

Transfer of heavy metals through terrestrial food webs: a review

Jillian E. Gall · Robert S. Boyd · Nishanta Rajakaruna

Abstract Heavy metals are released into the environment by both anthropogenic and natural sources. Highly reactive and often toxic at low concentrations, they may enter soils and groundwater, bioaccumulate in food webs, and adversely affect biota. Heavy metals also may remain in the environment for years, posing long-term risks to life well after point sources of heavy metal pollution have been removed. In this review, we compile studies of the community-level effects of heavy metal pollution, including heavy metal transfer from soils to plants, microbes, invertebrates, and to both small and large mammals (including humans). Many factors contribute to heavy metal accumulation in animals including behavior, physiology, and diet. Biotic effects of heavy metals are often quite different for essential and non-essential heavy metals, and vary depending on the specific metal involved. They also differ for adapted organisms, including metallophyte plants and heavy

metal-tolerant insects, which occur in naturally high-metal habitats (such as serpentine soils) and have adaptations that allow them to tolerate exposure to relatively high concentrations of some heavy metals. Some metallophyte plants are hyperaccumulators of certain heavy metals and new technologies using them to clean metal-contaminated soil (phytoextraction) may offer economically attractive solutions to some metal pollution challenges. These new technologies provide incentive to catalog and protect the unique biodiversity of habitats that have naturally high levels of heavy metals.

Keywords Ecosystem health · Metal toxicity · Metal hyperaccumulation · Bioaccumulation · Environmental pollution · Phytoremediation

Introduction

Polluting vast areas worldwide, heavy metals are highly reactive and toxic at low concentrations, posing severe risks to human and ecosystem health (Ensley 2000; Sánchez 2008; Wuana and Okieimen 2011). While many heavy metals are naturally present in the Earth's crust and atmosphere, humans may promote heavy metal pollution through activities such as mining, smelting, transportation, military operations, and industrial manufacturing, as well as applying metal-containing pesticides and fertilizers in commercial agriculture. These activities release metals into the environment through waste disposal, runoff, and application of heavy metal-laden chemical products, which then may enter

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terrestrial systems via aerial deposition, surface waters, or soil (Järup 2003; Boyd 2004; Pilon-Smits 2005; Chaffai and Koyama 2011). Unlike their organic pollutant counterparts, heavy metals cannot be degraded. As a result, heavy metals persist in the environment for years, well after point sources of pollution have been removed (Babin-Fenske and Anand 2011).

Categorized into essential and non-essential groups, heavy metals may interact directly with biomolecules, disrupting critical biological processes and resulting in toxicity. Essential heavy metals (i.e., micronutrients), including copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn), are required by organisms in small amounts (Epstein and Bloom 2004; Marschner 2012). Although organisms are generally able to regulate small amounts of essential metals, in excess these metals may become toxic (Kabata-Pendias and Pendias 2001; Chaffai and Koyama 2011). Contrastingly, non-essential metals such as aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are not required for normal biological function and may quickly lead to toxicity (see references within Boyd and Rajakaruna 2013). Both classes of metals may accumulate in tissues with potential to transfer to higher trophic levels (Peterson et al. 2003; Gall and Rajakaruna 2013; Neilson and Rajakaruna 2014; Bouriou et al. 2015).

While many studies of heavy metals focus on metal accumulation in individual taxa (e.g., Smith and Rongstad 1982; Mesjasz-Przybylowicz and Przybylowicz 2001; Miranda et al. 2009; Kupper and Leitenmaier 2011), fewer studies examine community-level effects (Peterson et al. 2003; Boyd et al. 2006; Bouriou et al. 2015) of heavy metals, including their ability to transfer through a food chain (Goodyear and McNeill 1999; Gray 2002; Langdon et al. 2003; Mann et al. 2011) and bioaccumulate in higher trophic levels. In this review, we discuss the literature on heavy metal transfer in terrestrial systems at each stage of a food web, including sources of heavy metals in the environment. We begin with studies of metal transfer from soils to microbes and producers (i.e., plants) and then discuss metal uptake and transfer in invertebrates, small animals, and large mammals (Fig. 1). Finally, we assess the risk of metal accumulation in humans. We also discuss some unique players along the way: plants, animals, and microorganisms that evade toxic effects because they have adaptations that allow them to exclude, tolerate, or even accumulate high concentrations of heavy metals in their tissues. Among such

physiologically distinct taxa, metal-tolerant and metal-accumulating plants (i.e., metallophytes) and microbes have attracted much attention, providing insights into adaptation (Yang et al. 2005; Haferburg and Kothe 2007; Gadd 2010) and speciation (Ernst 2006; O'Dell and Rajakaruna 2011) as well as leading to the development of certain green technologies, including phytoremediation and phytomining (Chaney et al. 2007; Wenzel 2009; Meier et al. 2012).

Microbes

Soil microbes have geoactive roles in the biosphere and are responsible for element biotransformations, biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and the formation of soil and sediments (Brussaard 1997; Haferburg and Kothe 2007; Gadd 2010). Among these important roles, microbes mineralize biocompounds, especially biopolymers like lignocellulose and chitin, by decomposing them (Haferburg and Kothe 2007). These microbial roles in biogeochemical processes and elemental cycles are summarized by Gadd (2010).

Although microbes have such important roles in the soil, the presence of heavy metals in soils can negatively affect microbe-mediated soil processes (Lee et al. 2002). Some heavy metals, such as Cu, Co, Fe, Mg, Mn, Ni, and Zn, are essential micronutrients for microbes because they are involved in regulating osmotic pressure, play a role in redox processes that stabilize molecules through electrostatic interactions, and are key components of certain enzymes (Gadd 2010; Boshoff et al. 2014). While microbes can tolerate larger quantities of these essential heavy metals, in excess both essential and non-essential heavy metals (e.g., Al, As, Cd, Hg, Pb) can adversely affect microbial communities (Gadd 2010). In general, heavy metals reduce the amount of soil microbial biomass (SMB) and lower enzyme activity (Gadd 1990; Chander et al. 1995; Giller et al. 1998), which, in turn, decrease diversity in soil ecosystems and change microbial structure (Bååth 1989; Giller et al. 1998; Pennanen et al. 1998; Giller et al. 2009). Furthermore, when microbes are adversely affected, soil organic matter decomposes more slowly and soil respiration decreases (Giller et al. 2009). Such toxic effects may occur from natural geochemical events, but are more often associated with anthropogenic metal contamination (Gadd 2010). However, these toxic effects

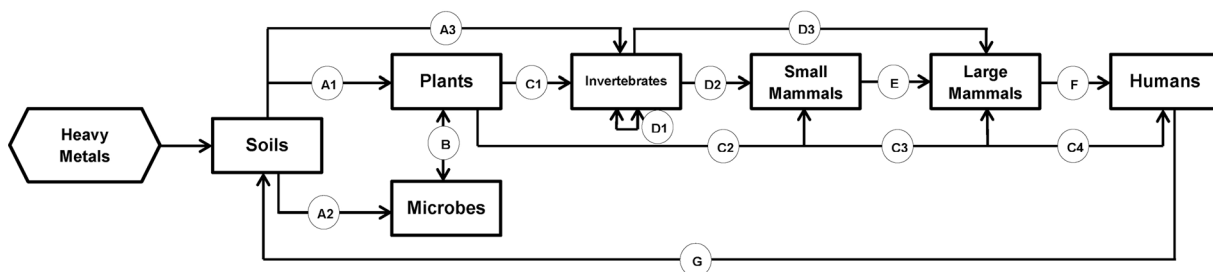


Fig. 1 Pathways of metal transfer in the terrestrial food web and select references for each pathway (A1–G). (A1) Gall and Rajakaruna (2013); Van der Ent et al. (2013); Pollard et al. (2014); (A2) Giller et al. (2009); Gadd (2010); Boshoff et al. (2014); (A3) Heikens et al. (2001); Hobbelen et al. (2006); (B) Wenzel (2009); Hol et al. (2010); Kothe and Büchel (2014); (C1) Janssens et al. (2009); Migula et al. (2011); Nica et al. (2012); Meindl and Ashman (2013); Bouriouq et al. (2015); (C2) Lopes et al (2002); Beernaert et al. (2007); Sánchez-Chardi et al. (2007a);

(C3) Reglero et al. (2009); Phillips and Tudoreanu (2011); Roggeman et al. (2013); (C4) Cao et al. (2010); Sahoo and Kim (2013); Street (2012); (D1) Peterson et al. (2003); Green et al. (2010); Cheruiyot et al. (2013); (D2) Sánchez-Chardi et al. (2007b); Moriarty et al. (2012); Drouhot et al. (2014); (D3) Reglero et al. (2009); (F) Chary et al. (2008); (G) Luo et al. (2009); Atafar et al. (2010); McClellan and Halden (2010); Jiao et al. (2012)

may be ameliorated by applying organic matter or other amendments to soils, thereby increasing soil pH and decreasing the mobility of metals (Pérez-de-Mora et al. 2006; Friesl-Hanl et al. 2009).

Both living and dead microbial biomass is capable of accumulating metals (Gadd 1990) and metal accumulation in microbes is often determined by two main characteristics of the cell: the sorptivity of the cell envelope and the capacity for taking up metals into the cytosol (Haferburg and Kothe 2007). Of the microbes, bacteria are usually the most numerous organisms in the soil and have a high surface-area-to-volume ratio. This high ratio allows not only for efficient uptake of nutrients and release of metabolic wastes, but also provides a large area for heavy metals to interact with microbial cell walls. Furthermore, microbe cell envelopes have a net negative charge, making microbes more prone to accumulate metal cations from the surrounding environment, and microbe transporters cannot differentiate between essential trace elements and toxic heavy metals (Haferburg and Kothe 2007; Gadd 2010).

Despite the negative effects of metals on microbes and the general microbe affinity for heavy metals, microbes utilize different intra- and extra-cellular mechanisms to limit metal toxicity (Gadd 1990; Haferburg and Kothe 2007; Gadd 2010; Haferburg and Kothe 2010). Most of these microbial survival mechanisms rely on changing the metal speciation (i.e., redox state), thereby decreasing or increasing the mobility of a particular metal (Gadd 2010). In one strategy, microbes stop metals from even entering a cell by releasing metal-binding compounds such as oxalates outside the cell to

chelate metals into a form that cannot be absorbed. However, other microbes (like the fungi *Aspergillus niger* and *Penicillium* spp.) actually solubilize metals (rather than immobilize them) by releasing organic acids into the soil; these fungi have been used to leach metals from a variety of materials (Brandl 2001; Santhiya and Ting 2005). In the soil, these metals would then be available for plant uptake. The structure of microbial cell walls and membranes themselves also causes metals to bind to the external surface, preventing them from entering the cell (Gadd 1990; Goyal et al. 2003; Haferburg and Kothe 2007). If metals do pass through the microbial cell wall, microbes provide efflux transporters that excrete toxic or over-concentrated metals from the cell into the outside environment (e.g., *Cupriavidus metallidurans*), thereby keeping levels of metals in the cytosol low. Another strategy to detoxify internalized metals is to sequester them from the cytosol into stable internal inclusion bodies such as polyphosphate granules. Once sequestered, these metal bodies provide no harm to the microbe (Haferburg and Kothe 2007).

In some cases, the presence of heavy metals has led to metal-tolerant microbial populations that exhibit the resistance strategies above and grow and flourish in metal-laden environments (Schmidt and Schlegel 1989; Ellis et al. 2003). In fact, microbial resistance to metals is common, with frequencies ranging from a few percent in pristine environments to nearly 100 % in heavily polluted environments (Silver and Phung 2009). Even small amounts of metal contamination can induce metal resistance in microbes (Witter et al.

2000). These metal-tolerant microbes can influence metal speciation, solubility, bioavailability, toxicity, and mobility, as well as mineral formation, dissolution, and deterioration (Gadd 2010). In metal-accumulating Indian mustard (*Brassica juncea*; Brassicaceae), heavy metal-tolerant microbes in the rhizosphere stimulate root elongation and could potentially be used to improve the growth of metal-accumulating plants for phytoremediation and phytostabilization (Belimov et al. 2005). Thus, microbes have an intimate relationship with plants, and plant-microbe pairs should be considered when bioremediating metal-polluted sites (Idris et al. 2004; Brandl and Faramarzi 2006; Wenzel 2009; Kothe and Büchel 2014).

Although these metal-resistant microbes may become dominant in metal-laden soils, they may not perform the same ecological functions as other metal-sensitive species (Boshoff et al. 2014). Changes in soil microbial communities may cause chain-reaction effects throughout the soil ecosystem, interrupting regular soil processes and reducing overall soil productivity (Gadd 2010; Boshoff et al. 2014). When heavy metals slow microbial decomposition of organic matter, elements turn over less rapidly (reducing nutrient pools), soils are less porous (causing poor drainage), and restricted amounts of nutrients are available to plants (Boshoff et al. 2014). Other soil organisms, such as micro-, meso-, and macro-fauna, are also affected by heavy metals, exhibiting decreased feeding activity, abundance, and diversity (Vargha et al. 2002; Kools et al. 2009; Boshoff et al. 2014). Furthermore, the disappearance of certain rare microbes with heavy metal exposure can even affect trade-offs in plant-herbivore interactions. For example, Hol et al. (2010) reported that, in soils where rare microbe species were reduced, crop plant tissues of *Brassica oleracea* (Brassicaceae) and *Beta vulgaris* (Amaranthaceae) had higher nutritional content and biomass and this made them more susceptible to root-feeding nematodes and shoot-feeding aphids. Alternatively, when rare microbes were present, the crop nitrogen, sugar, and amino acids were lower, but plant defense compounds were higher (Hol et al. 2010). Thus, the presence of heavy metals in soils can change microbial communities in ways that have repercussions throughout the soil ecosystem.

Soils to Plants

Metals may enter soils through several pathways, including runoff from roads and industrial sites, metallo-

pesticides, phosphate fertilizers, application of treated and untreated sewer sludge, and atmospheric deposition of metal-containing particles (Scott et al. 2004; Nziguheba and Smolders 2008; Luo et al. 2009; Chaffai and Koyama 2011; Efremova and Izosimova 2012). However, the risk of metals entering a food chain depends on the mobility of the metal and its availability in the soil. In the soil, metal cations are bound to negatively charged particles such as clay and organic matter (Rajakaruna and Boyd 2008). When metals detach from these soil particles and enter the soil solution, they become bioavailable with potential to accumulate in plants and other soil-dwelling organisms (Neilson and Rajakaruna 2012). Several studies reveal the effects of various physical, chemical, and biological processes on the bioavailability of metals in soils (Jabeen et al. 2009; Peralta-Videa et al. 2009; Maestri et al. 2010). In particular, Peralta-Videa et al. (2009) discuss the mobility of Cd, Cr, Hg, As, and Pb in soils and their potential for uptake by plants, a process that is also mediated by rhizospheric microbial activity (Fitz and Wenzel 2002; Wenzel 2009). At low pH, certain metals, such as Cd, Cu, Hg, and Pb, become more available for plant uptake (Blaylock and Huang 2000). Soil bacteria, including those in the rhizosphere, also may release compounds into soil, such as antibiotics, antifungals, organic acids, hormones, and metal chelators, which may increase or decrease metal bioavailability to other organisms (Xiong et al. 2008; Fitz and Wenzel 2002; Wenzel 2009). Many studies stress the importance of bioavailability assays of metals when assessing the risk of metal accumulation and transfer in terrestrial food webs (e.g., Gupta et al. 1996; Dai et al. 2004; Hobbelen et al. 2006), but the effectiveness of these bioavailability assay methods are debated (Menzies et al. 2007).

Although some heavy metals, such as Ni, Cu, Fe, Mn, and Zn, are important to particular biological processes in plants (Epstein and Bloom 2004), when they occur in excess these metals can disrupt critical physiological processes and result in toxicity. Thus, most plants exclude excess bioavailable metals at their roots by binding them to organic acids or ligands or storing them within vacuoles where they cannot interfere with important physiological processes (Hossain et al. 2012; Gall and Rajakaruna 2013). However, not all plants exclude heavy metals: certain plants can tolerate and accumulate bioavailable metals into aboveground tissues (Unterbrunner et al. 2007; Utmazian et al. 2007; Gall and Rajakaruna 2013). The amount of a heavy metal that enters a plant

from its substrate is expressed by the Transfer Factor (TF), also known as the *bioaccumulation factor* (Ghosh and Singh 2005; Bitterli et al. 2010):

$$TF = \frac{C_{plant}}{C_{soil}}$$

where C_{plant} is the concentration of a heavy metal in a plant and C_{soil} is the concentration of that heavy metal in the soil. Metal hyperaccumulators are plants with an unusually high ability for metal uptake (Wenzel and Jockwer 1999; van der Ent et al. 2013; Pollard et al. 2014). Hyperaccumulators of Cd, selenium (Se), or thallium (Tl) accumulate $>100 \mu\text{g g}^{-1}$ in dry leaf tissue, hyperaccumulators of cobalt (Co), Cr, or Cu accumulate $>300 \mu\text{g g}^{-1}$, and hyperaccumulators of Pb, As, antimony (Sb), or Ni accumulate $>1,000 \mu\text{g g}^{-1}$. Hyperaccumulators of Zn accumulate $>3,000 \mu\text{g g}^{-1}$ while those of Mn accumulate $>10,000 \mu\text{g g}^{-1}$ (van der Ent et al. 2013). For recent reviews of metal hyperaccumulation, see Krämer (2010), van der Ent et al. (2013), and Pollard et al. (2014).

Many authors have hypothesized reasons for metal hyperaccumulation in plants, but few of the hypotheses have been experimentally tested (Boyd and Martens 1998; Boyd 2007, 2010). As recently reviewed by Boyd (2014), these reasons include: drought resistance (Baker and Walker 1989), elemental allelopathy (Boyd and Jaffré 2001), inadvertent uptake (Cole 1973), metal tolerance and/or disposal from a plant (Baker 1981) and, the most widely tested, pathogen/herbivore defense (Reeves et al. 1981). This last hypothesis, termed the *elemental defense hypothesis* (Boyd 2012), suggests that metal accumulation in plant tissue defends a plant against insect herbivory and/or infection by pathogens (Boyd and Martens 1998; Strauss and Boyd 2011). Regardless of the reason for metal accumulation by plants, herbivorous and detritivorous invertebrates that feed on these plants or their litter may be exposed to heavy metals, potentially resulting in toxicity. In the sections below we discuss the transfer of metals into both primary and secondary consumer invertebrates, small mammals, and large mammals (including humans).

Invertebrates

Invertebrates may inadvertently ingest metals contained in soils and plants or, when in direct contact with metals, may absorb them through their exoskeletons or other

body coverings (Heikens et al. 2001; Hobbelen et al. 2006). Such metal accumulation has been well studied in certain taxa, including mollusks (Notten et al. 2008; Nica et al. 2012; Bourioug et al. 2015), earthworms (Heikens et al. 2001; Hobbelen et al. 2006), orthopterans (Devkota and Schmidt 2000), coleopterans (Migula et al. 2011), and ants (Grzes 2010). However, some taxa are more likely to accumulate metals than others, especially if they are in direct contact with metaliferous soils (e.g., soil-dwelling invertebrates) or have a diet composed of metal-rich food such as plants, detritus, or animals containing high metal concentrations. For example, detritivorous Isopoda that live in metal-contaminated leaf litter and feed on organic matter often have higher levels of metals than other taxa, such as Coleoptera, which have less-intensive contact with metals (Heikens et al. 2001). Heikens et al. (2001) compiled studies of Cd, Cu, Pb, and Zn accumulation in invertebrates from 1993 to 1998, reporting that all metals were highest in Isopoda, intermediate in Lumbricidae, and low in Coleoptera, reflecting the habitat and diets of members of these taxa. Some taxa had higher levels of a specific metal: Cu and Zn were just as high in Diplopoda as in Isopoda, and Cd was high in Formicidae and Lumbricidae, whereas Cu was high in Diplopoda and Collembola. These results suggest that due to their habitat, diets, and physiological responses, certain invertebrate orders and families may be more likely to accumulate one metal over another.

Even when in direct contact with metals, behavioral responses may limit metal accumulation in invertebrate tissues. Several studies show that, after an initial taste of metal-rich plant material, herbivorous invertebrates develop an aversion and decrease ingestion rates (Behmer et al. 2005; Notten et al. 2006; Scheirs et al. 2006b; Rathinasabapathi et al. 2007; Mathews et al. 2009). Laboratory studies by Rathinasabapathi et al. (2007) of As-hyperaccumulating Chinese Brake Fern (*Pteris vittata*, Pteridaceae) showed that high levels of As deterred both natural pest Caribbean black scale insect, *Saissetia neglecta* (Mathews et al. 2009), and a generalist herbivore grasshopper, *Schistocerca americana* (Orthoptera, Acrididae). However, the concentrations of metals that induced aversion differed for each invertebrate. While 15–30 mg/kg As was enough to deter scale insect herbivory (with some fallen *S. neglecta* containing 194 mg/kg), the larger *S. americana* was deterred by 46 mg/kg As. Similar concentration-dependent responses also have been observed for other

heavy metals. Plants of *Holcus lanatus* (Poaceae) treated with Cd deterred the grass miner, *Chromatomyia milii* (Diptera, Agromyzidae), collected from non-polluted sites, in a concentration-dependent way (Scheirs et al. 2006b). Similarly, Zn-hyperaccumulating *Thlaspi caerulescens* (now *Noccaea caerulescens*, Brassicaceae) deterred herbivory by desert locusts (*Schistocera gregaria*, Orthoptera, Acrididae) at Zn concentrations as low as 0.5–5 mg/g, an order of magnitude less than commonly found in *T. caerulescens* foliage (Behmer et al. 2005). However, as with other herbivores, the desert locust was undeterred by Zn on first contact: it developed an aversion to Zn from post-ingestive feedback with associative learning (Behmer et al. 2005). Thus, even when metals are bioavailable in the soil and accumulate in plants, metal transfer to invertebrates depends largely on the behavioral responses of the invertebrates involved.

Some insects may avoid contaminated sites, especially during oviposition. Females of *Drosophila melanogaster* (Diptera, Drosophilidae), *Plutella xylostella* (Lepidoptera, Plutellidae), *Pieris rapae* (Lepidoptera, Pieridae), and the land snail *Cepaea nemoralis* (Helicidae) avoided ovipositing on metal-rich plant material (Freeman et al. 2006; Notten et al. 2006; Bahadorani and Hilliker 2009). Alternately, some insects do not distinguish between high- or low-metal plants when ovipositing (e.g., Martens and Boyd 1994), potentially decreasing the fitness of their offspring. The adult ground beetle, *Pterostichus cupreus* (Carabidae), suffered impaired locomotion when exposed to 50 mg/g Cu as larvae (Bayley et al. 1995). For a recent review of the impacts of heavy metals on insect behavior, refer to Mogren and Trumble (2010).

Studies of unadapted insects feeding on hyperaccumulator plants generally support the *elemental defense hypothesis* mentioned in the previous section, showing that hyperaccumulators are relatively protected from herbivory. However, as with other herbivore defenses, elemental defenses are subject to herbivore counterdefenses that can allow herbivores to circumvent metal toxicity (Boyd 2009). For example, some insects can tolerate heavy metals in plant foliage and some are monophagous on metal-rich plants. These invertebrates, in particular, are likely to accumulate metals in their tissues, with potential to pass those metals on to higher trophic levels. Martens and Boyd (2002) studied herbivory on the Ni-hyperaccumulator, *Streptanthus polygaloides* (Brassicaceae), and found that elevated levels of Ni did not always prevent herbivore attack. These findings are similar to those of Wall

and Boyd (2002) who reported high levels of Ni in some arthropods at a Ni-rich site in the Red Hills of California. They also discovered the insect *Melanotrichus boydi* (Hemiptera, Miridae) to be monophagous on the Ni-hyperaccumulator *S. polygaloides* (Wall and Boyd 2006), with the insect accumulating Ni at a mean concentration of 780 $\mu\text{g/g}$ dry mass (the minimum requirement for a high-Ni insect is 500 $\mu\text{g/g}$ Ni; Boyd 2009). The *elemental defense hypothesis* suggests that *M. boydi* may specialize on *S. polygaloides* to potentially deter potential arthropod predators through the defensive effect of its high whole-body Ni concentration. A test of this hypothesis, using three arthropod predator species (two spiders and an insect), showed that the crab spider *Misumena vatia* (Araneae, Thomisidae) suffered significantly higher mortality when fed high-Ni *M. boydi* than when fed low-Ni prey (Boyd and Wall 2001). Another study (Boyd 2002) testing whether the high Ni concentration of *M. boydi* protected it from two entomopathogenic nematodes or an entomopathogenic fungus showed no protective benefit of the Ni. Other studies have documented insects feeding on high-metal plants, including *Chrysolina pardalina* (Coleoptera, Chrysomelidae) and *Epilachna nylanderi* (Coleoptera, Coccinellidae) feeding on Ni-hyperaccumulating *Berkheya coddii* (Asteraceae) in South Africa (Mesjasz-Przybyłowicz and Przybyłowicz 2001; Migula et al. 2011). To our knowledge, no other studies have examined whether other insect herbivores that feed on Ni hyperaccumulators are protected against enemies by high tissue Ni levels in the herbivore's bodies.

When ingested, an excess of metals can have a variety of impacts on invertebrate fitness, including decreased immune response (Sun et al. 2011), extended development time (Behmer et al. 2005), and loss of mass (Behmer et al. 2005; Noret et al. 2007). However, many invertebrates have evolved metal tolerance or detoxification mechanisms (Janssens et al. 2009). Some invertebrates excrete metals in their feces, limiting their metal uptake and therefore decreasing metal transfer into higher trophic levels (Przybyłowicz et al. 2003). Others accumulate metals in their tissues (Migula et al. 2011), including in the hepatopancreas, an organ of the invertebrate digestive system (Nica et al. 2012). The Roman Snail *Helix pomatia* (Helicidae) in the Banat area of Romania accumulates Cu, Zn, and Cd in its hepatopancreas (Nica et al. 2012) and similar accumulation has been observed in terrestrial isopods (Prosi et al. 1983). Invertebrates that ingest metals may

pass them on to predators, especially if their digestive tract contains metals at the time of predation or metals have accumulated in their tissues. Metals can also affect plant-pollinator interactions, and pollinators that visit plants growing on contaminated soils are at risk of ingesting potentially toxic metals. A recent study (Meindl and Ashman 2013) showed that metal-enriched nectar can alter foraging behavior of bumblebees, suggesting that metals—or metalloids like selenium (Se)—found in pollen and nectar (Hladun et al. 2011; Quinn et al. 2011; Meindl et al. 2014) could negatively affect pollinators in contaminated environments.

While there is a wealth of knowledge on plant-invertebrate and soil-invertebrate metal transfer, fewer studies have examined the potential for invertebrate-invertebrate metal transfer. Green et al. (2010) documented the transfer of Cd and Zn from soils amended with sewer sludge through a wheat-aphid-ladybird food chain in the UK. Similarly, a study of the Ni-hyperaccumulator *Alyssum pintodalsilvae* in Portugal suggested that Ni was transferred from grasshoppers and other invertebrates to spiders (Peterson et al. 2003). Boyd and Wall (2001) found similar results suggesting that Ni could be passed from herbivorous to carnivorous insects. A laboratory study by Cheruiyot et al. (2013), using caterpillars of *Spodoptera exigua* (Lepidoptera: Noctuidae) raised on metal-amended artificial diet and then fed to the predator *Podisus maculiventris* (Hemiptera: Pentatomidae), found that Cu and Zn were bioaccumulated whereas Co and Ni were not. These studies show that metals may be transferred between invertebrates; however, more research should focus on such transfers, as metals may continue to pose a risk to invertebrates by moving through food webs well after point sources of metal pollution have been closed (Babin-Fenske and Anand 2010, 2011).

Small Mammals

Small mammals primarily gain metals through ingestion (Beyer et al. 1994), but they also may accumulate them through inhalation, grooming, skin absorption, or placental transfer during pregnancy (Hunter and Johnson 1982; van den Brink et al. 2010). Studies of metal accumulation in small mammals have included raccoons (Procyonidae: Khan et al. 1995; Lord et al. 2002), wood mice, field mice, and yellow-necked mice (Muridae: Roberts and Johnson 1978; Mertens et al. 2001; Damek-Poprawa

and Sawicka-Kapusta 2003; Sánchez-Chardi et al. 2007a; Rogival et al. 2007; van den Brink et al. 2010; Tête et al. 2014), voles (Cricetidae: Roberts and Johnson 1978; Ma et al. 1991; Appleton et al. 2000; Mertens et al. 2001), moles (Talpidae: Komarnicki 2000), and shrews (Soricidae: Ma et al. 1991; Mertens et al. 2001; Hamers et al. 2006; Sánchez-Chardi and Nadal 2007; Sánchez-Chardi et al. 2007b; Moriarty et al. 2012; Drouhot et al. 2014). Most studies examine metal accumulation in various mammalian tissues, such as the stomach, liver, kidneys, lungs, femurs, hair, and teeth (Ma et al. 1991; Appleton et al. 2000; Damek-Poprawa and Sawicka-Kapusta 2003; Beernaert et al. 2007; Sánchez-Chardi et al. 2007b; Drouhot et al. 2014), while others examine whole-body metal concentrations (Veltman et al. 2007).

The extent of metal transfer in mammals depends not only on the degree of exposure, but also on the mammal species, diet, season, sex, age, and the metal being considered (Hunter et al. 1987; van den Brink et al. 2010). Hunter and Johnson 1982 studied Cd and Cu concentrations in insectivorous shrews (*Sorex araneus*; Soricidae), granivorous voles (*Microtus agrestis*; Cricetidae), and herbivorous wood mice (*Apodemus sylvaticus*; Muridae) surrounding a refinery in the UK, reporting that levels of metal accumulation ranked as: shrews > voles > wood mice. The authors attributed this trend to the species' diets: soil-dwelling arthropods are more likely to accumulate metals than plants, thus placing mammals with an omnivorous or carnivorous diet at a higher risk of accumulating metals.

This finding is consistent with other studies of small mammals in The Netherlands (Ma et al. 1991; Hamers et al. 2006; Veltman et al. 2007; Wijnhoven et al. 2007), North Wales (Roberts and Johnson 1978), Spain (Sánchez-Chardi et al. 2007b) and Canada (Smith and Rongstad 1982); these studies report higher levels of metal accumulation in carnivorous or omnivorous mammals than in exclusively herbivorous ones. In the heavily polluted floodplains of Biesboch, The Netherlands, earthworm-eating common shrews (*S. araneus*) were at higher risk of accumulating Cd, Cu, and Pb than primarily herbivorous bank voles (*Myodes glareolus*; Cricetidae); both are abundant and are important prey species for predatory birds (Hamers et al. 2006). Similarly, omnivorous deer mice (*Peromyscus maniculatus*; Cricetidae) were better indicators of Cu, Cd, Pb, and Zn pollution than herbivorous meadow voles (*Microtus pennsylvanicus*) near a Zn/Cu mine near Timmins, Ontario, Canada (Smith and Rongstad 1982).

Animal age and sex can also influence the level of metal accumulation in small mammals, but these trends are species- and metal-specific. For example, in both male and female insectivorous mammals, such as shrews, levels of Pb and Cd increase with age while Cr decreases (Pankakoski et al. 1994; Sánchez-Chardi et al. 2007b; Sánchez-Chardi and Nadal 2007). The authors attribute these trends to differences in metal exposure and physiological responses: for example, to detoxify Cd, shrews created stable Cd-Metallothionein complexes in their kidneys which accumulated over time. Similarly, prolonged exposure to Pb increased the amount of Pb in shrew livers and bones with age (Sánchez-Chardi et al. 2007b; Sánchez-Chardi and Nadal 2007). Cr may decrease with age due to initial high intestinal absorption in juvenile shrews, but low absorption in adults. Hg, however, may accumulate with age for male shrews, but not females. Females are thought to reduce Hg burdens through placental transfer to fetuses, or through milk to offspring (Sánchez-Chardi et al. 2007b). Studies of other small mammals show no age-related differences in metal accumulation (Smith and Rongstad 1982; Sánchez-Chardi et al. 2007a). For example, while adult omnivorous deer mice (*P. maniculatus*) accumulated more Cd and Pb than juveniles, herbivorous meadow voles (*M. pennsylvanicus*) did not show this age-related response (Smith and Rongstad 1982).

Studies of wood mice show the opposite effect, with juveniles accumulating more metals than adults and metal accumulation decreasing with age (Lopes et al. 2002; Sánchez-Chardi et al. 2007a; Scheirs et al. 2006a). Juvenile wood mice have a higher energy requirement than adults, and thus have a higher intake of food and (inadvertently) metals. Adult mice also exhibit lower intestinal absorption of certain metals (such as Cr), which may further explain the decrease with age (Sánchez-Chardi et al. 2007a). Some studies have reported sex-dependent variations in metal accumulation in wood mice (Lopes et al. 2002; Scheirs et al. 2006a; Beernaert et al. 2007), while others have not (Sánchez-Chardi et al. 2007a). This variability in age- and sex-dependent metal accumulation in small mammals may be due to different site conditions, metal exposure, and foraging ranges. Thus, when using small mammals as bioindicators of heavy metal pollution, studies should consider the variable effects of age and sex on metal accumulation.

Finally, the specific metal must be considered when examining accumulation in small mammals. Mammals are more likely to regulate and excrete excesses of essential metals (e.g., Zn and Cu are required for mammalian physiology) than non-essential metals. As a result, non-essential metals such as As, Cd, and Pb are more likely to accumulate in small mammal tissues, bound to metal-binding proteins or incorporated into insoluble granules, and, over time, this accumulation may lead to toxicity (Smith and Rongstad 1982; Kabata-Pendias and Pendias 2001; Veltman et al. 2007).

Many studies stress the importance of metal availability in predicting mammal and food chain metal concentrations (e.g., Gupta et al. 1996; Hamers et al. 2006; van den Brink et al. 2010), although total soil metal concentrations are often used to assess metal risks for wildlife (van den Brink et al. 2010). However, the extractants used to determine available metal concentration in soils largely dictate the effectiveness and accuracy of the reading (Menzies et al. 2007). Currently, there appears to be no consensus in the literature about which extractant most accurately demonstrates the phytoavailability of metals in soils (Menzies et al. 2007). Generally, it appears that available metal concentrations determined from complexing agents (such as DTPA or EDTA) or acid extractants (0.1 M HCL or Mehlich 1 extractant) correlate poorly with plant phytoavailability; instead neutral salt extractants (such as 0.01 M CaCl₂ or 0.1 M NaNO₃) seem more useful indicators of metal phytoavailability across a range of metals, but there is still no consensus in the literature (Menzies et al. 2007). This controversy highlights the urgent need for more research focused on the methods of determining metal availability in soils, as this measurement is often used for wildlife risk assessments and bioremediation plans.

Large Mammals

Much of the literature regarding heavy metal accumulation in large mammals includes mammals important to the human diet, especially grazers such as cattle (Alonso et al. 2003; Cai et al. 2009; Miranda et al. 2009; Mahajan et al. 2012; Roggeman et al. 2013), deer (Pokorny and Ribaric-Lasnik 2002; Reglero et al. 2008, 2009), and sheep (Phillips and Tudoreanu 2011), as well as omnivores such as wild boar (*Sus scrofa*; Suidae) (Reglero et al. 2009). Exposure to non-

essential metals (e.g., Cd, Pb, and As) can cause mutagenesis, carcinogenesis, teratogenesis, immunosuppression, and may inhibit growth, lower fertility and impair reproduction (Swarup and Dwivedi 2002; Alonso et al. 2002a; 2002b; Mahajan et al. 2012). As with small mammals, large mammals often ingest polluted vegetation (van der Fels-Klerx et al. 2011), small amounts of soil, or contaminated drinking water, but may also be exposed to metals through inhalation or grooming (Miranda et al. 2009). The extent of accumulation in large mammals depends on the metal and its concentration in herbage and soil, the age of the animal, and how quickly the metal passes through the gastrointestinal tract (Wilkinson et al. 2003; Miranda et al. 2009). Large grazers also forage at a larger scale than small herbivores, consuming metals from larger geographic ranges and from a greater range of vegetation types. Thus, plant compositions and variations in diet over a season may also play a major role in large mammal exposure to heavy metals (Pokorny and Ribaric-Lasnik 2002; Brekken and Steinnes 2004).

Metal accumulation in large mammals is tested through both non-destructive samples, such as hair, milk, and blood (Patrashkov et al. 2003; Kramarova et al. 2005; Rashed and Soltan 2005) and destructive samples such as liver, kidney, and muscle (Cai et al. 2009; Miranda et al. 2009; Reglero et al. 2009). Blood metal concentrations generally reflect more recent exposure and are therefore more useful when the exposure period is short (Wittman and Hu 2002). Liver and kidney accumulations also reflect short-term exposure, while metals accumulated in bone or hair reflect long-term exposure (Pragst and Balikova 2006; Reglero et al. 2009). For example, Reglero et al. (2009) found that deer and wild boar near a Pb mine had more Pb in their bones than their livers, indicating long-term Pb exposure. Furthermore, some authors suggest that kidneys are the most appropriate target organ to test for metals, because they are less likely to be contaminated by lead bullets during hunting season, in contrast to liver, which is more likely to be hit by bullets (Blottner et al. 1999; Pokorny and Ribaric-Lasnik 2002; Reglero et al. 2009). Several studies (e.g., Buchwalter et al. 2007; Sample et al. 2014; Smith et al. 2014) have proposed models that can predict risk of metal exposure from environmental factors (e.g., plant and soil metal concentration, plant composition, and animal diet). However, these predictions are most accurate when animal feeding ranges are localized and diet composition is simple.

This may not be the case for large mammals whose feeding ranges are larger and may include food from both polluted and non-polluted areas (van den Brink et al. 2010).

Several studies show that the diet of large mammals is the primary pathway for metal accumulation, reporting elevated levels of heavy metals in the kidneys, livers, bones, hair, and blood of these mammals (Alonso et al. 2003; Cai et al. 2009; Reglero et al. 2009; van der Fels-Klerx et al. 2011; Mahajan et al. 2012). Near a Pb-Zn smelter in Guizhou, China, Cai et al. (2009) found elevated levels of Zn, Pb, and Cd in the soil, feed, and kidneys and livers of cattle reared near the site. Concentrations of Zn and Cd in the soil (10 mg/kg Cd, 1100 mg/kg Zn), feed (6.6 mg/kg Cd, 895 mg/kg Zn), cattle kidney (6.64–38.3 mg/kg Cd, 36 mg/kg Zn) and cattle liver (1.31–2.47 mg/kg Cd, 54 mg/kg Zn) all surpassed the Chinese standards (soil: 1.0 mg/kg Cd, feed: 0.5 mg/kg Cd, liver: 0.5 mg/kg, kidney: 1.0 mg/kg). Although Pb concentrations in the soil (467 mg/kg) were just below the Chinese standard (500 mg/kg), Pb in feed (132 mg/kg) was 40-fold higher than the standard (8 mg/kg) and cattle kidneys (0.8 mg/kg) and livers (1.0 mg/kg) were just above the Chinese standard (0.5 mg/kg for cattle kidney and liver). Contrastingly, Cu was within the Chinese standards for all substrates. Despite high levels of metals in the environment, levels of Pb, Cd, Zn, and Cu in bovine muscle were all below the Chinese standards, suggesting that although these cattle accumulate heavy metals in other tissues, they are still safe for human consumption. However, the source of heavy metal pollution may also influence accumulation in mammals. For example, cattle reared by a thermal energy plant in India that were exposed to fly ash (trace metal-laced particulate matter that is the byproduct of coal combustion) had high levels of Hg in their blood, milk, and urine (Mahajan et al. 2012), while cattle raised near mining sites in northwest Spain, did not show any major accumulation of Hg in tissues other than the kidney (Alonso et al. 2003).

Studies from other parts of the world reflect similar trends: metal-rich diets lead to tissue metal accumulation (Miranda et al. 2009; Reglero et al. 2009; Mahajan et al. 2012). Reglero et al. (2009) analyzed the bones and livers of red deer and wild boar in a Pb mining area in the Sierra Madrona Mountains of southern Spain, reporting higher levels of Pb in animals near the mining area than those from an unpolluted site. Furthermore, boar from the mine site accumulated more Pb than the deer, but these levels were well below the level

associated with clinical signs of Pb poisoning (30 mg/kg dry weight). The authors attribute this trend to the animals' diets; boar consume more roots, bulbs, and tubers, and may inadvertently ingest soil, whereas deer graze aboveground parts of plants and so likely ingested less Pb.

A similar trend has also been observed for micronutrient metals such as Ni, found in high concentration in serpentine soils (Vithanage et al. 2014). In ruminants such as cattle, Ni is an important component of metalloenzymes like urease, which aids in ruminant metabolism (Spears et al. 1986). Because of this, the Ni requirement tends to be higher in ruminants but, in excess, Ni may be toxic. Twenty percent of cattle grazing on serpentine soils in northwest Spain had toxic levels of Ni in their kidneys and 32 % had Cu levels in their liver above an acceptable range. However, as with other studies of large and small mammals, metal concentrations in cattle muscle were well below detectable levels (Miranda et al. 2009).

Metals in both plant tissues and mammalian diets may vary over a season, influencing the risk of metal exposure in large mammalian grazers (Pokorny and Ribaric-Lasnik 2002; Brekken and Steinnes 2004; Miranda et al. 2005). Large grazers may be at risk for metal accumulation when metal levels in grazed plants are elevated. A Norwegian study of moose (*Alces alces*; Cervidae) in a metal-polluted natural area showed that grazed plants (primarily *Populus* and *Salix*; Salicaceae) significantly reduced levels of Cd and Zn in their tissues between spring and mid-summer, potentially posing less metal exposure to moose during that time (Brekken and Steinnes 2004). However, the authors only examined the concentrations in the plants and not in the moose organs, highlighting the difficulty of using plant metal concentration and diet composition data to predict the risk of metal exposure.

Pokorny and Ribaric-Lasnik (2002) reported a similar seasonal trend for Roe Deer in Slovenia, but examined deer kidneys directly, rather than the metal concentrations of the grazed plants. Deer kidneys contained significantly higher amounts of Hg and Pb in the late summer and early autumn with a similar, but less pronounced, seasonal pattern for Cd and Zn (Pokorny and Ribaric-Lasnik 2002). Food intake by roe deer was highest in August and September, when they consume on average ~0.06 kg of dry plant matter per day. The authors suggest that this elevated food intake, combined with a diet of metal-accumulating mushrooms, may

explain the seasonal difference in metal accumulation. Again, seasonal variations in large mammal metal concentrations are species-specific and depend on the availability of food, diet compositions, and metal variability within food sources throughout a particular season (Reglero et al. 2008; Vermeulen et al. 2009; Fritsch et al. 2011; Roggeman et al. 2013).

Humans

Humans may be exposed to heavy metals through several pathways, including dietary intake, occupational exposure, or from the environment such as inhalation of metal-laden dust (Amaya et al. 2013). Upon exposure, heavy metals cause a variety of adverse health effects (Anderson et al. 2005; Järup 2003). For example, exposure to Cd may cause kidney damage and acute exposure to Hg may damage lungs and kidneys, as well as cause neurological and psychological problems such as changes in personality, restlessness, anxiety, sleep disturbance, and depression. Lead poisoning may result in headaches, irritability, abdominal pain, and nervous system disorders (Järup 2003). Furthermore, heavy metal-contaminated foods pose potential barriers for international trade of produce (Cui et al. 2004). For more information on the human health effects of heavy metals and the history of anthropogenic heavy metal pollution, see Järup (2003) and Anderson et al. (2005).

Food and water are the most common sources of heavy metal exposure for humans, often resulting from crops grown on heavy metal-contaminated agricultural soils (Gimeno-García et al. 1996; Luo et al. 2009). Sources of heavy metals in agriculture include atmospheric deposition, phosphate-based fertilizers, livestock manures, wastewater irrigation, metallo-pesticides, and long-term applications of both treated and untreated sewage sludge (Nicholson et al. 2003; Cai et al. 2009; Chary et al. 2008; Luo et al. 2009; see references within Neilson and Rajakaruna 2014).

Some of the most consistent sources of heavy metals in agricultural systems are phosphate-based fertilizers, including single nutrient fertilizers like superphosphates, binary fertilizers like monoammonium phosphate and diammonium phosphate, and some combined NPK fertilizers (Otero et al. 2005; Nziguheba and Smolders 2008; Javied et al. 2009; Atafar et al. 2010; Jiao et al. 2012). While superphosphate fertilizers contain high levels of Cd, Co, Cu, and Zn, copper and iron

sulfate fertilizers often have high levels of Pb and contain Ni (Gimeno-García et al. 1996). Mined across the globe, phosphate rock is naturally high in heavy metals and serves as the base for all phosphate fertilizers (Mortvedt 1996; Cao et al. 2004; Nziguheba and Smolders 2008). However, the amount of metals in phosphate rocks varies with its origin; heavy metal content of igneous phosphate rocks is generally lower than that of sedimentary phosphate rocks (Van Kauwenbergh 2002). But even metal content within igneous and sedimentary phosphate rocks varies: for example, sedimentary phosphate rock extracted from areas in Pakistan has lower levels of heavy metals than sedimentary phosphate deposits imported from areas of Egypt, Jordan, and Morocco (Javied et al. 2009). In addition, the extent of heavy metal pollution from fertilizers not only depends on the phosphate rock from which the fertilizer was derived, but also on the amount of fertilizer applied (Nziguheba and Smolders 2008).

For economic reasons, phosphate fertilizers may not be sufficiently purified during processing and only a limited fraction of metals is lost, leaving trace heavy metals such as Cd, Co, Cu, Pb, Mn, and Zn in the final product fertilizer (Gimeno-García et al. 1996; Nziguheba and Smolders 2008). Not only are phosphate-based fertilizers a source of heavy metals in agricultural systems, but the processes of mining and milling phosphate rock are also key sources of heavy metal air and soil pollution (Javied et al. 2009). Pan et al. (2010) reported that 55 % of Cd in Europe comes from phosphate fertilizers, while 40 % arises from atmospheric deposition, with overall Cd input in Europe at $1.6 \text{ g ha}^{-1} \text{ year}^{-1}$ (Nziguheba and Smolders 2008). Nziguheba and Smolders (2008) estimated heavy metal input by phosphate fertilizers and atmospheric deposition for each country in the European Union. Similarly, Lugon-Moulin et al. (2006) provided a several-country survey of Cd in phosphate fertilizers used for tobacco production.

In pesticides such as fungicides, herbicides, and insecticides, heavy metals are often active compounds (Gimeno-García et al. 1996). However in certain countries like the UK and China, As-, Hg-, and Pb-based pesticides are banned and only a few approved pesticides contain other heavy metals (Nicholson et al. 2003). In particular, Zn is a minor part of fungicides used on wheat and potatoes, while Cu and sometimes As are used largely as a fungicide for viticulture in countries like France and Italy (Eckel et al. 2008).

Heavy metals are also present in many livestock diets at background concentrations, but may also be added to certain feeds as supplementary trace elements to promote health and growth (Nicholson et al. 2003; Luo et al. 2009). For example, Cu is often added to pig diets as a potential anti-bacterial agent in the gut (Rosen and Roberts 1996). However, most heavy metals consumed by livestock are excreted in urine or feces, which will then be present in manure that is applied to land (Nicholson et al. 2003).

As a byproduct of sewage treatment, sewage sludge is produced in vast amounts in Japan (70 million tons), China (30 million tons), and the USA (6 million tons) (Matsubara and Itoh 2006; McClellan and Halden 2010; Kelessidis and Stasinakis 2012; Bouriou et al. 2015). With such quantities of sewage sludge available and the need to dispose of it, many farmers turn to sewage sludge to fertilize and irrigate cropland (Nicholson et al. 2003; Cai et al. 2009; Muchuweti et al. 2006; Luo et al. 2009). In some nations, such as parts of India and China, spreading untreated or partially treated sewage effluents over agricultural land is often practiced because it is economically feasible when compared with costly effluent treatment (Cai et al. 1990; Scott et al. 2004). However, heavy metals may also be present in treated sewage sludge as a result of domestic inputs, road runoff, and industrial contributions to urban wastewater collection systems (Nicholson et al. 2003; Rattan et al. 2005; Luo et al. 2009; McClellan and Halden 2010). For example, while restrictions and changes in industrial practices in developed nations have limited discharges of heavy metals into sewage, heavy metals may still arise from domestic sources, such as Cu leaching from Cu pipes and Zn from body care products (Comber and Gunn 1996; Nicholson et al. 2003; McClellan and Halden 2010). Furthermore, repeated applications of wastewater to agricultural land may also cause heavy metals to build up in soils, with the potential for metals to leach into the soil solution and groundwater (Scott et al. 2004; Chary et al. 2008; Khan et al. 2008).

From these various sources, heavy metals may build up in soils and, under the right conditions (e.g., low pH), become bioavailable and accumulate in food crops such as wheat, rice, carrots, and lettuce (Hough et al. 2004; Muchuweti et al. 2006; Javied et al. 2009; Zhuang et al. 2009; Atafar et al. 2010; Cao et al. 2010). Multiple studies have examined the effects of these polluted food crops and medicinal plants on human health (e.g., Järup

2003; Cui et al. 2004; Chary et al. 2008; Cao et al. 2010; Amaya et al. 2013; Street 2012; Sahoo and Kim 2013). For example, lifetime exposure to low levels of Cd has led to renal dysfunction in residents living near contaminated sites in Japan (Ryan et al. 1982) and China (Cai et al. 1990; Nordberg et al. 1997; Watanabe et al. 2000; Jin et al. 2002; Cui et al. 2004). Residents may also inadvertently consume polluted soils while eating vegetable crops, especially when consuming root crops (Cui et al. 2004).

Many studies also have examined the transfer of heavy metals from food crops to humans (e.g., Cui et al. 2004; Hough et al. 2004; Pruvot et al. 2006; Chary et al. 2008; Khan et al. 2008; Zhuang et al. 2009). There are different methods to assess this transfer risk such as the hazard quotient (HQ), daily dietary index (DDI), daily intake of metals (DIM), health risk index (HRI), and hazard index (HI). The HQ provides a ratio of the average daily dose of a metal to the reference dose, where $[W_{plant}]$ is the dry weight of contaminated plant material consumed (mg/day), $[M_{plant}]$ is the concentration of metal in the vegetable(s) (mg/kg), RfD is the food reference dose (the maximum acceptable oral dose of a toxic substance) for the metal (mg/day), and B is human body mass (kg) (Oves et al. 2012):

$$HQ = \frac{[W_{plant}] \times [M_{plant}]}{RfD \times B}$$

A hazard quotient under 1 is considered to be safe for humans, whereas a quotient ≥ 1 indicates that hazardous health effects are possible. The hazard quotient does not provide a probability that adverse health effects will occur, but does indicate the presence of a health risk (Chary et al. 2008).

The daily dietary intake index (DDI) determines the daily amount of a metal consumed by a person, where X is the metal concentration of the vegetable, Y is the dry weight of the vegetable, Z is the approximate daily intake, and B is the average body mass of the consumers.

$$DDI = \frac{X \times Y \times Z}{B}$$

The daily intake of metals (DIM) may also be used, where C_{metal} is the heavy metal concentration in plants (mg/kg), C_{factor} is the conversion factor (typically 0.085 to convert fresh vegetable weight to dry weight: Rattan et al. 2005), $D_{food\ intake}$ is the daily intake of vegetables

and $B_{average\ weight}$ is the average weight of the consumers (Oves et al. 2012).

$$DIM = \frac{C_{metal} \times C_{factor} \times D_{food\ intake}}{B_{average\ weight}}$$

Using DIM values and the food reference dose together, the health risk index (HRI) can be calculated:

$$HRI = \frac{DIM}{RfD}$$

Generally, if the HRI value is less than 1, a population exposed to heavy metals is considered safe (Oves et al. 2012).

To assess the health risks from multiple metals, the Hazard Index may be used, where HQ_n is the hazard quotient for one particular metal:

$$HI = HQ_1 + HQ_2 + \dots + HQ_n$$

A HI greater than one indicates potential health risks from combined metals. However, HI does not take into account any chemical interactions among the metals. Without the ability to determine these interactions, the HI equation could underestimate health risks if interactions are present (Wilbur et al. 2004).

To analyze metal exposure in humans, researchers typically examine metal accumulation in human blood, hair, and urine (Chary et al. 2008). For example, along the Musi River in India, levels of Pb, Zn, Cr, and Ni were high in *Amaranthus*, spinach (both *Amaranthaceae*), and buffalo milk, and levels in humans were higher than permissible limits. The hazard quotient was high for Zn (1.9–5.3), followed by Cr (2.2–3.05) and then Pb (0.02–0.11), indicating that Zn and Cr posed potential health risks to residents (Chary et al. 2008). Examining rice and garden vegetables in developed regions of Jiangsu, China, Cao et al. (2010) found less exaggerated metal effects. The HQ of individual metals were all lower than 1, but when all six metal-intake values were combined, the Hazard Index value was close to 1 (0.95). This suggests that, when combined, metals pose a greater health risk than any lone metal (Cao et al. 2010).

Heavy metals may also transfer from mother to fetus, and several studies have associated early exposure to Pb, Hg, As, and Cd with infant health effects such as neurological, developmental, and endocrine disorders (Oken et al. 2005; Wright et al. 2006; Gollenberg et al. 2010; Gundacker et al. 2010; Amaya et al. 2013). Fetuses and neonates are especially vulnerable to heavy

metals because their detoxification systems are not fully mature. Some heavy metals may cross the placental barrier, interfering with placental transport systems (Amaya et al. 2013).

Metallophytes as a Potential Solution

Arising from anthropogenic and natural sources, both essential and non-essential heavy metals enter food webs through soils. From soils, heavy metals may transfer into plants, invertebrates, and grazers, and may ultimately accumulate in humans, posing severe health risks. However, many organisms may be used to monitor heavy metals in the environment and assess the risk of metal transfer within a food web (Stankovic et al. 2014). Despite negative impacts of heavy metals on health of many ecosystems, the presence of heavy metals in some natural environments has led to evolution of plants with the ability to resist, tolerate, or even thrive on metalliferous soils (Rajakaruna et al. 2014). Known as metallophytes, these plants have evolved mechanisms to cope with heavy metal stress (Wenzel and Jockwer 1999; Whiting et al. 2004) and thus can be readily grown on metal-contaminated soils. Metallophytes may be utilized in conjunction with their unique rhizospheric microbial communities to revegetate or clean up areas contaminated by heavy metals (Ensley 2000; Adriano et al. 2004; Wenzel 2009; Haferburg and Kothe 2010; Wuana and Okieimen 2011). Use of metallophytes to remove metals from soil (phytoextraction) is being investigated as an alternative to traditional methods (e.g., Chaney et al. 2007; Tang et al. 2012): traditional methods remove, incinerate, or chemically treat contaminated soil and thus destroy or severely disrupt biotic communities. These methods are also expensive, costing \$25 to \$50 billion worldwide annually (Neilson and Rajakaruna 2012). The USA alone spends \$6 to \$8 billion each year cleaning up metal-contaminated sites (Tsao 2003; Pilon-Smits 2005), an extensive investment that many developing nations cannot afford (Rajakaruna et al. 2006). Thus, metallophytes may be used for erosion control, revegetation, and potential cleanup of abandoned industrial sites and mine tailings and, if managed properly, may limit the opportunities for heavy metals to spread outside the area of contamination and cause toxicity in non-adapted species (Angle and Linacre 2005; Neilson and Rajakaruna 2012).

Given the potential benefits of green technologies, it is important to continue searching for undiscovered metallophytes (Gall and Rajakaruna 2013) and continue studying their unique rhizospheric interactions with microbial communities (Wenzel 2009). There are potentially many more 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). Thus, vegetation 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 (Whiting et al. 2004). The discovery, conservation, and study of such species from around the world can also make restoration of metal-contaminated sites more efficient and easily manageable (i.e., by using native species adapted to local conditions), thereby reducing the spread of metals outside the areas of contamination.

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