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Chapter 6

THE PHYSIOLOGY, FUNCTIONAL GENOMICS, AND APPLIED ECOLOGY OF HEAVY METAL-TOLERANT BRASSICACEAE

Jillian E. Gall and Nishanta Rajakaruna*
College of the Atlantic, Bar Harbor, ME, US

ABSTRACT

Globally, $25-50 billion is spent each year cleaning up sites contaminated with heavy metals. Because traditional cleanup methods such as incineration, chemical treatment, and excavation and removal are costly and can damage the environment, metal-hyperaccumulating plants (plants that accumulate >0.1% heavy metals in leaves or other tissues) may be a more cost-effective, less-intrusive option for remediating such sites. Members of the Brassicaceae comprise 25% of metal-hyperaccumulating species worldwide discovered to date and are potential candidates for phytoremediation technologies. Here we describe the diversity of metal-hyperaccumulating species in the Brassicaceae and discuss the physiological mechanisms of metal uptake and tolerance, the genetic basis for the metal tolerance mechanisms, ecological consequences of metal hyperaccumulation, and the role of the Brassicaceae species in remediating contaminated sites worldwide.

Keywords: Green technology, hyperaccumulation, metal tolerance, phytoremediation, ultramafic soils

INTRODUCTION

Heavy metals are highly reactive, toxic at low concentrations, and persist in the environment for years, posing severe risks to human and ecosystem health worldwide (Pilon-Smits, 2005; Neilson and Rajakaruna, 2012). Lead, for instance, may remain in the soil for

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* Email: nrajakaruna@coa.edu.

150 to 5,000 years (Kumar *et al*., 1995) and is known to cause cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, and renal impairment in humans (Patrick, 2006). Although heavy metals are naturally present in the Earth's crust, human activities such as transportation, industrial manufacturing, commercial agriculture, mining, smelting, and military operations contribute largely to heavy metal pollution, releasing metals into the environment through waste disposal, runoff, and heavy metal-laden chemicals (Chaffai and Koyama, 2011; Pilon-Smits, 2005).

Vast areas of the world are contaminated with heavy metals (Ensley, 2000; Wuana and Okieimen, 2011). Traditional cleanup methods remove, incinerate or chemically treat contaminated soil, disrupting biotic communities and damaging the environment. These methods are also expensive, costing $25 to $50 billion worldwide annually (Neilson and Rajakaruna, 2011). The United States alone spends $6 to $8 billion each year cleaning up metal-contaminated sites (Tsao, 2003; Pilon-Smits, 2005), a steep investment that many developing nations cannot afford (Rajakaruna *et al*., 2006).

Given the expense of conventional cleanup, there is much interest in seeking ecologically friendly, low-cost technologies to remove heavy metals from contaminated soils. One such alternative uses plants, a green technology known as phytoremediation (Krämer, 2005). Gaining popularity over the past few decades (Pilon-Smits and Freeman, 2006), phytoremediation utilizes metal hyperaccumulators, plants that can absorb, detoxify, and store high levels of heavy metals in their tissues. Hyperaccumulators take up high concentrations of heavy metals from the soil and translocate them into above-ground biomass at concentrations exceeding, in most cases, 0.1% of total dry leaf tissue mass (Baker *et al*., 2000; Van der Ent *et al*., 2012). The aboveground biomass can then be harvested and disposed of in a landfill or further processed for metal extraction (*i.e*. phytomining; Ghosh and Singh, 2005; Pilon-Smits, 2005).

Although some heavy metals, such as nickel (Ni), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) regulate various biological processes in plants (Epstein and Bloom, 2004), when they occur in excess these metals may interact directly with biomolecules, disrupting critical biological processes (Kabata-Pendias, 2001; Chaffai and Koyama, 2011). Thus, most plants exclude metals at the roots by binding them to organic acids or ligands or storing them within vacuoles in the roots where they cannot interfere with important physiological processes (Hossain *et al*., 2012).

Although metal-hyperaccumulators have the ability to detoxify and accumulate metals in their tissues, they do have limits to their extraordinary capacity to deal with metals, and the threshold for hyperaccumulation depends on the metal under consideration. Hyperaccumulators of cadmium (Cd), selenium (Se), and thallium (Tl) accumulate >100 µg g^{-1} in their dry leaf tissue, cobalt (Co), chromium (Cr), and Cu accumulate >300 µg g^{-1} in their dry leaf tissue, whereas lead (Pb), arsenic (As), antimony (At), and Ni accumulate >1,000 µg g^{-1} in their dry leaf tissue. Hyperaccumulators of Zn accumulate >3,000 µg g^{-1} while those of Mn accumulate >10,000 µg g^{-1} in their dry leaf tissue (Reeves and Baker, 2000; Van der Ent *et al*. 2012). Although Se is a metalloid, we incorporate it into our discussion because it is a major environmental pollutant (Terry *et al*., 2000). For recent reviews of metal-hyperaccumulation see Krämer (2010) and Van der ent *et al*. (2012).

Of the approximately 582 species of metal-hyperaccumulators from 50+ families of vascular plants worldwide, approximately 25% belong to the Brassicaceae, making it a model family for studying metal tolerance and hyperaccumulation (Rascio and Navari-Izzo, 2011;

Van der ent *et al*. 2012). In this chapter we introduce the metal-hyperaccumulating species in the Brassicaceae, outline the physiological mechanisms underlying their tolerance of heavy metals, and summarize the genetic basis for their remarkable physiology. We also discuss the ecological consequences of utilizing metal-hyperaccumulating species for phytoremediation, including the potential for metal transfer through trophic levels, the likelihood for invasiveness when employing non-native species, and the concerns of using genetically modified hyperaccumulators.

OVERVIEW OF METAL TOLERANCE IN THE BRASSICACEAE

The 93 documented species of metal-hyperaccumulating Brassicaceae (Table 1; Figure 1) provide substantial opportunity to study the physiological and genetic mechanisms behind metal tolerance and hyperaccumulation as well as the ecological implications of these mechanisms. Some of the most well-studied genera of hyperaccumulators in this family include *Arabidopsis*, *Brassica*, *Alyssum*, *Noccaea* (formerly *Thlaspi*), *Stanleya*, and *Streptanthus* (Bhargava *et al*., 2012; Boyd *et al*., 2009; Freeman *et al*., 2010; Vamerali *et al*., 2010; Verguggen *et al*., 2009; Figure 1). Below we briefly introduce the most studied metal-hyperaccumulating Brassicaceae taxa.

Noccaea caerulescens (formerly *Thlaspi caerulescens*) is, perhaps, the most well-studied metal-hyperaccumulator (Milner and Kochian, 2008), accumulating up to 36,900 µg g^{-1} Zn and 1800 µg g^{-1} Cd without signs of toxicity (Bhargava *et al*., 2012). Because *N. caerulescens*—like most other model taxa in the Brassicaceae—grows easily in the lab, it has been extensively studied, revealing several mechanisms for metal uptake, transport, and localization (Cosio *et al*., 2004). However, its small biomass limits its potential as a candidate for phytoremediation (Bhargava *et al*., 2012).

Arabidopsis thaliana, although not a naturally metal-accumulating species, is a popular model organism for plant-based research (Bevan and Walsh, 2005). *Arabidopsis thaliana*'s genome is mapped (Weigel and Mott, 2009) and its sequence is very similar to its metal-accumulating congener *A. halleri* (Becher *et al*., 2004; Meyer and Verbruggen, 2012; Weber *et al*., 2004). For this reason, both *A. thaliana* and *A. halleri* are commonly used to study the genetic basis for metal tolerance and hyperaccumulation (Bevan and Walsh, 2005; Cho *et al*., 2003; Chaffai and Koyama, 2011; Courbot *et al*., 2007; Hanikenne *et al*., 2008).

A common condiment crop in North America and Europe, *Brassica juncea* (Indian mustard) is a popular choice for phytoremediation (Lim *et al*., 2004; Neilson and Rajakaruna, 2012). Although not a hyperaccumulator, with the ability to accumulate Cd, Zn, Se, and Pb and a biomass at least 10-fold greater than that of *N. caerulescens*, *B. juncea* has been used with success in several phytoremediation studies and trials (Bhargava *et al*., 2012; Szczygłowska *et al*., 2011; Warwick, 2011).

The molecular mechanisms responsible for selenium (Se) tolerance and hyperaccumulation have been investigated in the Se hyperaccumulator *Stanleya pinnata* by comparing it with its Se-tolerant congener, *S. albescens*, using a combination of physiological, structural, genomic, and biochemical approaches (Freeman *et al*., 2010). Additionally, the ecological functions and implications of Se hyperaccumulation in *Stanleya* and other plants (El Mehdawi and Pilon-Smits, 2012) and the potential for Se phytoremediation have also been investigated (Bañuelos, 2001).

Table 1. Brassicaceae species known to hyperaccumulate heavy metals based on an extensive review of the literature and personal communication with Dr. Roger D. Reeves. Nomenclature follows International Plant Names Index [website (http://www.ipni.org/index.html); accessed Oct 2012]. The two taxa with * have questionable nomenclature in light of recent taxonomic revisions in the group (personal communication Ihsan A. Al-Shehbaz, Senior Curator, Missouri Botanical Garden, USA)

Species	**Metal Hyperaccumulated**	**Reference**
Aethionema spicatum Post	Ni	Reeves *et al*., 2001
Alyssum akamasicum Burtt	Ni	Brooks *et al*., 1979
A. alpestre L.	Ni	Brooks and Radford, 1978
A. anatolicum Hausskn. ex Nyár.	Ni	Brooks *et al*., 1979
A. argenteum All.	Ni	Brooks and Radford, 1978
A. baldaccii Vierh. ex Nyár.	Ni	Brooks and Radford, 1978
A. bertolonii Desv. subsp. *scutarinum* Nyár.	Ni	Minguzzi and Vergnano, 1948; Brooks *et al*., 1979; Reeves *et al*., 1983
A. bracteatum Boiss. & Buhse	Ni	Ghaderian *et al*., 2007a
A. callichroum Boiss. & Bal.	Ni	Brooks *et al*., 1979
A. caricum T.R. Dudley & Hub.-Mor.	Ni	Brooks *et al*., 1979
A. cassium Boiss.	Ni	Brooks *et al*., 1979
A. chalcidicum Janka	Ni	Brooks and Radford, 1978
A. cholorocarpum Hausskn	Ni	Brooks and Radford, 1978
A. chondrogynum Burtt	Ni	Brooks *et al*., 1979
A. cilicicum Boiss. & Bal.	Ni	Brooks *et al*., 1979
A. condensatum Boiss.	Ni	Brooks *et al*., 1979
A. constellatum Boiss.	Ni	Brooks *et al*., 1979
A. corsicum Duby	Ni	Brooks *et al*., 1979
A. crenulatum Boiss.	Ni	Brooks *et al*., 1979
A. cypricum Nyár.	Ni	Brooks *et al*., 1979
A. davisianum T.R. Dudley	Ni	Brooks *et al*., 1979
A. discolor T.R.Dudley & Hub-Mor.	Ni	Brooks *et al*., 1979
A. dubertretii Gomb.	Ni	Brooks *et al*., 1979
A. dudleyi N. Adigüzel & R.D. Reeves	Ni	Adigüzel and Reeves, 2002
A. eriophyllum Boiss. & Hausskn.	Ni	Brooks *et al*., 1979
A. euboeum Halácsy	Ni	Brooks and Radford, 1978
A. floribundum Boiss. & Bal.	Ni	Brooks *et al*., 1979
A. giosnanum Nyár.	Ni	Brooks *et al*., 1979
A. heldreichii Hausskn	Ni	Brooks and Radford, 1978
A. huber-morathii T.R.Dudley	Ni	Brooks *et al*., 1979
A. inflatum Nyár.	Ni	Ghaderian *et al*., 2007b
A. lesbiacum Candargy (Rech. f.)	Ni	Brooks *et al*., 1979
A. longistylum Grossh.	Ni	Ghaderian *et al*., 2007b
A. markgrafi O.E. Schulz	Ni	Brooks and Radford, 1978
A. masmenaeum Boiss.	Ni	Brooks *et al*., 1979
A. murale Waldst. & Kit. subsp. *haradjianii* (Rech.) T.R.Dudley subsp. *pichleri* (Velen.) Stoj. & Stef	Ni	Doksopulo, 1961; Reeves *et al*., 2001; Reeves and Adıgüzel, 2008; Reeves *et al*., 1983

Species	**Metal Hyperaccumulated**	**Reference**
A. obovatum (C.A. Meyer) Turcz.	Ni	Brooks *et al*., 1979
A. oxycarpum Boiss. & Bal.	Ni	Brooks *et al*., 1979
A. pateri Nyár.	Ni	Reeves and Adigüzel, 2008
A. peltarioides Boiss.	Ni	Reeves and Adigüzel, 2008
subsp. *virgatiforme* (Nyár.) T.R.Dudley		Reeves *et al*., 1983; Reeves and Adıgüzel, 2008
A. penjwinensis T.R. Dudley	Ni	Brooks *et al*., 1979
A. pinifolium (Nyár.) T.R.Dudley	Ni	Brooks *et al*., 1979
A. pintodasilvae T.R.Dudley	Ni	Gonçalves *et al*., 2007
A. pterocarpum T.R.Dudley	Ni	Brooks *et al*., 1979
A. robertianum Bernard ex Gren. & Godr.	Ni	Brooks and Radford, 1978
A. samariferum Boiss. & Hausskn.	Ni	Brooks *et al*., 1979
A. serpyllifolium Desf.	Ni	Brooks and Radford, 1978
subsp. *lusitanicum* T.R. Dudley & Pinto da Silva		Brooks *et al*., 1981
subsp. *malacitanum* Rivas Goday		
A. sibiricum Willd.	Ni	Brooks *et al*., 1979
A. singarense Boiss. & Hausskn.	Ni	Brooks *et al*., 1979
A. smolikanum Nyár.	Ni	Brooks and Radford, 1978
A. syriacum Nyár.	Ni	Brooks *et al*., 1979
A. tenium Halácsy	Ni	Brooks and Radford, 1978
A. trapeziforme Bornm. Ex Nyár.	Ni	Brooks *et al*., 1979
A. troodii Boiss.	Ni	Brooks *et al*., 1979
A. virgatum Nyár.	Ni	Brooks *et al*., 1979
Arabidopsis halleri (L.) O'Kane & Al-Shehbaz	Zn	Ernst, 1968
	Cd	Zhao *et al*., 2000; Bert *et al*., 2002
Arabis gemmifera (Matsum.) Makino	Zn	Kubota and Takenaka, 2003
A. paniculata Franch.	Zn Cd Pb	Tang *et al*., 2009
Bornmuellera baldacci (Degen Heywood)	Ni	Reeves *et al*., 1983
subsp. *baldacci*		Reeves *et al*., 1983
subsp. *markgrafi* (Schulz ex Markgraf) Dudley		Reeves *et al*., 1983
subsp. *rechingeri* Greuter		
B. glabrescens (Boiss. & Bal.) Cullen & T.R. Dudley	Ni	Reeves *et al*., 1983
B. kiyakii Aytaç & A.Aksoy	Ni	Reeves & Adıgüzel 2009
B. tymphaea (Hausskn.) Hausskn.	Ni	Reeves *et al*., 1983
B. x petri Greuter, Charpin & Dittrich	Ni	Reeves *et al*., 1983
Brassica oleracea L.	Ti	Al-Najer *et al*., 2005
Cardamine resedifolia L.	Ni	Vergnano Gambi and Gabbrielli, 1979
Iberis intermedia Guers.	Tl	Leblanc *et al*., 1999
Leptoplax emarginata (Boiss.) O.E. Schulz	Ni	Reeves *et al*., 1980
Masmenia rosularis (Boiss. & Bal.) F.K. Meyer	Ni	Reeves, 1988
Microthlaspi perfoliatum (L.) F.K.Mey. (as *T. perfoliatum* L.)	Ni	Reeves *et al*., 2001
Noccaea caerulescens (J.Presl & C.Presl) F.K.Mey. (as *T. caerulescens* J.Presl & C.Presl)	Zn Ni Cd	Sachs, 1865; Ernst, 1966, 1968, 1974; Reeves and Brooks, 1983; Lombi *et al*., 2000; Escarré *et al*., 2000; Reeves *et al*., 2001

Table 1. Continued

Species	**Metal Hyperaccumulated**	**Reference**
N. cariensis (Carlström) Parolly, Nordt & Aytaç (as *T. cariense* A. Carlström)	Ni	Reeves *et al*., 2001
N. cepaefolia (Wulfen) Rchb. (as *T. rotundifolium* (L.) Gaudin subsp. *cepaeifolium* (Wulfen) Rouy & Fouc.)	Zn	Rascio, 1977; Reeves and Brooks, 1983
N. cochleariforme (as *T. japonicum* H.Boissieu)	Ni	Reeves 1988; Mizuno *et al*., 2003, 2005
N. epirota (Halácsy) F.K.Mey. (as *T. epirotum* Halácsy)	Ni	Reeves and Brooks, 1983
N. fendleri subsp. *californica* (S. Watson) Al-Shehbaz & M. Koch (*T. montanum* var. *californicum* (Watson) P.K.Holmgren)	Ni	Reeves *et al*., 1983
N. fendleri subsp. *fendleri* (A. Gray) Holub (as *Thlaspi montanum* var. *fendleri* (A.Gray) P.K.Holmgren)		
N. fendleri subsp. *siskiyouensis* (P.K. Holmgren) Al-Shehbaz & M. Koch (as *T. montanum* var. *siskiyouense* P.K.Holmgren)		
N. goesingense (Halácsy) F.K.Mey. (as *T. goesingense* Halácsy)	Ni	Reeves and Baker, 1984
N. graeca (Jord.) F.K.Mey. (as *T. graecum* Jord.)	Ni	Reeves and Brooks, 1983
N. kovatsii (Heuff.) F.K.Mey. (as *T. kovatsii* Heuffel)	Ni	Bani *et al*., 2010
N. ochroleuca (Boiss. & Heldr.) F.K.Mey. (as *T. ochroleucum* Boiss. & Heldr.)	Ni	Reeves and Brooks, 1983
N. praecox (Wuljen) F.K.Mey. (as *T. praecox* Wulfen in Jacq.)	Ni	Bani *et al*., 2010
N. pindica (Hausskn.) Holub (as *T. pindicum Hausskn.*)	Ni	Taylor and Macnair, 2006
N. tymphaea (Hausskn.) F.K.Mey. (as *T. tymphaeum* Hausskn. and *T. goesingense* Halácsy)	Ni	Reeves and Brooks, 1983
Pseudosempervivum aucheri (Boiss.) Pobed. (as *Cochlearia aucheri* Boiss.)	Ni	Reeves, 1988
P. semprevivum Boiss. & Bal.) Pobed. (as *Cochlearia sempervivum* Boiss. & Balansa)	Ni	Reeves, 1988
Raparia bulbosa (Spruner) F.K.Mey. (as *Thlaspi bulbosum* Spruner)	Ni	Reeves and Brooks, 1983
Stanleya bipinnata Greene		Moxon *et al*., 1950; Rosenfeld and Beath 1964
S. pinnata (Pursh) Britton	Se	Rosenfeld and Beath, 1964
Streptanthus polygaloides A. Gray	Ni	Reeves *et al*., 1981
**Thlaspi jaubertii* Hedge	Ni	Reeves and Brooks, 1983
**T. rosulare* Boiss. & Bal.	Ni	Reeves and Adigüzel, 2008
Thlaspiceras eigii (Zohary) F.K.Mey. subsp. *samuelssonii* F.K.Mey. (as *Thlaspi eigii* (Zohary) Greuter & Burdet subsp. *samuelssonii* (F.K.Mey.) Greuter & Burdet)	Ni	Reeves and Adigüzel, 2008
T. elegans (Boiss.) F.K.Mey. (as *T. elegans* Boiss.)	Ni	Reeves and Brooks, 1983
T. oxyceras (Boiss.) F.K.Mey. (as *T. oxyceras* (Boiss.) Hedge)	Ni	Reeves and Adigüzel, 2008

Figure 1. Metal-hyperaccumulating species. A, *Alyssum bertolonii* (Brassicaceae) was first reported by Caesalpino (1583) as confined to Ni-rich serpentine outcrops near Florence Italy. Minguzzi and Vergnano (1948) discovered that this plant had an extraordinarily high Ni content of about 10,000 ppm [l%] in dried matter which translated to well over 10% of Ni in the ash. Photo Credit: Dr. A. J. M. Baker; B, *Noccaea caerulescens* (Brassicaceae) hyperaccumulates Zn, Cd, and Ni and is the 'lab rat' for metal-hyperaccumulating research. Photo Credit: Dr. A. J. M. Baker; C, *Streptanthus polygaloides* (Brassicaceae), a Californian serpentine endemic, is one of only two Ni-hyperaccumulating species found in North America. Photo Credit: Dr. Robert S. Boyd; D, *Stanleya pinnata* (Brassicaceae) is a Se-hyperaccumulating perennial species native to Southwestern United States. Photo Credit: Malia Volke; E, *Bornmuellera tymphaea* (Brassicaceae) is a Ni-hyperaccumulating species native to serpentine soils in Greece. Photo Credit: Dr. Roger D. Reeves; F, *Alyssum murale* (Brassicaceae) is a widespread and polymorphic Ni-hyperaccumulating species native to eastern Mediterranean Europe, Turkey and adjacent parts of SW Asia. Photo Credit: Dr. Roger D. Reeves.

Endemic to ultramafic (serpentine) soils along the western side of the Sierra Nevada in California, *Streptanthus polygaloides* is a small annual that hyperaccumulates Ni (Pope *et al*., 2013) in concentrations ranging from 1100 to 16,400 µg g^{-1} dry mass in its leaves, stems, roots, flowers, and fruits (Reeves *et al*., 1981). *Streptanthus polygaloides* is one of two native Ni hyperaccumulators confirmed from continental North America (Reeves *et al*., 1981; Boyd *et al*., 2009); the other, *Noccaea fendleri*, is also from the Brassicaceae (O'Dell and Rajakaruna, 2011). The relatively small aboveground biomass of these species makes *S. polygaloides* and *N. fendleri* poor candidates for phytoremediation. However, *S. polygaloides* has been investigated for its potential for phytoremediation and phytomining (Anderson *et al*., 1999). Additionally, its ecology (Jhee *et al*., 2005; Boyd *et al*., 2009; Pope *et al*., 2013), including the role it may play in the transfer of metals through the food web (Wall and Boyd, 2002), has also been studied. Wall and Boyd (2002) discovered an insect, *Melanotrichus boydi* (Hemiptera: Miridae), which is monophagous on *S. polygaloides*. They reported that *M. boydi* accumulates Ni up to nearly 800 µg g^{-1} of dry tissue, raising concerns about the potential for metals to transfer from hyperaccumulating plants to the insects that feed on them (Peralta-Videa *et al*., 2009).

PHYSIOLOGICAL MECHANISMS OF METAL TOLERANCE

In the soil, metal cations are bound to negatively charged particles such as clay and organic matter. Hyperaccumulators take up metals (Figure 2a) only after the cations have detached from these soil particles due to mass ion effect and become bioavailable in the soil solution (Neilson and Rajakaruna, 2012). The bioavailability of a metal depends on the interaction of various physical, chemical, and biological processes within the soil (Maestri *et al*., 2010; Jabeen *et al*., 2009). At low pH, certain metals such as Cd, Cu, Hg, and Pb become more available for plant uptake (Blaylock and Huang, 2000). Some hyperaccumulators release protons or metal-chelators such as mugenic and aveic acids (Jabeen *et al*., 2009) which acidify the rhizosphere, freeing metals into the soil solution (Salt *et al*., 1994). Soil bacteria may also release a number of compounds into the soil such as antibiotics, antifungals, organic acids, hormones, and metal chelators, which may increase the bioavailability of metals (Xiong *et al*., 2008). Puschenreiter *et al*. (2005) found that metals were more bioavailable in the rhizosphere of hyperaccumulators than in non-hyperaccumulators, suggesting that hyperaccumulators may actively alter their rhizospheric environment to increase the availability of metals (Neilson and Rajakaruna, 2012). The root structure of hyperaccumulators also appears to differ from that of non-hyperaccumulators. Mench *et al*. (2009) identified a zone in the roots of *N. caerulescens,* external to the endodermis near the root tip, with thickened inner tangential cell walls which may assist metal uptake and transport.

When bioavailable metals come into contact with roots (either through diffusion or bulk flow), they enter the root apoplast. Metal ions may remain in the apoplast, traveling passively from cell wall to cell wall, or they may cross the plasma membrane into the symplast through a number of embedded ion transport proteins (Figure 2b) including pumps, channels, or carriers (Jabeen *et al*., 2009; Salt *et al*., 1995). In addition to regulating metal ions within the cell, ion transporter proteins are critical for metal uptake. Plants have several classes of metal-transporters: the heavy metal (or CPX-type) ATPases, the natural resistance-associated macrophage-proteins (Nramp), cation-diffusion facilitator (CDF) proteins, zinc-iron permeases (ZIP), cation exchangers (CAXs), and copper transporters (COPTs) (Chaffai and Koyama, 2011; Jabeen *et al*., 2009). ZIP family proteins have been shown to be particularly important for metal uptake in *N. caerulescens* (Chaffai and Koyama, 2011; Maestri *et al*., 2010).

Once a metal ion has entered the symplast of a root cell via a metal transporter, it can either be sequestered into the root vacuoles or transported to the leaves via the xylem. In either situation, chelators must bind to the metals to protect the internal environment of the cell from metal-induced damage (Memon and Schroder, 2008). Chelators include organic acids such as citrate, malate, and malonate, and proteins such as histidine, metallothioneins, and phytochelatins (Maestri *et al*., 2010; Pilon-Smits and Pilon, 2002). Metallothioneins and phytochelatins are thiol-rich ligands which donate electrons more readily than oxygen, thus forming stable complexes with first-row transition metals (Baker *et al*., 2000). Organic acids and ligands bind metals differentially throughout the plant. Verbruggen *et al*. (2009) found that histidine accompanied Zn in the roots of *N. caerulescens*, whereas organic acids accompanied Zn in the shoots. They also found Cd bound to sulfur ligands in the leaves of *N. caerulescens*. Organic acids are also prevalent both in the cytosol and in the acidic vacuoles of root and leaf cells. Verbruggen *et al*. (2009) observed Zn-malate complexes in epidermal cell vacuoles of *N. caerulescens* and in mesophyll vacuoles of *A. halleri*. Malate also transports Zn from the cytosol to the vacuole, where it transfers Zn to sulfur-containing mustard oils before being transported back to the cytosol to retrieve more Zn (Baker *et al*., 2000).

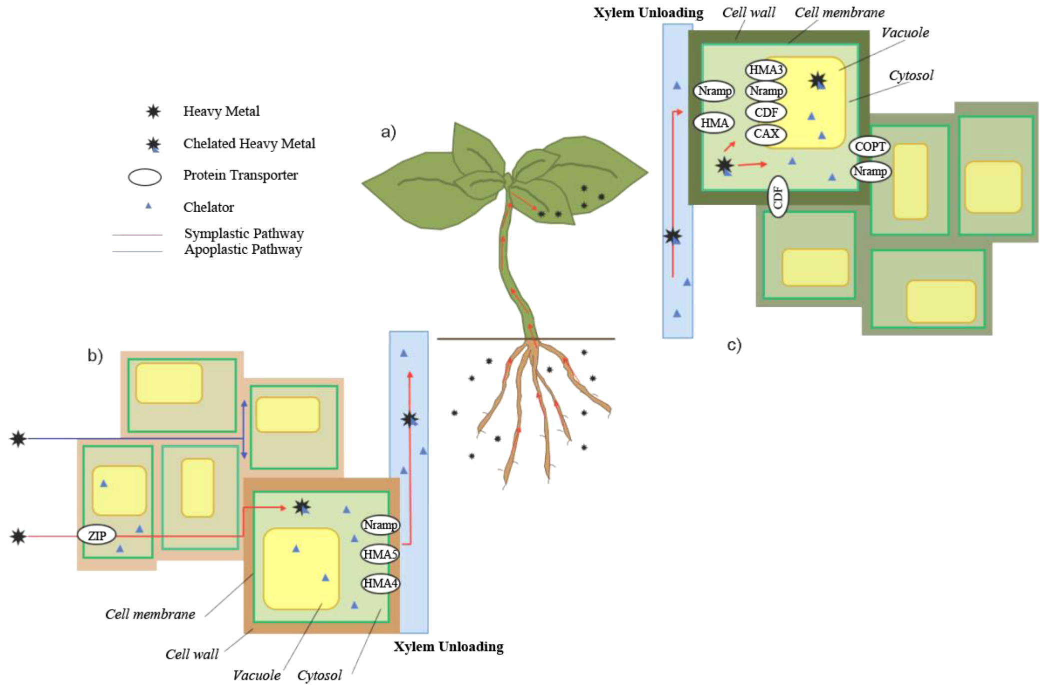

Figure 2. Mechanism of metal hyperaccumulation and tolerance. a) Translocation of heavy metals from roots to leaves in hyperaccumulator plants b) Heavy metals enter the roots and travel cell to cell through an apoplastic or symplastic pathway. In the symplastic pathway, heavy metals pass through specialized transporter proteins and are chelated, eventually loading into the xylem. c) Heavy metals in the xylem unload into leaf cells and either enter the vacuole or move to neighboring cells. Adapted from Maestri *et al*., 2010.

For metals to end up in the leaves of a hyperaccumulator, they must be loaded into the xylem, moved up the shoot, and deposited in the vacuoles of the leaf cells (Figure 2). Such movement requires passing through at least three plasma membranes: the plasma membrane of the root cell, the plasma membrane of the leaf cell, and the tonoplast of the leaf cell vacuole. The rate at which metals move through the xylem depends on the metal concentration in the root, with higher concentrations of metals in the roots resulting in faster loading into the xylem (Jabeen *et al*., 2009). ATPase and Nramp class transporters are particularly important for xylem loading; many members of ATPase have been identified in both *A. halleri* and *A. thaliana* (Maestri *et al*., 2010; Jabeen *et al*., 2009).

Once in the xylem, low molecular weight chelators (such as malate, citrate, phytochelatins, and free histidine) bind to metal ions and transpiration pulls the complexed metals up the xlyem to the leaves (Jabeen *et al.*, 2009). High levels of histidine in roots of hyperaccumulators may also increase translocation of metals from roots to leaves. Richau *et al*. (2009) found that as concentrations of histidine increased in roots, Ni-histidine complexes decreased in root vacuoles. They also found that the concentration of histidine in roots of *N. caerulescens* (reported as *T. caerulescens*) was 10-fold higher than in the non-metal-hyperaccumulating congener *N. arvense* (reported as *T. arvense*), although the amount of histidine found in the leaves was only slightly greater in *N. caerulescens*. Richau *et al*. (2009) also exposed three hyperaccumulating *Alyssum* species to Ni and reported an increase of histidine in the xylem sap of all three species, a phenomenon not observed in the non-hyperaccumulating species of the Brassicaceae.

To exit the xylem, metals must pass through the leaf cell wall and cell membrane (Figure 2c), a process regulated by ATPase and Nramp proteins (Jabeen *et al*., 2009). Once in the cytosol of the leaf, proteins such as ATPases and phytochelatins transport metals to the vacuoles where they are bound to organic acids or anthocyanins and are stored until senescence (Chaffai and Koyama, 2011; Pilon-Smits and Pilon, 2002). Hyperaccumulators often preferentially store more metals in shoot vacuoles than root vacuoles, with the opposite being the case for non-hyperaccumulators. In the hyperaccumulator *N. caerulescens*, Ni is higher in shoot tonoplast vesicles than in root tonoplast vesicles with the opposite pattern in the non-hyperaccumulator, *N. arvense* (Richau *et al.*, 2009). These findings suggest that the mechanisms of metal tolerance are species-specific.

Although hyperaccumulators sequester metals in the vacuoles of their leaves, the exact location of this sequestration within leaves varies by species. Broadhurst *et al*. (2004) found that in five *Alyssum* species, Ni was stored in either leaf epidermal cell vacuoles or in basal portions of stellate trichomes, reporting that the Ni in trichomes comprised a remarkable 15% to 20% of the plant’s dry weight. However, Ghasemi *et al*. (2009) did not find any more Ni in trichomes than in shoots of *A. inflatum*, a Ni-hyperaccumulating species of *Alyssum* native to Iran. However, after immersing whole *A. inflatum* leaves in Ni-indicating dimethylglyoxime (DMG), staining of trichomes increased with Ni exposure, showing that trichomes of this species are capable of accumulating high levels of Ni.

Seasonality has also been shown to affect where hyperaccumulators sequester metals (Bidar *et al*., 2008). Galeas *et al*. (2006) observed that Se was transported from roots to young leaves of *Stanleya pinnata* in the spring, from old leaves to flowers in the summer, and back to the roots in the fall. Such seasonal patterns may complicate phytoremediation efforts, especially if a phytoremediating plant is harvested during a season when metals are not present in above-ground biomass.

GENETICS OF METAL TOLERANCE

Hyperaccumulation often results from the overexpression of genes which code for specialized protein transporters and chelators (Chaffai and Koyama, 2011; Rascio and Navari-Izzo, 2011; Maestri *et al*., 2010; Verbruggen *et al*., 2009; Jabeen *et al*., 2009; Cobbett and Goldsbrough, 2002; Figure 2b, c). Below we describe what is known about the genetic basis of metal transporters and chelators from studies conducted on species of the Brassicaceae. Genes currently known to code for metal transporters and chelators in the Brassicaceae are listed in Table 2.

Table 2. The metal transporter genes characterized from Brassicaceae species

Species	Gene	Metal Transported	Reference
Arabidopsis halleri	hma4	Cd	Courbot *et al*. 2007
	nas2, nas3	Zn	Talke *et al*. 2006
A. thaliana	zip1-12	Zn	Weber *et al*., 2004; Roosens *et al*., (2008a,b)
	irt1	Fe	Kerkeb *et al*., 2008
	mtp1	Zn	Kawachi *et al*., 2008
	hma3	Co, Zn, Cd, Pb	Morel *et al*., 2008
	copt1	Cu	Sancenon *et al*., 2004; Andres-Colas *et al*., 2010
	yls2	Fe, Cu	DiDonato *et al*., 2004
Noccaea caerulescens (as *Thlaspi caerulescens*)	znt1-2	Zn	van de Mortel *et al*., 2006
	irt1-2	Fe	Schikora *et al*., 2006; Plaza *et al*., 2007
	ysl3	Fe, Ni	Gendre *et al*. 2006
N. cochleariforme (as *Thlaspi japonicum*)	nramp4	Fe	Mizuno *et al*., 2005
N. goesingense (as *Thlaspi goesingense*)	mtp1	Zn, Ni	Kim *et al*., 2004

Transporters

The ZIP protein transporter family was one of the first metal transporter groups identified in plants. ZIP transporters take up cations, particularly Zn and Fe, in different plant species including *Arabidopsis* (Rascio and Navari-Izzo, 2011). In *A. thaliana*, 15 genes have been documented to code for transporters of various metals (Chaffai and Koyama, 2011). Interestingly, non-hyperaccumulating *Arabidopsis* only expressed ZIP transporters when deficient in Zn, whereas hyperaccumulating *Arabidopsis* expressed ZIP transporters independent of Zn levels (Verbruggen *et al*., 2009). This suggests that ZIP transporters are constitutively expressed in the hyperaccumulator and not in the non-hyperaccumulator. Similarly, both hyperaccumulating *Noccaea caerulescens* and metal-excluding *Noccaea arvense* (as *Thlaspi caerulescens* and *T. arvense*) have a ZIP protein with similar affinities for Zn. Because the affinities for Zn do not differ, this protein is likely expressed at higher rates in hyperaccumulating *N. caerulescens* than in *N. arvense*, resulting in a greater number of membrane proteins that transport Zn (Lasat and Kochian, 2000).

ATPase protein transporters use ATP to transport cations within and between cells, especially between root and shoot cells. In hyperaccumulating *A. halleri*, HMA4 and HMA5 transporters move metal ions from root to xylem cells, increasing tolerance of metals in the roots (Chaffai and Koyama, 2011). When AtHMA4 in *A. thaliana* was artificially overexpressed, higher levels of Zn and Cd were translocated from the root to the shoot (Verret *et al*., 2004). In *A. halleri*, the HMA4 gene is consistently overexpressed, accounting for the greater Cd-tolerance of *A. halleri* (Hanikenne *et al*. 2008). In contrast with HMA4 and

HMA5, which are predominantly expressed in the roots, HMA3 transporters reside in the vacuolar tonoplast and AtHMA3 regulates Zn levels in the vacuole in shoots of *A. thaliana* (Gravot *et al*., 2004).

Similar to ATPase proteins, COPT proteins transport Cu^{2+} within and between cells. Because COPT proteins deal exclusively with Cu^{2+}, they have a higher specificity than some ZIP family transporters. Non-hyperaccumulating *Arabidopsis thaliana* has five genes that encode COPT proteins. Over-expression of COPT1 proteins in *Arabidopsis* appears to increase Cu tolerance in the roots (Kobayashi *et al*., 2008; Puig *et al*., 2007).

The Nramp family of transporter proteins contains at least seven members, five of which have been characterized. *Arabidopsis thaliana* encodes six Nramp-like proteins located in different parts of the cell which regulate metal homeostasis. AtNRAMP3 is located in the tonoplast and is responsible for transporting Fe, Cd, Mn, and Zn between the vacuole and the cytosol (Chaffai and Koyama, 2011; Thomine *et al*., 2003). AtNRAMP3 also appears to be controlled by the presence of Fe. In Fe-sufficient conditions, over-expressing or disrupting AtNRAMP3 does not change the metal content of the cell. However, in Fe-starved conditions, overexpressing AtNRAMP3 decreases overall plant Zn and Mn concentrations, while disrupting AtNRAMP3 invokes the opposite response (Thomine *et al*., 2003). This suggests that AtNRAMP3's function is tightly linked with Fe and together they regulate concentrations of Mn and Zn within the cell. AtNRAMP4 and AtNRAMP6 proteins transport ions within the cell, with AtNRAMP4 responsible for transporting Fe, Mn, Cd, and Zn and AtNRAMP6 responsible for transporting Cd and regulating the distribution and availability of Fe and Mn.

Embedded primarily in tonoplast and cell plasma membranes, CDF and CAX transporter proteins regulate the concentration of metal ions in the cytoplasm of hyperaccumulators. Divided into Mn^{2+}, Zn^{2+}, and Fe^{2+}/Zn^{2+} groups, CDF transporters pump H^+ or K^+ either outside of the cell or into the vacuole, regulating the concentration of heavy metals in the cytoplasm (Chao and Fu, 2004). Similarly, CAX transporters pump H^+ or Na^+ outside of the cell or into the vacuole, regulating the concentration of heavy metals in the cytoplasm (Hall and Williams, 2003).

Chelators

Cytosine-rich and with a low molecular weight, metallothionein chelators form mercaptide bonds with a range of metals (Maestri *et al*., 2010). Metallothioneins comprise four subfamilies: MT1, MT2, MT3, and MT4. The expression of each group varies between hyperaccumulators and non-hyperaccumulaters. When non-hyperaccumulators such as *A. thaliana* are exposed to Cd, Cu, or Zn, they express MT1a and MT1b at high levels in the roots, whereas in hyperaccumulators these genes are expressed at higher levels in the leaves (Maestri *et al*., 2010). Overexpression of MT2 is associated with Cu-tolerance in *A. halleri* and *N. caerulescens*. In *N. caerulescens* metallothionein MT3 is also associated with Cu-tolerance; however, this transporter is expressed only when Cd is present. MT4 chelators have been shown to maintain Cu homeostasis in the seeds of *A. thaliana* (Maestri *et al*., 2010).

Like metallothioneins, phytochelatins (PCs) detoxify heavy metals in a number of species, binding with metals in the cytosol of the roots and leaves to form stable heavy metal complexes that are deposited in the vacuoles (Cobbett, 2000). These cytesine-rich phytochelatins are synthesized by PC synthase, an enzyme that binds to protein substrate glutathione (GSH). Several studies confirm that *Arabidopsis* mutants deficient in GSH are also deficient in PC synthase (Lee *et al*., 2003). In *A. thaliana*, the AtPCS1 and CAD1 genes encode PC synthase, and CAD2 encodes GSH (Cobbett and Goldsbrough, 2002). PCs have been shown to play a major role in Cd detoxification, as mutant lines of *A. thaliana* with defective PC synthase are intolerant of Cd. PC production can be induced in plants and cultured cells exposed to metal ions, particularly Cd. To increase the level of these metal-binding peptides and enhance heavy metal tolerance, PC synthase genes from *A. thaliana* (Ha *et al*., 1999; Vatamaniuk *et al*., 1999) and *Brassica juncea* (Heiss *et al*., 2003) have been inserted and overexpressed in a number of plant species (Lee *et al*., 2011).

ECOLOGICAL IMPLICATIONS

Hypotheses for Metal Hyperaccumulation

Many authors have hypothesized about the reasons for metal hyperaccumulation in plants (Boyd, 2010). However, only a few of these hypotheses have been tested experimentally (Boyd and Martens, 1998; Boyd, 2007). Some of the reasons hypothesized for metal-hyperaccumulation include the ability to tolerate the metal and dispose it from the plant body (Baker, 1981), drought resistance (Baker and Walker, 1989), elemental allelopathy (Boyd and Jaffré, 2001), inadvertent uptake (Cole, 1973), and the most widely tested, pathogen/herbivore defense (Reeves *et al*., 1981). Species of Brassicaceae have often been utilized to shed light on these various hypotheses.

In the *metal tolerance hypothesis*, it is believed that plants sequester metals in their cell walls and vacuoles to avoid toxicity, keeping metals away from metabolically active sites in the cell (Kruckeberg and Reeves, 1995). The *disposal hypothesis* suggests that plants either store metals in tissues which are about to be shed by the plant or in the epidermal cells of the leaf where the metals may be washed out by rainfall (Farago and Cole, 1988). Similarly, the *elemental allelopathy hypothesis* suggests that hyperaccumulators shed metal-laden leaves to increase the metal concentration of the soil (Boyd and Jaffré, 2001), thus keeping metal-intolerant competitors at bay. The *drought resistance hypothesis* (Baker and Walker, 1989) suggests that plants may use metals to prevent drought by increasing the concentration of ions within the roots, thus creating negative water potential which draws water into the plant. In the *Mimulus guttatus* complex (Phrymaceae), drought tolerance also appears to provide tolerance to metal-enriched serpentine soils (Hughes *et al*., 2001). The *inadvertent uptake hypothesis* assumes that hyperaccumulation is a by-product of another adaptive function (Boyd and Martens, 1992). The most experimentally tested and commonly accepted hypothesis for metal hyperaccumulation is the *elemental defense hypothesis*, suggesting that metal sequestration in the leaf tissue defends plants against insect herbivory and infection by pathogens (Strauss and Boyd, 2011; Boyd and Martens, 1998).

Martens and Boyd (2002) tested the effects of herbivory on the Ni-hyperaccumulating *S. polygaloides* and found that elevated levels of Ni did not always prevent herbivore attack. They suggested, instead, that Ni-hyperaccumulation may only defend plants against some herbivores and that herbivores can overcome these plant defenses through the evolution of metal-tolerance or detoxification mechanisms. These findings are similar to those of Wall and

Boyd (2002) who examined the arthropods associated with the flora of an ultramafic site in the Red Hills of California. They found elevated levels of Ni in arthropods of serpentine sites relative to levels of Ni in arthropods of non-serpentine sites and discovered the insect *M. boydi* to be monophagous on *S. polygaloides*. Boyd defined this insect as a high-Ni insect (Boyd, 2009), accumulating Ni at levels up to 777 µg g^{-1} (the minimum requirement for a high-Ni insect is 500 µg Ni g^{-1} dry tissue). Whereas the *elemental defense hypothesis* suggests that *S. polygaloides* may hyperaccumulate Ni to deter herbivores, *M. boydi* may have specialized on *S. polygaloides* to potentially deter predators.

To date, Ni is the most explored element in terms of the *elemental defense hypothesis*, with most studies focusing on Ni-hyperaccumulators (Strauss and Boyd, 2011). Boyd (2007), in a recent review of the elemental defense hypothesis, calls for further experiments with a wider array of metals (see Barillas *et al*., 2011 for studies on Se). Boyd (2004) noted that elemental defense may also occur in plants which accumulate metals below the threshold for hyperaccumulation and that studies should be directed at plant-biota interactions even in plants accumulating metals below the threshold necessary to be considered hyperaccumulation (Van der Ent *et al*., 2012; Krämer 2010).

Transfer of Metals into the Food Chain

Regardless of the reason for metal hyperaccumulation in plants, we must consider the implications of this trait in the environment around such plants. In particular, herbivores that feed on metal accumulating plants may transfer these metals into the food web, with potential for metals to bioaccumulate in higher trophic levels (Boyd, 2009; Cai *et al*., 2009; Boyd, 2004; Peterson *et al*., 2003; Wall and Boyd, 2002). Peterson *et al*. (2003) surveyed the arthropods of a serpentine outcrop in Portugal, where the Ni-hyperaccumulator *Alyssum pintodasilvae* is present, and found that Ni was being mobilized into the food chain. Similarly, Wall and Boyd (2002) found elevated levels of Ni in arthropods collected from a serpentine outcrop in the Red Hill formation of California, with the greatest concentrations of Ni being found in insects associated with the Ni-hyperaccumulator *S. polygaloides*. Outridge and Scheuhammer (1993) showed that vertebrates can be negatively affected if they consume >500 µg g^{-1} of Ni, suggesting that a diet rich in metal-laden insects may be harmful to birds and other animals.

Galeas *et al*. (2007) assessed arthropod abundance and diversity over two growing seasons in Se-enriched habitats in Colorado, comparing Se-hyperaccumulator species (including *S. pinnata*) with non-hyperaccumulator species. The Se-hyperaccumulators, with Se at 1,000 to 14,000 μg $g^{–1}$ dry weight, harbored significantly fewer arthropods (approximately two-fold lower) and fewer arthropod species (approximately 1.5-fold lower) compared with non-hyperaccumulator species which contained Se at <30 μg $g^{–1}$. Arthropods collected on Se-hyperaccumulating plants contained three- to ten-fold higher concentrations of Se than arthropods found on non-hyperaccumulating species but less than ten-fold lower concentrations of Se than their hyperaccumulator host plants. Several arthropod species contained Se at >100 μg $g^{–1}$, indicating a relatively great tolerance to Se.

Some animals avoid metal-enriched tissue, lowering the chance that metals may enter the food chain (Boyd, 2007). Isopods fed with leaf litter from the hyperaccumulator *A. pintodasilvae* showed 83% mortality compared with isopods that were fed with leaf litter from non-hyperaccumulatoring plants. When given a choice in diet, the isopods preferred leaf litter from non-hyperaccumulating plants (Goncalves *et al*., 2007). This finding supports the *elemental defense hypothesis* which suggests that certain animals may avoid eating metal-enriched plant tissue. Furthermore, a generalist diet in herbivorous and predacious insects may dilute any metals that the insect may have consumed from hyperaccumulating plants (Boyd, 2009). Further discussion of herbivory and other cross-kingdom interactions in metal-enriched environments can be found in Strauss and Boyd (2011).

CHALLENGES OF PHYTOREMEDIATION

Although phytoremediation using metal hyperaccumulators may be a low-cost, eco-friendly alternative to traditional cleanup methods, this technology is not without its limitations (Pilon-Smits, 2005). Firstly, hyperaccumulators must be able to grow on a contaminated site; the soil properties, concentration of metals, and climate cannot inhibit plant growth. Secondly, many naturally-occurring hyperaccumulators are small, with shallow root systems and minimal above-ground biomass, limiting the depth and amount of land that can be cleaned using a given plant. Thirdly, some metals may not be bioavailable in the soil and the soil may need to be chemically treated to make the metal bioavailable before introducing a hyperaccumulator for clean-up. Fourthly, most hyperaccumulators are specific for one metal only and many sites are contaminated with multiple metals. As discussed above, there is a risk that herbivores may transfer metals into the food chain. This risk, therefore, should be assessed on a site-by-site basis before implementing a phytoremediation effort (Neilson and Rajakaruna, 2012). Furthermore, the public may be concerned that non-native, fast-growing hyperaccumulators may escape from remediation sites and become invasive (Whiting *et al*., 2004). As in a recent case in the town of O’Brien, Oregon, USA, *Alyssum murale* and *A. corsicum*—species from Mediterranean Europe—appear to have naturalized and become invasive in nearby serpentine outcrops, potentially threatening native plants: (http://www.oregon.gov/ODA/PLANT/WEEDS/edrr.shtml). Thus, it is vital to understand the biology and ecology of the plants to be used, in as much detail as possible, before undertaking field-based phytoremediation or phytomining operations using non-native species.

Given that many of the metal-hyperaccumulating species in the Brassicaceae are small-statured, there is an interest in genetically modifying hyperaccumulators to have greater biomass, deeper root systems, or an enhanced ability to uptake metals (Bhargava *et al*., 2012; Cherian and Oliveira, 2005; Doty *et al*., 2008; Pilon-Smits and Pilon, 2002; Rugh, 2004). A number of concerns have been raised regarding these designer hyperaccumulators (Ellstrand, 2001; Pilon-Smits and Freeman, 2006; Angle and Linacre, 2005; Eapen and D’Souza, 2005). As with naturally occurring hyperaccumulators, genetically modified (GM) hyperaccumulators may escape from their remediation sites and become invasive in other habitats (Pilon-Smits, 2005). Furthermore, pollen from GM and non-GM hyperaccumulators may be transferred off-site via wind or insects, landing on wild and agronomic relatives with the potential for metal-tolerant genes to become fixed in close relatives (Whiting *et al*., 2004). Given that many hyperaccumulators and crop plants belong to the Brassicaceae (Warwick, 2012), this is a serious concern in need of further study (Neilson and Rajakaruna, 2012;

Whiting *et al*., 2004). Species of the Brassicaceae that have been genetically modified to tolerate or hyperaccumulate heavy metals are listed in Table 3.

Table 3. Brassicaceae species that have been genetically modified to increase heavy metal accumulation

Species	Metals Hyperaccumulated	Reference
Arabidopsis spp.	As, Cd, Cu, Hg, Ni, Pb, Se, Zn	Li *et al*., 2005 (As, Hg, Cd); Lee *et al*., 2003 (Cd); Xu *et al*., 2009 (Cu); Bizily *et al*., 2003 (Hg); Pianelli *et al*., 2005 (Ni); Song *et al*., 2003 (Pb, Cd); Leduc *et al*., (2004) (Se); Haydon and Cobbett, 2007 (Zn)
Brassica juncea	As, Cd, Pb, Se, Zn	Wangeline *et al*., 2004 (As); Reisinger *et al*., 2008 (Cd); Zhu *et al*., 1999 (Cd); Bhuiyan *et al*., 2011a,b (Pb); Gleba *et al*., 1999 (Pb); Bañuelos *et al*. (Se), 2005; Bennett *et al*., 2003 (Zn);
Brassica napus	As, Ni, Zn	Stearns *et al*., 2005 (As); Brewer *et al*. (Ni), 1999; Nie *et al*., 2002 (Zn)

One alternative to genetically modifying hyperaccumulators is to continue our search for undiscovered hyperaccumulators. There are potentially many more hyperaccumulators waiting to be discovered on both naturally occurring and anthropogenically created metal-enriched sites worldwide (Whiting *et al*., 2004; Boyd *et al*., 2009). Such sites are undergoing drastic changes due to ever-expanding development, deforestation, mining, exotic species invasions, and atmospheric deposition of various pollutants or previously limiting nutrients such as nitrogen (Williamson and Balkwill, 2006; Rajakaruna and Boyd, 2008; Harrison and Rajakaruna, 2011). Floristic surveys should be encouraged to document metal-tolerant and hyperaccumulating plants which may be at risk of being lost from these under-studied habitats worldwide.

CONCLUSION

The family Brassicaceae is extremely important for phytoremediation of heavy metals worldwide, both in the number of hyperaccumulating species found in this family and the knowledge they have supplied regarding metal tolerance at the molecular, cellular, and whole-plant level. The use of *Arabidopsis thaliana, A. halleri, Brassica juncea*, and *Noccaea caerulescens* as model species has revealed numerous physiological mechanisms contributing to metal uptake and has elucidated the genetics behind these mechanisms. Other species such as *Streptanthus polygaloides*, *Alyssum pintodasilvae*, and *Stanleya pinnata* have provided insight into the ecology of metal-tolerant species, helping assess the potential for transfer of metals into the food chain and to investigate the various hypotheses relating to the causes and consequences of metal hyperaccumulation. Despite extensive research over the last several decades, phytoremediation as a technique for cleaning metal contaminated sites needs further development. To improve this green technology, we must continue to research metal tolerance at the molecular, cellular, organismic, and ecosystem levels, address the risks these plants may pose to surrounding habitat, and actively search for new plant candidates for phytoremediation worldwide. Species from the Brassicaceae will no doubt continue to provide valuable insight on all aspects of metal-plant-ecosystem relationships.

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