of different genes. Whereas a strict separation was claimed in earlier publications (Macnair et al. 1999), a simultaneous evolution of both traits on heavy metal soils and the phenotypic expression of shared genes was favored by others at that time (Krämer et al. 1997) which is corroborated in more recent communications (Frérot et al. 2010). The ecological role of hyperaccumulation of heavy metals is not understood. It is stated that the accumulation of heavy metals much in excess of the concentration of polluted soils may make metallophytes resistant against attack by carnivores or pathogens. However, experimental evidence for this selective advantage is missing (Noret et al. 2005).

In *Cardaminopsis halleri*, the tolerance to Zn and Cd is constitutive, meaning that all populations from either polluted or noncontaminated soils share the properties to be tolerant and to be able to accumulate these two heavy metals (Pauwels et al. 2006). In contrast, Cd and Ni tolerance may not be constitutive in *Thlaspi caerulescens* (Verbruggen et al. 2009), but this issue does not seem to be finally resolved. Studies on the genetic basis of Zn and Cd tolerances of *C. halleri* are favored by the fact that it form fertile crosses with the closely related *Arabidopsis lyrata* ssp. *petraea* = *Cardaminopsis petraea* = *C. hispida* {Macnair et al. 1999}. This species did not develop tolerances to Zn and Cd and also does not occur on heavy metal soils. However, it also occurs in extreme habitats in nature. It is a typical element of dolomite soils in the Alps and has a disjunctive occurrence in the central European plains on gypsum soils where only few higher plant species can live. Thus, like *Arabidopsis halleri*, *Cardaminopsis (Arabidopsis) petraea* is also adapted to stress conditions such as drought and nutrient limitations in its natural habitats.

Earlier it was believed that tolerance to a range of heavy metals including Zn, Cu and As is controlled by a small number (one or two) of major genes, with additional

modifiers for the degree of tolerance (Schat et al. 1993; Smith and Macnair 1998). This view has recently been changed by quantitative trait loci (QTL, Perez-Figueroa et al. 2010) mapping. The gene regions of complex adaptive traits such as heavy metal tolerance can be identified on the chromosomes by analyzing the crosses between *Cardaminopsis halleri* and *C. petraea* (Macnair et al. 1999; Willems et al. 2007). Three regions on different chromosomes of *C. halleri* were mapped which are responsible for Zn tolerance, Zn hyperaccumulation, or both (Willems et al. 2007). Individual genes cannot be localized by such an approach, since all three QTL regions were several cM long, and 1 cM corresponds to about 250 kb or about 40 genes (Mauricio 2001; Willems et al. 2007). In a more recent study, even five QTL regions were identified by analyzing crosses between *C. halleri* and *C. petraea* grown in a soil polluted by low Zn-concentrations, and three QTL were mapped both in plants from either low or high Zn-polluted soil (Frérot et al. 2010). Similar data with essentially the same results were published by Filatov et al. (2007). At least 23 genes are known to be involved in metal homeostasis of *A. thaliana* (Roosens et al. 2008), and Zn tolerance and hyperaccumulation make the molecular biology in *C. halleri* even more complex. For Cd-hyperaccumulation by *C. halleri*, only one QTL was identified, and Cd hyperaccumulation and tolerance are not independent (Willems et al. 2010). As said before, the latter has now been described as likely to be the case, at least partially, also for Zn (Frérot et al. 2010).

Transcription studies indicated that more than 30 different genes associated with metal hyperaccumulation showed an enhanced expression level in *C. halleri* compared to the situation in *A. thaliana* (Hanikenne et al. 2008; Verbruggen et al. 2009). One QTL of *C. halleri* contains the *HMA4* gene encoding a plasma membrane heavy metal ATPase of the P-type superfamily. This ATPase is involved in loading transition metal ions (particularly Zn and Cd) into the xylem from the surrounding vascular tissues (Verbruggen et al. 2009) and is thus an important control element in long distance transport. RNA silencing (RNAi interferences) of this gene in *C. halleri* showed that the gene product is required for Cd and also – to a lesser extent – for Zn tolerance in *C. halleri* (Hanikenne et al. 2008; Krämer 2010). *HMA4* had much higher transcript abundance in *C. halleri* than in *A. thaliana*. These enhanced transcript levels are due to a combination of the expansion to three almost identical copies of the gene present in tandem in the genome of *C. halleri*, and promoter mutations enhancing promoter strength in all the three copies, together with altered cis-regulations of the expressions of these genes (Hanikenne et al. 2008; Krämer 2010). For comparison, *A. thaliana* contains only one ortholog of this gene. The *HMA4* gene seems to be a major gene with its product serving in the translocation of Zn and Cd from the root symplasm to the xylem vessels, although it is not sufficient for Zn tolerance and hyperaccumulation in *C. halleri* (Hanikenne et al. 2008). The function of different parts of this gene, in particular the essential role of the C-terminal domain in translocation heavy metals, has recently been assigned (Mills et al. 2010).

Similar duplications occurred with the gene *MTP1* of the cation diffusion facilitator family with its products controlling Zn transport into the vacuole (Desbrosses-Fonrouge et al. 2005). Whereas *A. thaliana* contains only one copy of *MTP1*,

C. halleri possesses five paralogues of it which have apparently evolved recently (Shahzad et al. 2010). These five different *MTP1* genes underwent different evolutionary fates such as neo-functionalization, nonfunctionalization, and sub-functionalization (Shahzad et al. 2010). Also in *Thlaspi caerulescens* and in the Ni-hyperaccumulator *T. goesingense*, transcript levels of *MTP1* as well as of *HMA4* are higher than in related nonhyperaccumulating members of the Brassicaceae (Krämer 2010).

Members of the ZIP family (zinc-regulated transporters) mediate the uptake of Zn from the root apoplast into the parenchyma cells at the cytoplasmic membrane. Of these, *ZNT1* is highly expressed in roots of *T. caerulescens* but not in *Thlaspi arvense* (Pence et al. 2000). As reviewed by Verbruggen et al. (2009) or Krämer (2010), *ZNT1* mediates high-affinity Zn-transport and low-affinity Cd-uptake and is also found to be highly expressed by microarray analyses of large parts of the genome of *T. caerulescens*. Its ortholog in *A. thaliana*, termed *ZIP4*, is expressed only under Zn-deficiency, whereas this gene is transcribed independently of the Zn-supply and at a high level in hyperaccumulators. The individual role of the other members of the ZIP-transporter family in hyperaccumulation has not been assessed as yet. This is the case for the orthologues of *ZIP3*, *ZIP6*, *ZIP9*, *ZIP10* and *IRT3* of *A. thaliana* which were shown to be overexpressed in *C. halleri* and *T. caerulescens* by microarray analysis (Verbruggen et al. 2009). In addition, the roles of transporters such as members of the N_{RAMP} family, of the ABC group, of the Ca^{2+}/H^{+} antiporters (CaCA superfamily) and others (Verbruggen et al. 2009) remain to be assigned in future investigations. To illustrate the complexity of the subject, more than 2,000 genes were more than five times upregulated in *C. halleri* compared with *A. thaliana*, and 1,147 of these had an unknown function (Van de Mortel et al. 2008).

Expression studies of different metallothioneins in *T. caerulescens* indicated that their forms contribute in variable ways to heavy metal tolerance but not so much to hyperaccumulation by this plant (Hassinen et al. 2009).

Suppression subtractive hybridization (SSH) approaches are a tool to reveal the genes expressed in plants grown under heavy metal stress but not in nonaffected control plants. In such trials, genes coding for enzymes detoxifying reactive oxygen radicals are in the priority list of expressed candidates (Ouziad et al. 2005; Dai et al. 2010). Glutathione-S-transferase is of paramount importance in detoxifying such radicals generated by exposure of the cells to excess heavy metals or salts (Ouziad et al. 2005) and is distinctly overexpressed under such conditions. The roles of such detoxifying enzymes and the differential expression of their encoding genes do not seem to be in the focus of the current research.

Fine control of heavy metal uptake may be modulated by signal molecules. There is some evidence that salicylic acid stimulates Cd-uptake in soybean (Drazic and Mihailovic 2009). Vitamin B6 has been implicated in defense against cellular oxidative stress caused by excess of heavy metals, and one gene of the vitamin B6 biosynthetic pathway has recently been identified in the arbuscular mycorrhizal fungus *Glomus intraradices* (Benabdellah et al. 2009). The field "role of signal molecules in heavy metal resistance of plants" is just emerging but might be extensively researched in the near future.

2.7 Aspects of the Use of Metallophytes to Remediate Soils Polluted by Heavy Metals

Phytoremediation has been reviewed (Salt et al. 1998; Pilon-Smits 2005); Maerques et al. 2009; Muthukumar and Bagyaraj 2010). It comprises stabilization of soils devoid of plants due to heavy metal toxicity and extraction of heavy metals by plants. Volatilization and degradations have a role in the removal of other pollutants but not in the case of heavy metals. However, Hg and the toxic metalloids As and Se may be chemically converted to more readily extractable or volatile compounds.

It is sometimes stated that metallophytes are often slow growing and are not productive enough to stabilize heavy metal soils against erosion, leaching, or runoff (Pilon-Smits 2005). This is, indeed, the case for most metallophytes. However, *Thlaspi goesingense* (Fig. 2.1) of South-Eastern European serpentine soils is fairly productive and offers good promises to achieve this goal. Edaphic factors of the soils to be stabilized play an important role in any attempt. It is in most cases not sufficient to sow or plant metallophytes in contaminated, almost vegetation-free soils. Such habitats are not only polluted by excess heavy metals, but are often also nutrient-limited (Becker and Dierschke 2008; Turnau et al. 2010; Bothe unpublished). Thus an extensive soil analysis is necessary before any stabilization projects can be started. Heavy rainfalls during a thunderstorm may destroy the laboriously planted and just growing metallophytes in a vegetation-free soil, as this author had to encounter in an aborted attempt some years ago. Likewise, sea-gulls from a neighboring garbage dump (or grazing animals) suddenly noticing a green spot of metallophytes within a larger vegetation-free area can also attack the site and ruin the best-planned experiment within minutes (also my own experience).

Phytoextraction of heavy metals by hyperaccumulating plants offers a perspective to enrich heavy metals in soils poor in these elements. As said before, hyperaccumulators enrich heavy metals such as Ni in their above-ground parts which can easily be harvested, and then the heavy metals could be concentrated by ashing. The Ni-hyperaccumulators *Alyssum bertolonii* and *Streptanthus polygaloides* have been suggested as best candidates for Ni-phytoextraction in the field (Li et al. 2003).

Outside of heavy metals, metallophytes or other plants, either alone or in combination with mycorrhizal fungi, could be used to enrich radioactive compounds such as ^{137}Cs , ^{90}Sr , or ^{238}U (Westhoff 1999). Plants take up Cs instead of K which is highly mobile and is mainly transferred to the shoot. In contrast, Sr can serve as a substitute for Ca which is deposited mainly in cell walls and is concentrated in roots. Whole plants must therefore be harvested to enrich radionuclides from soils and to collect the plants in heaps for ashing and concentrating the radioactive material. Such an idea seems to be attractive for soils around the Chernobyl reactor but it would be necessary to treat large areas and to convince local authorities of the need.

Plant life in soils is dependent on interactions with microorganisms which may better exploit water and nutrients than roots and may provide growth-promoting substances to the plants. The literature is filled with positive effects of rhizobacteria

on plant growth, and a review is required to summarize the subject. In general, bacterial inocula work fine in laboratory experiments, but they are frequently outcompeted by indigenous bacteria in the field. Arbuscular mycorrhizal fungi (AMF) exist that are better adapted to the harsh conditions in heavy metal soils than fungal isolates from nonpolluted soils (Weissenhorn and Leyval 1993). In our experiments, a mycorrhizal fungus, *Glomus intraradices* Br1, was isolated from the roots of the yellow zinc violet grown in a calamine soils near Aachen, Germany. This isolate was consistently more effective than a conventional AMF isolate in promoting growth of diverse plants in several heavy metal soils, provided fertilization was optimized (Hildebrandt et al. 1999; Kaldorf et al. 1999). Other specifically adapted AMF exist in heavy metal soils that could be exploited for phytoremediation purposes (Tonin et al. 2001). As said, metallophytes are concentrated within the Brassicaceae. Unfortunately, members of this family are generally AMF-negative or are at best poorly colonized (De Mars and Boerner 1996) which might exclude any application of these plants in combination with AMF. The zinc violets are strongly AMF-positive (Hildebrandt et al. 1999) and are fairly productive perennials. Due to the beauty of these plants, any phytoremediation attempt is faced with the risk of human rapture. Thus the best metallophyte/fungal combination has still to be found.

Phytoremediation appears to be an attractive field which is in infancy due to its complexity. Up to the present, remediation of soils polluted by heavy metals is governed mainly by chemists, and biologically based applications await future directives.

2.8 Conclusions

Plants of diverse families can grow in heavy metal soils, and their different biology offers fascinating research perspectives. Molecular studies of heavy metal tolerance and hyperaccumulation are currently focused on the two metallophytes *Cardaminopsis (Arabidopsis) halleri* and *Thlaspi caerulescens*. This is conceivable because of their close relatedness to the model plant *Arabidopsis thaliana*. The complete sequence of a metallophyte genome has not been published as yet. When such knowledge becomes available it will largely facilitate future research of heavy metal tolerance and hyperaccumulation. Publications in the last 3 years seem to indicate that any difference between heavy metal tolerance and hyperaccumulation is not so obvious as previously claimed. Another feature to emerge is that heavy metal tolerance might have arisen by gene duplications and the altered regulations of their expressions and not by the modifications of sequences of genes which originally had different functions in nonmetallophytes. The field of molecular biology of metallophytes is developing rapidly and exciting results are to be expected in the very near future.

The use of metallophytes, either alone or in combination with microorganisms, to stabilize heavy metal soils against erosion or to phytoextract heavy metals is an

attractive idea. For various reasons, the field is in its infancy despite the many thoughts about it and experimental attempts in the past. Broad-scale applications are conceivable, but the cost–benefit outcome has not yet been properly assessed.

References

- Adriaensen K, Vralstad T, Noben JP, Vangronsveld J, Colpaert JV (2005) Copper-adapted *Suillus luteus*, a symbiotic solution for pines colonizing Cu mine spoils. *Appl Environ Microbiol* 71:7279–7284
- Alford EA, Pilon-Smits EAH, Paschke MW (2010) Metallophytes – a view from the rhizosphere. *Plant Soil* 337:33–50
- Baker AJM (1981) Accumulators and excluders-strategies in the responses of plants to heavy metals. *J Plant Nutr* 3:643–654
- Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81–126
- Becher M, Talke IN, Krall L, Krämer U (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. *Plant J* 37:251–268
- Bechsgaard JS, Castric V, Charlesworth D, Vekemans X, Schierup MH (2006) The transition to self-compatibility in *Arabidopsis thaliana* and evolution within S-haplotypes over 10 Myrs. *Mol Biol Evol* 23:1741–1750
- Becker T, Dierschke T (2008) Vegetation response to high concentrations of heavy metals in the Harz Mountains. *Phytocoenologia* 38:255–265
- Benabdellah K, Azcon-Aguilar G, Valderas A, Speziga D, Fitzpatrick TB, Ferrol N (2009) *GintPDX1* encodes a protein involved in vitamin B6 biosynthesis that is up-regulated by oxidative stress in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 184:682–693
- Bert V, Macnair MR, DeLaguérie P, Saumitou-Laprade P, Petit D (2000) Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations in *Arabidopsis halleri* (Brassicaceae). *New Phytol* 146:225–233
- Bert V, Bonnin I, Saumitou-Laprade P, De Laguérie P, Petit D (2002) Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytol* 155:47–57
- Boominathan R, Saha-Chaudhury NM, Sahajwall V, Doran PM (2004) Production of nickel bio-ore from hyperaccumulator plant biomass: applications in phytomining. *Biotechnol Bioeng* 86:243–250
- Bothe H, Regvar M, Turnau K (2010) Arbuscular mycorrhiza, heavy metal and salt tolerance. In: Sherameti I, Varma A (eds) *Soil heavy metals*. Springer, Heidelberg, pp 87–111
- Bradshaw AD (1952) Populations of *Agrostis tenuis* resistant to lead and zinc poisoning. *Nature* 169:1098
- Broadley MR, White P, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. *New Phytol* 173:677–702
- Brooks RR (1998) *Plants that hyperaccumulate heavy metals*. CABI Publishing, Wallingford
- Brooks RR, Yang X-H (1984) Elemental levels and relationships in the endemic serpentine flora of the Great Dyke, Zimbabwe, and their significance as controlling factors for the flora. *Taxon* 33:392–399
- Chipeng FK et al (2010) Copper tolerance in the cuprophyte *Haumaniastrum katangense* (S. Moore) PA Duvign Plancke. *Plant Soil* 328:235–244
- Cicatelli A, Lingua G, Todeschini V, Biondi S, Torrigiani P, Castiglione S (2010) Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-

- contaminated soil, and this is upregulated of foliar methallothionein and polyamine biosynthetic gene expression. *Ann Bot* 106:791–802
- Cyranowski D (2001) Almost human. *Nature* 418:910–912
- Dai QL, Huang BF, Yang ZY, Yuan JG, Yang JZ (2010) Identification of cadmium-induced genes in maize seedlings by suppression subtractive hybridization. *Front Environ Sci Eng China* 4:449–458
- De Mars BG, Boerner REJ (1996) Vesicular arbuscular mycorrhizal development in the Brassicaceae in relation to plant life span. *Flora* 191:179–189
- Desbrosses-Fonrouge AG, Voigt K, Schröder A, Arrivault S, Thomine S, Krämer U (2005) *Arabidopsis thaliana* *MTP1* is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf accumulation. *FEBS Lett* 570:4165–4174
- Drazic G, Mihailovic N (2009) Salicylic acid modulates accumulation of Cd in seedlings of Cd-tolerant and Cd-susceptible soybean genotypes. *Arch Biol Sci* 61:431–439
- Elstner EF (1990) *Der Sauerstoff, Biochemie, Biologie. Medizin.* BI-Wissenschaftsverlag, Mannheim, Wien, Zürich
- Ernst WHO (1974) *Schwermetallvegetation der Erde.* Gustav Fischer, Stuttgart
- Ernst WHO (1982) *Schwermetallpflanzen.* In: Kinzel H (ed) *Pflanzenökologie und Mineral-Stoffwechsel.* Ulmer, Stuttgart, pp 472–506
- Ernst WHO (2005) Phytoextraction of mine wastes – options and impossibilities. *Chem Erde – Geochem* 65:29–42
- Ernst WHO, Verkleij JAC, Schat H (1992) Metal tolerance in plants. *Acta Bot Neerl* 41:229–248
- Ernst WHO, Krauss GJ, Verkleij JAC, Wesenberg D (2008) Interaction of heavy metals with sulphur metabolism in angiosperms from an ecological point of view. *Plant Cell Environ* 31:123–143
- Filatov V, Dowdle J, Smirnov N, Ford-Lloyd B, Newbury HJ, Macnair MR (2007) A quantitative trait loci analysis of Zn hyperaccumulation in *Arabidopsis halleri*. *New Phytol* 174:580–590
- Frérot H et al (2010) Genetic architecture of zinc hyperaccumulation in *Arabidopsis halleri*: the essential role of QTL × environment interactions. *New Phytol* 187:355–367
- Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *J Exp Bot* 53:1–11
- Hanikenne M et al (2008) Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. *Nature* 453:391–396
- Hassinen VH, Tuomainen M, Peraniemi S, Schat H, Karenlamp SO, Tervahauta AL (2009) Metallothioneins 2 and 3 contribute to the metal-adapted phenotype but are not directly linked to Zn accumulation in the metal hyperaccumulator *Thlaspi caerulescens*. *J Exp Bot* 60:187–196
- Hildebrandt U, Kaldorf M, Bothe H (1999) The zinc violet and its colonisation by arbuscular mycorrhizal fungi. *J Plant Physiol* 154:709–717
- Hildebrandt U et al (2006) The rare endemic zinc violets of Central Europe originate from *Viola lutea* Huds. *Plant Syst Evol* 257:205–222
- Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. *Phytochemistry* 68:139–146
- Humphreys MO, Nicholls MK (1984) Relationship between tolerance to heavy metals in *Agrostis-capillaris* L. (*Agrostis-tenuis* Sibth). *New Phytol* 98:177–190
- Ingle RA, Muford ST, Rees JD, Campbell MM, Smith JAC (2005) Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. *Plant Cell* 17:2089–2106
- Jaffré T, Brooks RR, Lee J, Reeves RD (1976) *Sibertia acuminata*: a hyperaccumulator of nickel from New Caledonia. *Science* 193:579–580
- Jedrzejszyk M, Rostanski A, Malkowski E (2002) Accumulation of zinc and lead in selected taxa from genus *Viola* L. *Acta Biologica Cracoviensia Series Botanica* 44:49–55
- Kaldorf MO, Kuhn AJ, Schröder WH, Hildebrandt U, Bothe H (1999) Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. *J Plant Physiol* 154:718–728

- Kinzel H, Weber M (1982) Serpentin-Pflanzen. In: Kinzel H (ed) Pflanzenökologie und Mineral-Stoffwechsel. Ulmer, Stuttgart, pp 381–410
- Klingner A, Bothe H, Wray V, Marnier FJ (1995a) Identification of a yellow pigment formed in maize roots upon mycorrhizal colonization. *Phytochemistry* 38:53–55
- Klingner A, Hundeshagen B, Kernebeck H, Bothe H (1995b) Localization of the yellow pigment formed in roots of gramineous plants colonized by arbuscular fungi. *Protoplasma* 185:50–57
- Koch MA, Matchinger M (2007) Evolution and genetic differentiation among relatives of *Arabidopsis thaliana*. *Proc Nat Acad Sci USA* 104:6272–6277
- Koch M, Mummenhoff K, Hurka H (1998) Systematics and evolutionary history of heavy metal tolerant *Thlaspi caerulescens* in Western Europe: evidence from genetic studies based on isoenzyme analysis. *Biochem Syst Ecol* 26:823–838
- Kovacic J, Klejdus B, Hedbavny J, Backor M (2010) Tolerance of *Silene vulgaris* to copper: population related comparison of selected physiological parameters. *Environ Toxicol* 25: 581–592
- Krämer U (2010) Metal hyperaccumulation in plants. *Annu Rev Plant Biol* 61:517–534
- Krämer U, Coster-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as metal chelator in plants that accumulate nickel. *Nature* 379:635–638
- Krämer U, Smith RD, Wenzel WW, Raskin I, Salt RD (1997) The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense*. *Plant Physiol* 115:1641–1650
- Lambinon J, Augier P (1964) La flore et la végétation des terrains calaminaires de la Wallonie septentrionale et de la Rhénanie axoïse. Types chorologiques et groupes écologiques. *Natura Monsana* 16:113–130
- Li Y-M et al (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. *Plant Soil* 249:107–115
- Liao XY, Chen TB, Lei M, Huang ZC, Xiao XY, An ZZ (2004) Root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.) from As-contaminated soils. *Plant Soil* 261:109–116
- Lucassen E, Van Kempen MML, Roelofs JGM, Van der Velde G (2010) Decline in metallophytes in tertiary polluted floodplain grassland in the Netherlands, experimental evidence for metal and nutritional changes in soil as driver factors. *Chem Ecol* 26:273–287
- Lüttge U (1975) Salt glands. In: Baker DA, Hall JL (eds) Ion transport in plant cells and tissues. North-Holland Publishing Comp, Amsterdam, Oxford, pp 335–376
- Macnair MR, Bert V, Huitson SB, Saumitou-Laprade P, Petit D (1999) Zinc tolerance and hyperaccumulation are genetically independent characters. *Proc R Soc Lond B Biol Sci* 266:2175–2179
- Maerques APGC, Rangel AOSS, Castro PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Crit Rev Environ Sci Technol* 30:622–654
- Massa N, Andreucci F, Poli M, Aceto M, Barbato R, Berta G (2010) Screening for heavy metal accumulators amongst autochthonous plants in a polluted site in Italy. *Ecotoxicol Environ Saf* 73:1988–1997
- Mauricio R (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nat Rev Genet* 2:370–380
- Meyer KF (2006) Kritische Revision der *Thlaspi*-Arten Europas, Afrikas und Vorderasiens. Spezieller Teil IX-Noccaea Moench. *Haussknechtia Beiheft*, Jena, p 343
- Meyer CL, Vitalis R, Saumitou-Laprade P, Castric V (2009) Genomic pattern of adaptive divergence in *Arabidopsis halleri*, a model species for tolerance to heavy metal. *Mol Ecol* 18:2050–2062
- Meyer CL et al (2010) Variability of zinc tolerance among and within populations of the pseudometallophyte species *Arabidopsis halleri* and possible role of directional selection. *New Phytol* 185:130–142
- Mikkelsen TS et al (2005) Initial sequences of the chimpanzee genome and comparison with the human genome. *Nature* 437:69–87

- Mills RF et al (2010) Functional significance of *AtHMA4* C-terminal domain in planta. *PLoS One* 5(10):e13388
- Muthukumar T, Bagyaraj DJ (2010) Use of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Proc Natl Acad Sci India Sect B Biol Sci* 80:103–121
- Nauenburg JD (1986) Untersuchungen zur Variabilität, Ökologie und Systematik der *Viola tricolor*-Gruppe in Mitteleuropa, Thesis, The University of Göttingen, Germany, pp 126
- Noret N, Meerts P, Tolrà RP, Poschenrieder C, Barceló D, Escarré J (2005) Palatability of *Thlaspi caerulescens* for snails: influence of Zn and glucosinolates. *New Phytol* 165:763–773
- Noret N, Josens G, Escarré J, Lefévre C, Panichelli S, Meerts P (2007) Development of *Issoria lathonia* (Lepidoptera: Nymphalidae) on zinc-accumulating and nonaccumulating *Viola* species (Violaceae). *Environ Toxicol Chem* 26:565–571
- Orłowska E, Zubek S, Jurkiewicz A, Szarek-Lukaszewska G, Turnau K (2002) Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. *Mycorrhiza* 12:153–160
- Ouziad F, Hildebrandt U, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. *J Plant Physiol* 162:634–649
- Patzke W, Brown G (1990) *Festua aquisgranensis* sp. nova ein neuer Vertreter der Kollektivart *Festuca ovina* L. *Decheniana* 143:194–195
- Pauwels M, Saumitou-Laprade P, Holl AC, Petit D, Bonnin I (2005) Multiple origin of metalcolous populations of the pseudometallophyte *Arabidopsis halleri* (Brassicaceae) in central Europe: the cpDNA testimony. *Mol Ecol* 14:4403–4414
- Pauwels M, Frérot H, Bonnin I, Saumitou-Laprade P (2006) A broad-scale analysis of population differentiation for Zn tolerance in an emerging model species for tolerance study: *Arabidopsis halleri* (Brassicaceae). *J Evol Biol* 19:1838–1850
- Pence NS et al (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc Nat Acad Sci USA* 97:4956–4960
- Perez-Figueroa A, Garcia-Pereira MJ, Saura M, Rolan-Alvarez E, Caballero A (2010) Comparing three different methods detect selective loci using dominant markers. *J Evol Biol* 23:2267–2276
- Pilon-Smits E (2005) Phytoremediation. *Annu Rev Plant Biol* 56:15–39
- Prasad MNV, Hagemeyer J (eds) (1999) Heavy metal stress in plants – from molecules to ecosystems. Springer, Heidelberg
- Ramirez-Rodriguez V, Lopez-Bucio J, Herrera-Estrella L (2005) Adaptive responses in plants to nonoptimal soil pH. In: Jenks MA, Hasegawa PM (eds) Plant abiotic stress. Blackwell, Oxford, pp 145–170
- Regvar M et al (2003) Colonization of pennycresses *Thlaspi* sp. of the Brassicaceae by arbuscular mycorrhizal fungi. *J Plant Physiol* 160:615–626
- Robinson RH, Lombi E, Zhao FJ, McGrath SP (2003) Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. *New Phytol* 158:279–285
- Roosens N, Willems G, Gode C, Courseaux A, Saumitou-Laprade P (2008) The use of comparative genome analysis and systemic relationships allows extrapolating the position of Zn tolerance QTL regions from *Arabidopsis halleri* into *Arabidopsis thaliana*. *Plant Soil* 306:105–116
- Rozema J, Gude H, Pollak G (1981) An ecophysiological study of the salt excretion of four halophytes. *New Phytol* 89:201–207
- 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, Dordrecht
- Salt DE, Smith RD, Raskin RD (1998) Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol* 49:643–668
- Schat H, Tenbookum VM (1992) Genetic control of copper tolerance in *Silene vulgaris*. *Heredity* 68:219–229
- Schat H, Vooijs R (1997) Multiple tolerance and co-tolerance to heavy metals in *Silene vulgaris*: co-segregation analysis. *New Phytol* 136:489–496

- Schat H, Kuiper E, TenBookum WM, Vooijs R (1993) A general model for the genetic control of copper tolerance in *Silene vulgaris*: evidence from crosses between plants from different tolerant populations. *Heredity* 70:142–147
- Scheloske S, Maetz M, Schneider T, Hildebrandt U, Bothe H, Povh H (2004) Element distribution in mycorrhizal and nonmycorrhizal roots of the halophyte *Aster tripolium* determined by proton induced X-ray emission. *Protoplasma* 223:183–189
- Schwickerath M (1944) Das Hohe Venn und seine Randgebiete. Gustav Fischer, Jena
- Shahzad Z et al (2010) The five *AhMTP1* Zinc transporters undergo different evolutionary fates towards adaptive evolution to zinc tolerance in *Arabidopsis halleri*. *PLoS Genet* 6(4): e1000911
- Slomka A et al (2011) Violets of the section Melanium, their colonization by arbuscular mycorrhizal fungi and their occurrence on heavy metal heaps. *J Plant Physiol* 168:1191–1199
- Smith SE, Macnair MR (1998) Hypostatic modifiers cause variations in degree of copper tolerance in *Mimulus guttatus*. *Heredity* 80:760–768
- Sun Q, Ye ZH, Wang X-R, Wong MH (2007) Cadmium hyperaccumulation leads to an increase of glutathione rather than phytochelatins in the cadmium hyperaccumulator *Sedum alfredii*. *J Plant Physiol* 164:1489–1498
- Tonin C, Vandenkoornhuysen P, Joner EJ, Strzcek J, Leyval C (2001) Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza* 10:161–168
- Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza* 13:185–190
- Turnau K, Ostachowicz B, Wojtczak G, Anielska T, Sobczyk L (2010) Metal uptake by xerothermic plants introduced into Zn-Pb industrial wastes. *Plant Soil* 337:299–311
- Van de Mortel JE et al (2008) Expression differences for genes involved in lignin, glutathione and sulphate metabolism in response to cadmium in *Arabidopsis thaliana* and the related *Thlaspi caerulescens*. *Plant Cell Environ* 31:301–324
- Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. *New Phytol* 181:759–776
- Vogel-Mikuš K, Pongrac P, Kump P, Necemer M, Regvar M (2006) Colonization of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal mixture induces changes in heavy metal and nutrient uptake. *Environ Pollut* 139:362–371
- Weissenhorn I, Leyval C (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant Soil* 157:247–256
- Westhoff A (1999) Mycorrhizal plants for phytoremediation of soils contaminated with radionuclides. *Restor Reclam Rev (Student On-line Journal)* 5(4)
- Willems G, Drager DB, Courbot M, Gode C, Verbruggen N, Saumitou-Laprade P (2007) The genetic basis of zinc tolerance in the metallophyte *Arabidopsis halleri* ssp. *halleri* (Brassicaceae) An analysis of quantitative trait loci. *Genetics* 176:659–674
- Willems G, Frérot H, Gennen J, Salis P, Saumitou-Laprade P (2010) Quantitative trait loci analysis of mineral element concentration in an *Arabidopsis halleri* × *Arabidopsis lyrata petraea* F₂-progeny grown on cadmium-contaminated soil. *New Phytol* 187:368–379
- Wu L, Bradshaw AD, Thurman DA (1975) Potential for evolution of heavy-metal tolerance in plants. 3. Rapid evolution of the copper tolerance in *Agrostis stolonifera*. *Heredity* 34:165–172
- Wu CA, Liao B, Wang SL, Zhang J, Li JT (2010) Pb and Zn accumulation in a Cd-hyperaccumulator (*Viola baoshanensis*). *Int J Phytoremediation* 12:574–585
- Zelko I, Lux A, Czibula K (2008) Difference in the root structure of hyperaccumulator *Thlaspi caerulescens* and non-hyperaccumulator *Thlaspi arvense*. *Int J Environ Pollut* 33:123–132

