Factors Influencing the Evolution of Heavy Metal Hyperaccumulation in Plants

Introduction

Although many European countries have significantly reduced their heavy metal emissions since 1990, emission levels have remained relatively constant since 2004 (EEA 2015) and continue to be an important issue in developing nations that lack the resources for effective monitoring and remediation. Chemically, heavy metals are defined as any metal with a density greater than 5 g cm^{-3} (Järup 2003). However, when discussed in relation to pollution the term is used more broadly in reference to any metal or metalloid that is highly toxic at low concentrations (Järup 2003). Heavy metals are naturally present in the environment and input is dependent on volcanoes and continental dusts (Schützendübel and Polle), but there are also numerous anthropogenic sources such as industrial activities, mine tailings, waste disposal, leaded gasoline and paints, fertilizers, animal manure, sewage sludge, pesticides, wastewater irrigation, coal combustion, petrochemicals, and atmospheric deposition (Wuana and Okieimen 2011). Once released into the environment, heavy metals do not decompose, resulting in accumulation of the metals in ecosystems and allowing them to travel up the food chain.

In humans, exposure to heavy metals is known to have serious negative health effects, including cancer and renal tubular damage (Järup 2003). In plants, metals can be characterized as nonessential or essential. Nonessential metals like As, Cd, Hg, Pb and Se do not have any known functional role in plants and essential metals like Co, Cu, Fe, Mn, Mo, Ni, and Zn are required in small amounts for the plant to function normally (Rascio and Navari-Izzo 2011). Higher concentrations of essential or nonessential metals are toxic to the plant and result in stunting, chlorosis, and/or a variety of other symptoms (Foy et al. 1978). Yet in many areas that have been contaminated with heavy metals or that have naturally high levels of metal in the soil, plants are still able to grow (Wang et al. 2003). Some of these plants have evolved adaptations that have allowed them create an ecological niche in regions with contaminated soils. One adapted mechanism is accumulation. Accumulation allows the plant to concentrate metals in different parts of its tissues (Baker 2008). Plants capable of accumulating extraordinarily high levels of metals in their tissues are known as hyperaccumulators. This review describes the proposed selective pressures that may have given adaptive advantages to hyperaccumulators, ultimately resulting in the evolution of hyperaccumulation.

Hyperaccumulator Plants

Before discussing the evolution of metal hyperaccumulation, hyperaccumulator plants must be further defined. Plants that are able to successfully colonize an area with a high concentration of metals can be categorized as excluders or accumulators. Excluder plants restrict uptake of metals by exploring less contaminated soils. Their symbiosis with mycorrhizal fungi allows them to reduce uptake or change metal speciation, reducing the bioavailability of the metal by exuding chemicals to immobilize it, or downregulating transporter activity that may inadvertently cause uptake of metals (Ernst 2006; Mehes-Smith et al. 2013). In contrast, accumulator plants actively take up metals from the soil (Rascio and Navari-Izzo 2010). Hyperaccumulator plants take up exceedingly large amounts of metals without showing any signs of phytotoxcity (Rascio and Navari-Izzo 2010). Characteristically, the metals taken up by hyperaccumulators are translocated to the shoot and accumulated in above-ground organs, where they can reach concentrations 100-1000 times higher than those in non-hyperaccumulating species (Rascio and Navari-Izzo 2010).

Over 450 plant species have been identified as heavy metal hyperaccumulators (Verbruggen et al. 2009). These species are distributed over a range of distantly related families, suggesting that hyperaccumulation has evolved independently more than once (Rascio and Navari-Izzo 2010). A large portion of the known hyperaccumulators belong to the Brassicaceae family (Verbruggen et al. 2009), and *Arabidopsis halleri* and *Thlaspi caerulescens* are frequently used as model systems for studying metal hyperaccumulation (Kramer 2010). The classification of a plant as a hyperaccumulator is dependent on its ability to hyperaccumulate any given metal to a concentration above a defined threshold value without suffering phytotoxic damage when grown on native soil. The threshold values are >10 mg g^{-1} Mn or Zn, >1 mg g^{-1} As, Co, Cr, Cu, Ni, Pb, Sb, Se, or Ti, and >0.1 mg g^{-1} Cd (Verbruggen et al. 2009).

Selective Factors Causing the Evolution of the Hyperaccumulation Trait

The phylogeny of hyperaccumulator plants is still relatively unknown, but it is thought that metal hyperaccumulation offers an adaptive advantage to plants, although this and the type of advantage offered has not been conclusively proven. Many plants that are tolerant to soils with high concentrations of heavy metals exist, but they do not hyperaccumulate the metals (Ernst 2006; Mehes-Smith et al. 2013). Hyperaccumulation and heavy metal tolerance are genetically independent traits (Macnair et al. 1999), so the selective pressures affecting hyperaccumulation may not have been specifically related to heavy metal tolerance (Behmer et al. 2005). An early review listed five explanations for the evolution of metal hyperaccumulation in plants: increased metal tolerance, interference and allelopathy, disposal from plant body, drought tolerance, and pathogen and herbivore defense (Boyd and Martens 1998). A sixth explanation, inadvertent uptake, was also proposed, but it does not attribute a selective value to hyperaccumulation (Boyd and Martens 1998) and so will not be discussed in this paper.

Increased metal tolerance. Metal tolerance is coded independently of metal accumulation in a plant's genome (Macnair et al. 1999). Phylogenetic analysis of *Stanleya* Se hyperaccumulation yielded results suggesting that Se tolerance was a prerequisite for its hyperaccumulation (Cappa et al. 2015). Thus, it is likely that the evolution of metal hyperaccumulation in plants may have been driven by an enhanced ability to tolerate soils with high concentrations of metal. Because the plants that are unable to tolerate high concentrations of metals would be selected against, plants with a better ability to tolerate metals would populate contaminated areas and would have less competition for resources. Metal tolerance is governed by only a few major genes (Ernst 2006) with minor genes enhancing their effect, increasing metal tolerance, and causing variation among tolerant species (Smith and Macnair 1998). Enhanced metal tolerance can occur through a variety of physiological mechanisms: rapid cellular compartmentalization, allocation to less metabolically active tissues, and/or allocation to seeds and to deciduous organs at senescence (Ernst 2006). It has been suggested that the genes for metal tolerance are present and expressed at a low frequency in some non-tolerant plant species (Gartside and McNeilly 1974), so random genetic mutations in a plant's genome could easily result in an enhanced ability to tolerate metal.

Interference and allelopathy. According to this hypothesis, plants capable of accumulating heavy metals in their tissues had the ability to use the metals as a form of allelopathy, interfering with the growth of competitor plants and ultimately outcompeting them. Theoretically, heavy metals would be more effective at allelopathy than the chemicals typically derived from photosynthate, due to toxicity stemming from an inorganic element unable to be broken down by the organic compounds produced as counterdefences by herbivores and other competitors (Rascio and Navari-Izzo 2010). However, very little evidence supporting this theory has been published. A more recent study produced evidence indicating that this theory is most likely not an accurate explanation for the evolution of hyperaccumulation (Zhang et al., 2007). The study examined the effects of Ni-rich *Alyssum murale* biomass and $Ni(NO_3)_2$ on the germination of eight species of plant seeds, and the results indicated that the biomass from the hyperaccumulator did not have an inhibitory effect on the germination or growth of the competing plants (Zhang et al. 2007).

Disposal from plant body. Closely related to the allelopathy and tolerance theories, this theory proposes that the disposal of metal-containing plant tissue, such as leaves, provides a selective pressure favoring the evolution of hyperaccumulation by interfering with the growth of competitor plants and increasing the concentrations of metals in the soil. The ability of a plant to dispose accumulated metals by shedding its leaves would also have functioned as a detoxification strategy, increasing the plant’s metal tolerance. Again, no significant evidence has been published supporting this theory and the study performed by Zhang et al. (2007) contradicts it. However, more research is needed before the theory can be completely discounted.

Drought tolerance. According to this theory, the hyperaccumulation trait provides a selective advantage by increasing the plant’s resistance to water stress. This could happen through two mechanisms: reduction of cuticular transpiration or increased osmolarity within the plant cell, which would allow the cell to maintain turgor and activity during the onset of water stress (Kachenko 2008). However, a study conducted by Whiting et al. (2003) found that hyperaccumulation of Ni and Zn in *Alyssum murale* and *Thlaspi caerulescens* did not enhance survival or whole-plant growth under drought conditions. Another study reached similar conclusions after testing drought resistance in Ni hyperaccumulator *Hybanthus floribundus* (Kachenko 2008). Kachenko (2008) found that Ni concentrations in *H. floribundus* did not significantly increase in response to water stress, suggesting that osmolarity did not play a role in the evolution of metal hyperaccumulation.

Pathogen and herbivore defense. This is the most popular theory regarding the evolution of the hyperaccumulation trait and consequently the one that has been most heavily researched. According to this theory, metal hyperaccumulation gives plants a selective advantage by acting as an elemental defense against pathogen attack and herbivory. Over time, this defensive benefit may have driven a continued increase in the accumulation ability of plants, resulting in the evolution of the hyperaccumulator species present today (Boyd 2007). This theory has been studied in a multitude of organisms, including between *Streptanthus polygaloides* and powder mildew (*Erysiphe polygonl*), the pathogen *Xanthomonas campestris*, and the fungus *Alternaria brassiciola* (Boyd et al. 1994); between *T. caerulescens* and the pathogen *Pseudomonas syringae* pv Maculicola (Fones et al. 2010); between *T. caerulescens* and the desert locust *Schistocerca gregaria* (Behmer et al. 2005); and between *Thlaspi montanum* and *Pieris rapae* larvae (Boyd and Martens 1994). An extensive experiment performed by Boyd et al. (1994) indicated that hyperaccumulated Ni significantly inhibited or prevented growth of all pathogenic organisms tested, suggesting that defense against pathogens may have played a role in the evolution of nickel hyperaccumulation. This conclusion was supported by the results of a study performed by Fones et al. (2010), which used leaf inoculation assays to show that the growth of *P. syringae* on *T. caerulescens* was in habited by the hyperaccumulation of Zi, Ni, and Cd by *T. caerulescens*. Fones et al. (2010) also showed that inhibition of *P. syringae* growth by the presence of metals was dependent on the bacteria's growth abilities as determined by mutations for increased or decreased Zi tolerance. Behmer et al. (2005) explored the aversion of desert locusts to high Zi levels in *T. caerulescens* by measuring growth and feeding rates and mass gain in response to high Zi levels in *T.* caerulescens. Their results indicated that the locusts were able to develop associated learning through a post-ingestive feedback mechanism, suggesting that the evolution of hyperaccumulators could have been driven by the ability of plants with high concentrations of metals in their tissues to influence the feeding behavior of insect herbivores (Behmer et al. 2005). These results supported earlier findings showing that hyperaccumulated Ni in *T. montanum* grown on serpentine soils poisons folivores like *P. rapae* larvae (Boyd and Martens 1994). A field experiment also supporting the defense hypothesis has been performed with *S. polygaloides* (Martens and Boyd 2001). Results from this experiment showed that hyperaccumulation of Ni by *S. polygaloides* resulted in an elemental defense for the plant against some insect herbivores and pathogens (Marten and Boyd 2001).

However, evidence contradicting the defense hypothesis has also been found. The field study performed by Martens and Boyd (2001) also showed that Ni hyperaccumulation in *S. polygaloides* did not deter consumption by larger herbivores. In a study that evaluated the relationship between *S. polygaloides* and the viral pathogen Turnip mosaic virus, it was found that the elevated Ni concentrations found in *S. polygaloides* actually enhanced the colonization ability of the virus (David et al. 2001). Another study documented the preference of *Melanotrichus boydi* for feeding on *S. polygaloides* (Wall and Boyd 2006). The beetle species *Chrysolina pardalina* is capable of feeding exclusively on Ni hyperaccumulator *Berkheya coddii* (Mesjasz-Przybylowciz and Przybylowicz 2001). The results of these studies suggest that hyperaccumulation does not provide a comprehensive defense and that herbivores may be able to evolve tolerance to the high metal concentrations in the tissues of hyperaccumulators. Additional research is needed to further clarify the relationship between hyperaccumulators and their predators. However, the current research appears to show that while hyperaccumulation may have evolved as a defense mechanism, the selective advantage it provides functions only against select bacteria and small herbivores. It is also possible that coevolution between hyperaccumulaters and metal tolerant herbivores may have promoted the evolution of hyperaccumulation in plants.

Implications of the Use Hyperaccumulater Plants for Phytoremediation on the Food Web

The scientific interest in hyperaccumulator plants stems from their potential to remediate environments contaminated with heavy metals from anthropogenic sources. However, heavy metal hyperaccumulation also has the potential to interfere with ecosystems and food webs. Due to their toxicity at low concentrations (Järup 2003), accumulation of heavy metals can negatively affect ecosystems. Because heavy metals can be very toxic even at low concentrations (Järup 2003), ecosystems can easily be negatively affected by metal accumulation. Studies have shown that metals can accumulate in higher organisms through their food sources. The transfer of Zn, Cu, Cd, and Pb from polluted soils to the plant *Urtica dioica* and from *U. dioica* to the snail *Cepaea nemoralis* was observed, and statistical analysis found positive relationships between the concentrations of all four metals in the snails and all four metals in *U. dioica* leaves (Notten et al. 2005). Accumulation is often compounded as it travels up trophic levels. This is exemplified by a study performed by Hunter and Johnson (1982), which found that shrews, the top predator in the ecosystem surrounding a refinery, had accumulated the highest levels of Cu and Cd. Consideration of the diet of large mammals is especially important because it is the primary pathway for metal accumulation (Gall et al. 2015). However, humans are more likely to be exposed to heavy metals from crops or water rather than contaminated meats (Gall et al. 2015). Thus, the use of hyperaccumulator plants for phytoremediation poses less of a risk to humans than it does to strict herbivores, provided the plants used for remediation are not consumed.

Conclusion

Because of the potential hyperaccumulator plants have for phytoremediation, much of the research surrounding these organisms is still focused on understanding the mechanisms of accumulation and the genes that control it. A more comprehensive understanding of the mechanisms and pathways involved in hyperaccumulation would allow for the creation of transgenic plants that are better able to perform phytoremediation. More recent studies have implicated that nicotinanamine synthase and a ZIP transporter play a role in zinc homeostasis and hyperaccumulation in *Arabidopsis halleri* (Weber et al. 2003, demonstrating that genetic mutations resulting in *cis*-regulatory changes and triplication of the heavy metal aptase 4 gene played a role in the evolution of metal hyperaccumulation (Hanikenne et al. 2008). A more comprehensive review of the recent major breakthroughs in the understanding of the genetic and molecular basis of metal hyperaccumulation and hypertolerance can be found from Hanikenne and Nouet (2011).

While hyperaccumulator plants have remediation potential, it is also important to consider the consequences of purposeful metal accumulation on ecosystems. Plants are direct and indirect food sources for microbes, invertebrates, and mammals. Because of this, the hyperaccumulation trait enables the transfer of toxic heavy metals through food webs, which has serious implications for ecosystems. Understanding why hyperaccumulation has evolved is also important in determining the effects of soil contamination from anthropogenic sources. Five theories have been proposed to explain the evolution of hyperaccumulation through selective factors: metal tolerance, allelopathy, disposal from plant body, drought tolerance, and defense against pathogens and herbivores (Boyd and Martens 1998). No strong evidence supporting the allelopathy, disposal, and drought tolerance hypotheses has been found. The defense theory is the most popular, and research indicates hyperaccumulation does provide a measure of defense against herbivores and bacteria. However, studies have produced evidence contradicting the defense hypothesis, suggesting that any elemental defense hyperaccumulation may provide is not comprehensive. These studies also hint at a relationship between hyperaccumulators and metal tolerant herbivores that is driven by coevolution. Future research on the evolution of hyperaccumulation and the genes and molecular pathways involved will likely lead to the development of transgenic plants highly capable of performing phytoaccumulation for remediation purposes. However, research into this topic is still in its infancy and whether these plants will be effective enough for widespread commercial use is yet to be determined.

References

Baker, A. J. M. 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. Journal of Plant Nutrition 3:643-654.

Behmer, S. T., C. M. Lloyd, D. Raubenheimer, J. Stewart-Clark, J. Knight, R. S. Leighton, F. A. Harper, and J. A. C. Smith. 2005. Metal hyperaccumulation in plants: mechanisms of defence against insect herbivores. Functional Ecology 19:55-66.

Boyd, R. S., J. J. Shaw, and S. N. Martens. 1994. Nickel hyperaccumulation defends *Streptanthus polygaloides* (brassicaceae) against pathogens. American Journal of Botany 81:294-300.

Boyd, R. S. and S. N. Martens. 1994. Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. Oikos 70:21-25.

Boyd, R. S. and S. N. Martens. 1998. The significance of metal hyperaccumulation for biotic interactions. Chemoecology 8:1-7.

Boyd, R. S. 2007. The defense hypothesis of elemental hyperaccumulation: status, challenges, and new directions. Plant and Soil 293:153-176.

Cappa, J. J., C. Yetter, S. Fakra, P. J. Cappa, R. De Tar, C. Landes, E. A. Pilon-Smits, and M. P. Simmons. 2015. Evolution of selenium hyperaccumulation in *Stanleya* (Brassicaceae) as inferred from phylogeny, physiology, and X-ray microprobe analysis. New Phytotechnology 205:583-95.

David, M. A., J. F. Murphy, and R. S. Boyd. 2001. Nickel increases susceptibility of a nickel hyperaccumulator to Turnip mosaic virus. Journal of Environmental Quality 30:85-90.

European Environment Agency. 2015. EEA 2015. Heavy metal emissions. <http://www.eea.europa.eu/data-and-maps/indicators/eea32-heavy-metal-hm-emissions-1/assessment-4>

Ernst, W. H. O. 2006. Evolution of metal tolerance in higher plants. Forest Snow and Landscape Research 80:251-274.

Fones, H., C. A. R. Davis, A. Rico, F. Fang, J. Andrew, C. Smith, and G. M. Preston. 2010. Metal hyperaccumulation armors plants against disease. Plos Pathogens.

Foy, C. D., R. L. Chaney, and M. C. White. 1978. The physiology of metal toxicity in plants. Annual Review of Plant Physiology 29:511-566.

Gall, J. E., R. S. Boyd, and N. Rajakaruna. 2015. Transfer of heavy metals through terrestrial food webs: a review. Environmental Monitoring and Assessment 187.

Gartside, D. W. and T. McNeilly. 1974. The potential for evolution of heavy metal tolerance in plants: II. Copper tolerance in normal populations of different plant species. Heredity 32:335-348.

Hanikenne, M. and C. Nouet. 2011. Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. Current Opinion in Plant Biology 14:252-259.

Hanikenne, M., I. N. Talke, M. J. Haydon, C. Lanz, A. Nolte, P. Motte, J. Kroymann, D. Weigel, and U. Kramer. 2008. Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. Nature 453:391-395.

Hunter, B. A. and M. S. Johnson. 1982. Food chain relationships of copper and cadmium in contaminated grassland ecosystems. Oikos 38:108-117.

Järup, L. 2003. Hazards of heavy metal contamination. British Medical Bulletin 68:167-182.

Kachenko, A. G. 2008. Ectophysiology and phytoremediation potential of heavy metal(loid) accumulating plants. PhD Thesis, University of Sydney, NSW, Australia.

Kramer, U. 2010. Metal hyperaccumulation in plants. Annual Review of Plant Biology 61:517-534.

Macnair, M. R., V. Bert, S. B. Huitson, P. Saumitou-Laprade, and D. Petit. 1999. Zinc tolerance and hyperaccumulation are genetically independent characters. Proceedings of the Royal Society B: Biological Sciences 266:2175-2179.

Martens, S. N. and R. S. Boyd. 2001. The defensive role of Ni hyperaccumulation by plants: a field experiment. American Journal of Botany 89:998-1003.

Mehes-Smith, M., K. Nkongolo, and E. Cholewa. 2013. Coping mechanisms of plants to metal contaminated soil. *In* Silvern, S. and S. Young, editors. Environmental change and sustainability. InTech.

Mesjasz-Przybylowicz, J. and W. J. Przybylowicz. 2001. Phytophagous insects associated with the Ni-hyperaccumulating plant *Berkheya coddii* (Asteraceae) in Mpumalanga, South Africa. South African Journal of Science 97:596-598.

Notten, M. J. M., A. J. P. Oosthoek, J. Rozema, and R. Aerts. 2005. Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. Environmental Pollution 138:178-190.

Prasad, M. N. V. and H. M. de Oliveira Freitas. 2003. Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology 6.

Rascio, N. and F. Navari-Izzo. 2010. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science 2:169-181.

Schützendübel, A. and A. Polle. 2001. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany 53:1351-1365.

Smith, S. E. and M. R. Macnair. 1998. Hypostatic modifies cause variation in degree of copper tolerance in *Mimululs guttatus*. Heredity 80:760-768.

Verbruggen, N., C. Hermans, and H. Schat. 2009. Molecular mechanisms of metal hyperaccumulation in plants. New Phytologist 181:759-776.

Wall, M. A. and R. S. Boyd. 2006. *Melanotrichus boydi* (Hemiptera: Miridae) is a specialist on the nickel hyperaccumulator *Streptanthus polygaloides* (Brassicaceae). The Southwestern Naturalist 51:481-489.

Wang, Q. R., Y. S. Cui, X. M. Liu, Y. T. Dong, and P. Christie. 2003. Soil contamination and plant uptake of heavy metals at polluted sites in China. Journal of Environmental Science and Health 38:823-838.

Weber, M., E. Harada, C. Vess, E. V. Roepenack-Lahaye, and S. Clemens. 2003. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. The Plant Journal 37:269-281.

Whiting, S. N., P. M. Neumann, and A. J. M. Baker. 2003. Nickel and zinc hyperaccumulation by *Alyssum murale* and *Thlaspi caerulescens* (Brassicaceae) do not enhance survival and whole-plant growth under drought stress. Plant, Cell and Environment 26:351-360.

Wuana, R. A. and F. E. Okieimen. 2011. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology 2011:1-20.

Zhang, L., J. S. Angle, and R. L. Chaney. 2007. Do high-nickel leaves shed by the nickel hyperaccumulator *Alyssum murale* inhibit seed germination of competing plants? New Phytologist 173:509-516.