Chapter 15
Heavy Metal Uptake and Tolerance Mechanisms of Serpentine Flora: Implications for Phytoremediation

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15.1 Introduction

Soil derived from ultramafic rocks or serpentinite (metamorphosed ultramafic rock) are termed as serpentine soils. These rocks cover about 1 % of the earth's surface. The term 'Ultramafic' is generally used to rocks which contain more than 70 % ferromagnesian materials [1–3]. Different pedogenesis processes lead to the formation of serpentine soil from the serpentinite rocks and these soils can be red, green, blue, or black in color, due to their chemical composition. The ultramafic rocks are known to contain high concentrations of potentially harmful elements including Ni, Cr, Mn and Co [4, 5]. Due to varying climatic conditions, nature of the parental material, tectonic activity, time and weathering processes, the heavy metal content of serpentine soils may differ from location to location [6–9].

The rocky granular textured soil, lack of organic material, low soil water-holding capacity, low Ca/Mg ratio, low nutrient content, and heavy metal toxicity in serpentine soils create an unfavorable environment for plant growth and development [10, 11]. Interestingly, there are several mechanisms in plants to withstand the heavy metal toxicity in serpentine soil. Due to the restricted soil chemical and physical characteristics, several plant species are evolved as serpentine endemics [12, 13]. Moreover, due to the chemistry of these soils, the serpentine associated groundwater and vegetation will differ from site to site with possible health inferences for

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humans [14]. Studies have conducted in order to estimate the impact of heavy metals release from the ultramafic rocks to soil and water [15, 16]. Due to the transport of weathered parent rock fragments from upslope ultramafic sources, high concentrations of lithogenic Ni and Cr were observed in agricultural top soils of Thiva Valley in Central Greece [17]. The serpentine soil contains higher Ni (up to 2640 mg/kg) compared to Cr (up to 856 mg/kg). Plants and soils were tested from central Euboea, for Cr (total), Cr(VI), Ni, Mn, Fe, and Zn. Cr(VI) concentrations in soils extracted by alkaline digestion solution were lower than phytotoxic levels. However, foliar contents of Cr and Ni in majority of plants were near or above toxicity levels [17]. Franciscan Complex of California produces serpentine soils containing high concentrations of Cr as well as Ni, Co, and Mn. It has recorded that chromium-bearing igneous and metamorphic silicates in the protolith and Cr-bearing clay minerals in the soil are the major sources of bioavailable Cr [18].

Since some of these areas are associated with agriculture, it may create hazardous effects to the biota. It has observed that the agricultural soil of the valley Atalanti in central Greece has affected by the physical and chemical weathering of mafic and ultramafic rocks outcropping in the surrounding mountainous area, giving rise to high concentrations of Cr, Ni, and Co [15]. Nickel concentrations in groundwater were significantly lower than Cr, especially in the alluvial aquifer of Atalanti, since primary silicate minerals containing Ni in ultramafic rocks are relatively unstable in the surface environment. However, the mobile fraction of Ni is subsequently incorporated into secondary clay minerals and Fe–Mn oxides. Both ligands and protons hasten the release of Ni and Mn from serpentine soil into surrounding environments. Nickel is the seventeenth element recognized as essential for plant growth and development (Liu 2001). Plants' Ni requirement is the lowest of all essential elements at < 0.5 mg per kg of dry weight, making it an essential plant micronutrient [9, 19].

Interestingly, even under such extreme conditions, plant life exists. Serpentine flora exhibits unique features to adapt to its extreme micro-environment. The endemics have developed molecular to macro-level features to withstand these unfavorable conditions [20]. These marvels of nature can be used for biotechnological applications in phytoremediation approaches. A comprehensive understanding of cellular and biochemical processes is essential to step forward in further research. Even though studies have been conducted on heavy metal chemistry and serpentine flora in different regions, there is a lack of research carried on Ni hyperaccumulation mechanisms, especially on Ni transport across the plasma membrane and sequestration, which can be used for development of phytoremediation via biotechnological modifications. Moreover, there are many other cellular processes that are yet to be revealed to answer why they uptake these heavy metals without developing mechanisms to avoid them. Furthermore, there is a lack of comprehensive reviews on the significance of plant life on serpentine soils. Hence, this chapter focuses a discussion on the serpentine flora distributed throughout the world with their metal uptake mechanisms and tolerance in the light of phytoremediation aspects.

15.2 Plants in Serpentine Soil

Due to high Ni, Cr, Mn, and Co contents in serpentine soils, plants possess several mechanisms to tolerate them. There are distinct differences between plants which are grown in serpentine and nonserpentine soils, even though in the same genus [21]. An extraordinary uptake of heavy metals has been observed in some plants grown in Ni-rich serpentine soils originated from ultramafic rocks in various parts of the world. It has been revealed that about 1000 times greater Ni accumulation has been reported in hyperaccumulators (about 190 species) grown in Ni-rich serpentine soils derived from ultramafic rocks in various parts of the world [22]. Family Buxaceae is recognized as a serpentine plant family in Cuba with 30 species of serpentine endemics [23]. Genus *Buxus* is an evergreen shrub or a small tree. Moreover, the genus *Buxus* is reported in serpentine areas in Japan as well. The largest community of *Buxus microphylla* is located in Sugashima Island in Japan. *Buxus microphylla* showed the highest Ni concentration among the plants collected with 400 mg kg^{-1} of Ni [24]. The genus *Leucocroton*, in family Euphorbiaceae is a common thorny shrub found in xeromorphic serpentine areas in Cuba and there are 27 *Leucocroton* serpentine endemic species in Cuba [22]. Almost all serpentine endemic species are identified as hyperaccumulators while nonserpentine plants in the same area possess lower Ni content [22].

Alyssum bertolonii is the first Ni hyperaccumulator plant discovered from Italy by Minguzzi and Vergnano [25]. They observed a 7900 mg kg^{-1} of Ni in *Alyssum* leaves. *Alyssum serpyllifolium* (Brassicaceae) shows an extensive growth on both limestone and serpentine soils of Iberian Peninsula and southern France. *Alyssum* species are also found in southern Europe, including Portugal, where several ultramafic outcrops are present [26]. Interestingly, the family Brassicaceae contains the highest number (90 taxa) of Ni hyperaccumulators in the temperate region [27]. Due to infertile soil conditions, grass species are prominent in most of the serpentine areas. *Agrostis pallens, Aira caryophyllea, Avena barbata, Bromus carinatus, Bromus madritensis, Cortaderia selloana, Cynosurus echinatus, Elymus glaucus, Festuca arundinacea, Festuca bromoides, Hordeum brachyantherumm, Hordeum marinum, Hordeum murinum, Melica Californcia, Polypogon monspeliensis, Stipa lepida*, and *Stipa lepida* are some of the grass species that have been found in California [28, 29].

15.3 Plant Heavy Metal Uptake Mechanisms in Serpentine Soil

15.3.1 Effect of pH on Plant Heavy Metal Uptake

Hyperaccumulators are the plants that are able to uptake high doses of heavy metals. According to [30], metal hyperaccumulator plants are able to uptake metals from the soil, translocate them to their shoots, and concentrate in a range of 100- to

1000-fold more than the nonhyperaccumulating plants. The xylem translocation of metals from the roots to aerial parts is a characteristic feature of hyperaccumulator phenotype [31]. Hyperaccumulator plants are able to accumulate heavy metals in their shoot tissues at high concentrations. Even though the soil contains a high or low amount of heavy metals, the hyperaccumulating plants show an extraordinary ability to uptake metals from soils [33]. The soil heavy metal content, pH, Eh, soil water content, soil organic matter, and other elements in the rhizosphere affect the bioavailability and plant uptake of heavy metals [34, 35].

Changes in pH and redox potential in the rhizosphere soil were studied in a Ni hyperaccumulator plant (*Alyssum murale*) and of a crop plant, radish (*Raphanus sativus*). Differences in rhizosphere pH and reducing activity were found between the lateral and the main roots of both species, but the pH changes in the rhizosphere were similar in both species. Changes in pH were associated with the relative uptake of cations and anions and the source of nitrogen. However, the reducing activity of *A. murale* was always smaller than that of radish. They conclude that the reduction of pH in the rhizosphere or the release of reductants from roots do not involve in the metal uptake mechanism in hyperaccumulators [36].

The organic acids secreted by both plant root system and rhizospheric organisms cause pH reduction thus leading to mobilization of heavy metals. The amount of low molecular weight organic acids (LMWOAs) present in the rhizosphere of durum wheat cultivars, *Arcola* (low Cd accumulator), and *Kyle* (high Cd accumulator) was investigated [37]. The plants were grown for two weeks in pot cultures and the amounts of organic acids were quantified in water extracts of rhizospheric soil. However, water extractable organic acids were unable to be found in the bulk soil. This indicates that the root exudates and/or microbial metabolites at the soil/root interface are the major sources of organic acids found in the rhizosphere soil. Acetic and succinic acids were prominent in the water extracts while oxalic, fumaric, succinic, L-malic, tartaric, citric, acetic, propionic, and butyric acids were found in minor quantities. The total amount of water extractable LMWOAs in the soils ranged from 50.6 to 5520.3 nmol g^{-1} dry weight. According to the study carried by [37], higher amounts of LMWOAs were present in the rhizosphere soil of high Cd-accumulating cultivar *Kyle* than the low accumulating cultivar *Arcola* irrespective of the soil type.

15.4 Heavy Metal Transport Through Plasma Membrane

The heavy metal transport across the plasma membrane is a significant feature of hyperaccumulators. Heavy metal uptake and transport to different tissues, delivery to metal-requiring proteins in different subcellular localizations, metal storage, and remobilization requires the assistance of transition metal transporters. Membrane transport proteins and intracellular high-affinity binding sites involve in the uptake of metals across the plasma membrane. The zinc-regulated transporter, iron-regulated transporter protein (ZIP) family, the cation diffusion facilitator (CDF) family, the

P_{1B}-type subfamily of P-type ATPases, the natural resistance-associated macrophage protein (NRAMP) family, the yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily, and three subfamilies of ATP-binding cassette (ABC) transporters play a vital role in metal transport across membranes [8, 38, 39].

High-affinity Zn^{2+} uptake, as well as low-affinity Cd^{2+} uptake was observed with *ZNT1* with Zn/Cd-hyperaccumulating *Thlaspi caerulescens*. Moreover, it was found that ZIP transporter is expressed at very high levels in roots and shoots of the hyperaccumulators. Furthermore, increased Zn influx in roots of the hyperaccumulating *Thlaspi* species was detected with respect to plant Zn status [40]. As in Cd/Zn hyperaccumulator, *Alyssum halleri* concentration showed a decreasing uptake of Cd by roots when supplied with increasing Zn. With respect to *A. halleri* and numerous ecotypes of *T. caerulescens*, it reveals that Cd influx is largely influenced due to Zn with a strong preference for Zn over Cd [41]. Moreover, the serpentine and the non-metallicolous populations of *Thlaspi caerulescens* showed low-affinity for Cd and Zn over Ni. This may be due to the Ni hyperaccumulation of the serpentine population in its natural environment. Moreover, this suggests that a Zn transport system might also lead to Ni entrance into roots [42].

Table 15.1 shows the membrane transport systems of heavy metals.

15.5 Heavy Metal Translocation from Root–Shoot

Nonhyperaccumulator plants retain most of the heavy metals in their root cells via detoxifying them by chelation in the cytoplasm or accumulating in vacuoles. Furthermore, hyperaccumulators are characteristically translocating heavy metals to the shoot through the xylem in an efficient manner [43]. It has been observed that lower sequestration into root vacuoles enhances the As translocation in hyperaccumulator plant *Pteris* sp. (*Pteris vittata* and *Pteris cretica*) compared with nonhyperaccumulator species such as *Nephrolepis exaltata* [44]. Due to the low association constants of malate and citrate (organic acids) toward the metals, their complexes make less bioavailable under cytosolic pH values. Moreover, they could be considered only within the acidic vacuolar environment [43]. Interestingly, a significant role in heavy metal hyperaccumulation seems to be played by free amino acids, such as histidine and nicotinamine, due to the formation of stable complexes with divalent cations [43, 45].

Nicotianamine is a metal-chelating, nonproteinogenous amino acid produced by the trimerization of *S*-adenosylmethionine catalyzed by nicotianamine synthase, which is essential in plant metal homeostasis. As nicotianamine synthase gene of *Arabidopsis thaliana* was expressed in transgenic tobacco plants, it led to a significant increase in iron levels in leaves and an increase in the tolerance ability against Ni (upto 1 mM) [46]. Furthermore, enhanced chelation of Ni(II) by nicotianamine in the xylem has been suggested as a mechanism of Ni tolerance in the hyperaccumulator *Thlaspi caerulescens*. Furthermore, Ni–NA complexes have been detected in roots of Ni-treated Zn-hyperaccumulator *Thlaspi caerulescens* [46]. Transgenic *Arabidopsis*

Table 15.1 Membrane transport systems of heavy metals

Transport system	Heavy metal	Plant	Reference
ZIP family	Zn/Cd	*Thlaspi caerulescens*	Pence et al. [40]
	Zn	*Arabidopsis thaliana*	Grotz et al. [69]
ATPase	Cd	*Lactuca sativa*	Costa and Morel [70]
	Cd	*Beta vulgaris*	Lindberg and Wingstrand [71]
	Cd	*Thlaspi caerulescens*	Bernard et al. [72]
	Cd	*Lupines albus*	Costa and Morel [73]
	Cd	*Zea mays*	Astolfi et al. [74], Pál et al. [75]
	Ni/Cd	*Oryza sativa*	Ros et al. [76]
	Cd	*Arabidopsis thaliana*	Courbot et al. [77]
	Zn/Cd	*Oryza sativa*	Satoh-Nagasawa et al. [78]
Nramp	Cd and Fe	*Arabidopsis thaliana*	Thomine et al. [79]
	Cd and Mn	*Oryza sativa*	Sasaki et al. [80]
	Cd	*Thlaspi caerulescens*	Zhao et al. [41]
	Cd	*Arabidopsis thaliana*	Vacchina et al. [46]
	Cd	*Lycopersicon esculentum*	López-Millán et al. [81]
	Ni	*Thlaspi japonicum*	Mizuno et al. [82]
CDF	Ni/Zn	*Thlaspi goesingense*	Kim et al. [83]
	Ni	*Alyssum lesbiacum*, *Brassica juncea*	Kerkeb and Krämer [49]
	Cd	*Arabidopsis halleri*	Zhao et al. [84]
ABC transporters	Cd	*Arabidopsis thaliana*	Benavides et al. [85]
	Cd/Pb	*Arabidopsis thaliana*	Kim et al. [86]
	As	*Arabidopsis thaliana*	Song et al. [87]
	Cd/Hg	*Arabidopsis thaliana*	Park et al. [88]

and tobacco plants showed an enhanced biosynthesis which granted a pronounced tolerance of high Ni levels. Moreover under high Ni concentrations, the activities of nicotianamine synthase genes in *Arabidopsis* were increased. Furthermore, transgenic tobacco plants with a high level of nicotianamine grew well in a Ni enriched serpentine soil without developing any Ni toxicity symptoms [47].

Free histidine (His) has been considered as one of the most important Ni-binding ligand that increases xylem transport of Ni. Due to the presence of carboxylate, amine, and imadazole function it acts as a tridentate ligand. Histidine has a relatively high association constant (8.7 lg K) toward Ni. High Ni concentrations have shown an activation of free histidine production in a larger proportion with hyperaccumulator *Alyssum lesbiacum* while there was no such indication in the nonaccumulator *Alyssum montanum* [48]. Moreover, Ni tolerance, Ni transport from roots to the shoots was enhanced with foliar spray or by addition to the root medium to nonaccumulator *Alyssum montanum* [49]. Similarly, an enhanced release of Ni into the xylem is coupled with the release of histidine from free histidine pool in roots of both

Alyssum lesbiacum and *Brassica juncea* [49]. Similarly Ingle et al. [50] has shown that the nonaccumulator *B. juncea* increased the root-to-shoot mobility of Ni with exogenous histidine. Furthermore, it has suggested that ATP-phosphoribosyltransferase (ATP-PRT) expression plays a major role in regulating the free histidine content, which causes the exceptional Ni tolerance of hyperaccumulator *Alyssum* species. It has suggested that the overexpression of genes coding for transport systems in non-hyperaccumulators is the cause efficient root-to-shoot heavy metal translocation hyperaccumulator plants.

YSL (Yellow Strip1-Like) family members are also considered as an important group involved in heavy metal translocation in plants. YSL family members assist the xylem loading and unloading nicotianamine metal (Ni) chelates [51]. Three *YSL* genes in *Thlaspi caerulescens* have been identified in hyperaccumulation of several heavy metals. Which were TcYSL7, TcYSL5 and TcYSL3. They were highly expressed respectively in the flowers, shoots and in all the organs tested. The transport activity of NA-metal chelates, upregulation, and the localization of the expression of these *TcYSL* genes around the vascular tissues are high and efficient in all organs. This feature of the *YSL* genes in *T. caerulescens* is important for the long-distance transport of metal ions (mainly Ni). The capacity of TcYSL3 to catalyze the influx of Ni–NA is vital for the entry of Ni–NA into the symplastic transport in the root for delivery to the xylem and for the unloading of the Ni–NA complexes from the xylem in the leaves to the final storage sites [52].

15.6 Storage and Detoxification of Heavy Metals and Excess Mg

As the heavy metals are taken up, they are directed for detoxification and storage. Detoxification and storage are major mechanisms that are shown by hyperaccumulators. Since the free form of heavy metals is toxic for plant biochemical and physiological activities, hyperaccumulators lead them to concentrate in aboveground organs without causing any phytotoxic effect [53]. The heavy metal detoxification sites in leaves are located in epidermis, trichomes, cuticles, and as latex to cause a minimal damage to the photosynthetic machinery [54, 55]. Accumulation of Ni in vacuoles of epidermal cells in *Hybanthus floribundus* (a hyperaccumulator of Ni) leaves was investigated at relatively high spatial resolution using energy-dispersive X-ray microanalysis (EDAX). Moreover, it was observed that Ni is concentrated on the outside of cell walls throughout the leaves, indicating that apoplastic compartmentation is also involved in Ni tolerance and accumulation in this plant [53].

CDF (cation diffusion facilitator) family members are proteins, which mediate bivalent cation transfer from the cytosol, across plasma membrane/tonoplast. It has been observed that CDF proteins are involved in mediating the vacuolar storage of Ni in *Thlaspi goesingense* shoots [56]. Organic acids are small ligands, which play a major role in detoxification. Where these ligands lead the bound form of metal cations and accumulate them in vacuoles. However, citrate is the main ligand of Ni in leaves of *Thlaspi goesingense* [37]. Phytochelatins (PC) are heavy metal binding peptides and it is another plant heavy metal immobilization mechanism [57]. PC synthase-deficient mutants of *Arabidopsis* have been used to extract the apparent role of PCs in heavy metal detoxification. It has revealed that, PCs play an important role in Cd and arsenate detoxification and there is no significant role shown in the detoxification of Zn, Ni, and selenite ions. During the detoxification process with respect to PC, the metal ion activates PC synthase followed by chelation by the PCs synthesized, and then transported to the vacuole and immobilize in the vacuole with sulfide or organic acids. Moreover, Zn and Ni are relatively ineffective activators of PC synthase in vitro [58].

Due to the high Mg:Ca in serpentine soils, the serpentine plants show tolerance towards high Mg and Ca levels. Most plants show a huge Mg requirement for maximum growth and some plants have several mechanisms to remove Mg from their leaves. The genetic basis of adaptation to serpentine soils in *Mimulus guttatus* was studied by [59]. It was observed that none—serpentine plants are unable to survive on serpentine soils. There was a significant difference in tolerance to low Ca:Mg in serpentine and nonserpentine populations of *M. guttatus*. The ecotypic differences in survival and tolerance were characterized using the genetic basis of quantitative trait locus (QTL) mapping. A single major QTL that controls both the ability to survive on serpentine soils and tolerance to low Ca:Mg ratio was identified. It suggests that the major QTL consists of different serpentine tolerance alleles which are not functionally equivalent [60].

It was clearly observed that the growth of crop plants retarded considerably as soil Ca levels dropped. However, the native serpentine species were not significantly affected by changing Ca concentrations. Analysis of plant tissues revealed that as both the plants were grown in soils with low Ca:Mg ratios, native serpentine species absorbed more Ca and less Mg than the crop plants. Because serpentine-tolerant species are able to absorb an adequate amount of Ca without absorbing an excessive amount of Mg [61]. Even though these plants absorb more Mg it is not toxic to the plant. Analysis of *Agrostis* spp. growing on serpentine soils shows that, as they uptake an excess amount of magnesium/calcium without causing negative effects on their survival [61]. As Ca plays a major role in plant cell wall stability and cell-to-cell adhesion [62], Ca deficiency causes lessening the strength of cell wall extension, breakdown of cell walls, and tissue destruction [32]. These symptoms are mostly seen in actively growing tissues. Serpentine plants need specialized physiological mechanisms to maintain a sufficient internal concentration of Ca under the higher Ca:Mg (<1) in soil [60].

High substratum concentrations of Mg in the serpentine soil are antagonistic to plant Ca uptake [32]. Due to immobilization nature of Ca, it shows a unidirectional transport which is from the root elongation zone to the shoot. Hence, the growing root tips should obtain the required Ca from its immediate environment [32]. High concentration of Mg in the serpentine soil causes induction of Ca deficiency in growing root tips, which leads necrosis of root tip [63].

15.7 Use of Serpentine Flora for Heavy metal Remediation

A number of species in the genus Alyssum has been recorded as Ni hyperaccumulators [50]. *Alyssum bertolonii* is a hyperaccumulator plant, which is grown in Ni-rich serpentine soil. It has an extraordinary Ni accumulating ability, which contains about 1 % Ni with respect to the dry matter. Experiments have carried out for the use of *Alyssum bertolonii* in phytomining of Ni. It was observed that there was no relationship between age of the plant and its Ni content. It has suggested that *Alyssum bertolonii* and other Alyssum species could be used for phytomining in Mediterranean area, Western Australia, and Western United States [64].

A number of species in the genus Alyssum has been recorded as Ni hyperaccumulators [50]. Ni phytoextraction was assessed in A. lesbiacum with phenanthrene and chrysene (polycyclic aromatic hydrocarbons). According to the results Alyssum lesbiacum has the ability to uptake Ni in polycyclic aromatic hydrocarbons contaminated soils [65]. *Thalspi* is also a plant, which is principally grown in Ni-rich contaminated soil. However, it has been indicated that *Thlaspi* is able to hyperaccumulate more than one metal. Principally, *Thlaspi* grows on Ni contaminated sites and accumulates about 3 % of its dry weight. Moreover *T. caerulescens* and *T. rotundifolium* can accumulate Cd, Ni, Zn and also Pb whereas *T. ochroleucum* hyperaccumulate Ni and Zn. Thereby species in the *Thlaspi* genus can be used to remediate heavy metal-contaminated sites [48, 66]. Moreover, *Berkheya coddii* is an asteraceous perennial plant grown in serpentine soils. It has reported as a hyperaccumulator, which accumulates about 1.16 % foliar Ni concentrations. Its higher Ni uptaking ability, high biomass, tolerance to cool climates render the hyperaccumulation ability to this plant in contaminated sites [67]. Furthermore, it has been revealed that the Ni-hyperaccumulator *Streptanthus polygaloides* could yield a 100 kg/ha of sulfur-free Ni [68].

15.8 Remarks

Serpentine soils derived from ultramafic rock depositions have given rise to a specialized vegetation, which shows tolerance to the heavy metal-rich less nutritious soil. To withstand against these harsh conditions a specialized flora has evolved associated with the serpentine areas, which distributed as patches in all over the world. This endemic vegetation is highly specialized to the particular regions with numerous mechanisms of heavy metal uptake and storage. The metal uptake is the most crucial part of the hyperaccumulators where un-heavy metal accumulators do not allow. Though numerous research has come up with heavy metal (As, Cd) transporters during recent decade, there is a gap to fill with respect to Ni transport. Interestingly, the xylem transport and vacuolar sequestration have studied in detail with important results. Moreover, the knowledge of cell biology of these plants can be used as biotechnological tools for further research in genetic engineering and phytomining activities.

Not like in normal soils serpentine soil plants are able to live under a higher Mg:Ca ratio. However, the mechanism that allows for greater growth rates and higher biomass in serpentine plants despite low calcium availability is not clear.

- Shoot translocation is one of the major features in serpentine vegetation. However, more research is needed for the clear understanding of mechanisms in metal chelation in long-distance transport of metal.
- Though many studies are conducted under other heavy metals such as Cd and As, research on Ni transporters across the plasma membrane is much lesser.
- More studies are suggested with the involvement of organic acids in heavy metal transport through the xylem.
- It has been revealed that hyperaccumulators create a high heavy metal concentration in their cytoplasm to keep a high osmotic potential to avoid excess metal uptake. However, it is not clear that osmotic potential is created via free ions or with bound forms. Moreover if it is in free-ion form the mechanisms to avoid heavy metal toxicity should be further revealed.
- These patches of ecosystems, which are distributed worldwide, consist of a wide genetic diversity. Numerous endemics, which consist vital biochemical compounds could be used as pharmaceuticals. More studies should be carried toward the biochemistry and genetic of such vegetations.
- Even though it is hypothesized that phytoaccumulation of heavy metals causes allelopathic effects on neighboring plants, there is not enough body of knowledge to confirm it.
- A comprehensive understanding of the metal transport processes in plants is essential for formulating effective strategies to develop genetically engineered plants that can accumulate specific metals.

References

1. Farough A, Moore D, Lockner D, Lowell R (2016) Evolution of fracture permeability of ultramafic rocks undergoing serpentinization at hydrothermal conditions: an experimental study. Geochem Geophys Geosyst. doi:10.1002/2015GC005973
2. Kruckeberg AR (1969) Soil diversity and the distribution of plants, with examples from western North America. Madroño 20:129–154
3. Robinson BH, Brooks RR, Kirkman JH, Gregg PE, Alvarez HV (1997) Edaphic influences on a New Zealand ultramafic ("serpentine") flora: a statistical approach. Plant and Soil 188:11–20
4. Cheng C-H, Jien S-H, Iizuka Y, Tsai H, Chang Y-H, Hseu Z-Y (2011) Pedogenic chromium and nickel partitioning in serpentine soils along a toposequence. Soil Sci Soc Am J 75:659–668
5. Oze C, Fendorf S, Bird DK, Coleman RG (2004) Chromium geochemistry of serpentine soils. Int Geol Rev 46:97–126
6. Garnier J, Quantin C, Guimarães EM, Vantelon D, Montargès-Pelletier E, Becquer T (2013) Cr (VI) genesis and dynamics in Ferralsols developed from ultramafic rocks: the case of Niquelândia, Brazil. Geoderma 193:256–264
7. Hseu Z-Y, Iizuka Y (2013) Pedogeochemical characteristics of chromite in a paddy soil derived from serpentinites. Geoderma 202:126–133

8. Sadegh MJM, Heidari A, Sarmadian F (2012) The role of pedogenic processes and soil characteristics on nickel distribution in some Oxiaquic Paleudalfs. Int Res J Appl Basic Sci 3(5):1032–1039
9. Vithanage M, Rajapaksha AU, Tang X, Thiele-Bruhn S, Kim KH, Lee S-E, Ok YS (2014) Sorption and transport of sulfamethazine in agricultural soils amended with invasive-plant-derived biochar. J Environ Manage 141:95–103
10. Rajakaruna N, Bradfield GE, Bohm BA, Whitton J (2003) Adaptive differentiation in response to water stress by edaphic races of Lasthenia californica (Asteraceae). Int J Plant Sci 164:371–376
11. Seneviratne M, Seneviratne G, Madawala H, Iqbal M, Rajakaruna N, Bandara T, Vithanage M (2015) A preliminary study of the role of bacterial–fungal co-inoculation on heavy metal phytotoxicity in serpentine soil. Aust J Bot 63:261–268
12. Bayer RJ, Figura PJ (2015) Antennaria sawyeri (Asteraceae: Gnaphalieae), a new serpentine endemic species from the Klamath mountains of Northern California. Syst Bot 40:620–626
13. Brady KU, Kruckeberg AR, Bradshaw H Jr (2005) Evolutionary ecology of plant adaptation to serpentine soils. Annu Rev Ecol Evol Syst 36:243–266
14. Cox SF, Chelliah MC, McKinley JM, Palmer S, Ofterdinger U, Young ME, Cave MR, Wragg J (2013) The importance of solid-phase distribution on the oral bioaccessibility of Ni and Cr in soils overlying Palaeogene basalt lavas, Northern Ireland. Environ Geochem Health 35:553–567
15. Kanellopoulos C, Argyraki A, Mitropoulos P (2015) Geochemistry of serpentine agricultural soil and associated groundwater chemistry and vegetation in the area of Atalanti, Greece. J Geochem Explor 158:22–33
16. Shchegolikhina A, Guadagnini L, Guadagnini A (2015) Genesis and transport of hexavalent chromium in the system ophiolitic rocks-groundwater, EGU General Assembly Conference Abstracts, p 754
17. Megremi I (2010) Distribution and bioavailability of Cr in central Euboea, Greece. Open Geosci 2:103–123
18. Oze C, Fendorf S, Bird DK, Coleman RG (2004) Chromium geochemistry in serpentinized ultramafic rocks and serpentine soils from the Franciscan complex of California. Am J Sci 304:67–101
19. Rajapaksha AU, Vithanage M, Oze C, Bandara W, Weerasooriya R (2012) Nickel and manganese release in serpentine soil from the Ussangoda Ultramafic Complex, Sri Lanka. Geoderma 189:1–9
20. Reeves RD, Laidlaw WS, Doronila A, Baker AJ, Batianoff GN (2015) Erratic hyperaccumulation of nickel, with particular reference to the Queensland serpentine endemic Pimelea leptospermoides. Aust J Bot 63:119–127
21. Chathuranga P, Dharmasena S, Rajakaruna N, Iqbal M (2015) Growth and nickel uptake by serpentine and non-serpentine populations of Fimbristylis ovata (Cyperaceae) from Sri Lanka. Aust J Bot 63:128–133
22. Reeves R, Baker A, Bgrhidi A, Berazain R (1996) Nickel-accumulating plants from the ancient serpentine soils of Cuba. New Phytol 133:217–224
23. Reeves R, Baker A, Borhidi A, Berazain R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. Ann Bot 83:29–38
24. Mizuno T, Kirihata Y (2015) Elemental composition of plants from the serpentine soil of Sugashima Island, Japan. Aust J Bot 63(4):252–260
25. Minguzzi C, Vergnano O (1948) Il contenuto di nichel nelle ceneri di Alyssum bertolonii. Atti Soc Tosc Sci Natur 55:49–77
26. Freitas H, Prasad M, Pratas J (2004) Analysis of serpentinophytes from north–east of Portugal for trace metal accumulation—relevance to the management of mine environment. Chemosphere 54:1625–1642
27. Ghaderian S, Mohtadi A, Rahiminejad R, Reeves R, Baker A (2007) Hyperaccumulation of nickel by two Alyssum species from the serpentine soils of Iran. Plant and Soil 293:91–97
28. Harrison S, Rice K, Maron J (2001) Habitat patchiness promotes invasion by alien grasses on serpentine soil. Biol Conserv 100:45–53

29. Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478–491
30. Brooks R, Lee J, Reeves RD, Jaffré T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57
31. Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315
32. Marschner H (2011) Marschner's mineral nutrition of higher plants. Academic, Amsterdam
33. Clemens S, Ma JF (2016) Toxic heavy metal and metalloid accumulation in crop plants and foods. Annu Rev Plant Biol 67:517–534
34. Adamczyk-Szabela D, Markiewicz J, Wolf WM (2015) Heavy metal uptake by herbs. IV. Influence of soil pH on the content of heavy metals in Valeriana officinalis L. Water Air Soil Pollut 226:1–8
35. Martínez-Alcalá I, Bernal MP, de la Fuente C, Gondar D, Clemente R (2016) Changes in the heavy metal solubility of two contaminated soils after heavy metals phytoextraction with Noccaea caerulescens. Ecol Eng 89:56–63
36. Bernal M, McGrath S, Miller A, Baker A (1994) Comparison of the chemical changes in the rhizosphere of the nickel hyperaccumulator Alyssum murale with the non-accumulator Raphanus sativus. Plant and Soil 164:251–259
37. Cieśliński G, Van Rees K, Szmigielska A, Krishnamurti G, Huang P (1998) Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant and Soil 203:109–117
38. Hall J, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613
39. McGrath SP, Zhao F-J (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282
40. Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator Thlaspi caerulescens. Proc Natl Acad Sci U S A 97:4956–4960
41. Zhao FJ, Hamon RE, Lombi E, McLaughlin MJ, McGrath SP (2002) Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator Thlaspi caerulescens. J Exp Bot 53:535–543
42. Assunção AG, Bleeker P, Wilma M, Vooijs R, Schat H (2008) Intraspecific variation of metal preference patterns for hyperaccumulation in Thlaspi caerulescens: evidence from binary metal exposures. Plant and Soil 303:289–299
43. Haydon MJ, Cobbett CS (2007) Transporters of ligands for essential metal ions in plants. New Phytol 174:499–506
44. Poynton CY, Huang JW, Blaylock MJ, Kochian LV, Elless MP (2004) Mechanisms of arsenic hyperaccumulation in Pteris species: root As influx and translocation. Planta 219:1080–1088
45. Callahan DL, Baker AJ, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12
46. Vacchina V, Mari S, Czernic P, Marquès L, Pianelli K, Schaumlöffel D, Lebrun M, Lobinski R (2003) Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation. Anal Chem 75:2740–2745
47. Kim S, Takahashi M, Higuchi K, Tsunoda K, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK (2005) Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiol 46:1809–1818
48. Charnock J, JAC JBAS (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638
49. Kerkeb L, Krämer U (2003) The role of free histidine in xylem loading of nickel in Alyssum lesbiacum and Brassica juncea. Plant Physiol 131:716–724
50. Ingle RA, Mugford ST, Rees JD, Campbell MM, Smith JAC (2005) Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. Plant Cell 17:2089–2106

51. Colangelo EP, Guerinot ML (2006) Put the metal to the petal: metal uptake and transport throughout plants. Curr Opin Plant Biol 9:322–330
52. Gendre D, Czernic P, Conéjéro G, Pianelli K, Briat JF, Lebrun M, Mari S (2007) TcYSL3, a member of the YSL gene family from the hyper-accumulator Thlaspi caerulescens, encodes a nicotianamine-Ni/Fe transporter. Plant J 49:1–15
53. Bidwell S, Crawford S, Woodrow I, Sommer-Knudsen J, Marshall A (2004) Sub-cellular localization of Ni in the hyperaccumulator, Hybanthus floribundus (Lindley) F. Muell. Plant Cell Environ 27:705–716
54. Freeman JL, Zhang LH, Marcus MA, Fakra S, McGrath SP, Pilon-Smits EA (2006) Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants Astragalus bisulcatus and Stanleya pinnata. Plant Physiol 142:124–134
55. Küpper H, Lombi E, Zhao F-J, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator Arabidopsis halleri. Planta 212:75–84
56. Persans MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in Thlaspi. Proc Natl Acad Sci U S A 98:9995–10000
57. Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486
58. Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832
59. Selby JP (2014) The genetic basis of local adaptation to serpentine soils in Mimulus guttatus. Duke University, Durham
60. Ghasemi R, Chavoshi ZZ, Boyd RS, Rajakaruna N (2015) Calcium: magnesium ratio affects environmental stress sensitivity in the serpentine-endemic Alyssum inflatum (Brassicaceae). Aust J Bot 63:39–46
61. Proctor J (1971) The plant ecology of serpentine: III. The influence of a high magnesium/calcium ratio and high nickel and chromium levels in some British and Swedish serpentine soils. J Ecol 59:827–842
62. Jarvis M, Briggs S, Knox J (2003) Intercellular adhesion and cell separation in plants. Plant Cell Environ 26:977–989
63. O'Dell RE, James JJ, Richards JH (2006) Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca: Mg than in tolerance of low N, low P, or heavy metals. Plant and Soil 280:49–64
64. Robinson B, Chiarucci A, Brooks R, Petit D, Kirkman J, Gregg P, De Dominicis V (1997) The nickel hyperaccumulator plant Alyssum bertolonii as a potential agent for phytoremediation and phytomining of nickel. J Geochem Explor 59:75–86
65. Singer AC, Bell T, Heywood CA, Smith J, Thompson IP (2007) Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant Alyssum lesbiacum: evidence of histidine as a measure of phytoextractable nickel. Environ Pollut 147:74–82
66. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PE (1998) The potential of Thlaspi caerulescens for phytoremediation of contaminated soils. Plant and Soil 203:47–56
67. Robinson B, Brooks R, Howes A, Kirkman J, Gregg P (1997) The potential of the high-biomass nickel hyperaccumulator Berkheya coddii for phytoremediation and phytomining. J Geochem Explor 60:115–126
68. Anderson C, Brooks R, Chiarucci A, LaCoste C, Leblanc M, Robinson B, Simcock R, Stewart R (1999) Phytomining for nickel, thallium and gold. J Geochem Explor 67:407–415
69. Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D (1998) Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. Proc Natl Acad Sci U S A 95:7220–7224
70. Costa G, Morel J (1994) Efficiency of H^+-atpase activity on cadmium uptake by four cultivars of lettuce. J Plant Nutr 17:627–637
71. Lindberg S, Wingstrand G (1985) Mechanism for Cd^{2+} inhibition of (K^{++} Mg^{2+}) ATPase activity and K^+ ($86Rb^+$) uptake join roots of sugar beet (Beta vulgaris). Physiol Plant 63:181–186

72. Bernard C, Roosens N, Czernic P, Lebrun M, Verbruggen N (2004) A novel CPx-ATPase from the cadmium hyperaccumulator Thlaspi caerulescens. FEBS Lett 569:140–148
73. Costa G, Morel J (1993) Cadmium uptake by Lupinus albus (L.): cadmium excretion, a possible mechanism of cadmium tolerance. J Plant Nutr 16:1921–1929
74. Astolfi S, Zuchi S, Passera C (2005) Effect of cadmium on H^+ ATPase activity of plasma membrane vesicles isolated from roots of different S-supplied maize (Zea mays L.) plants. Plant Sci 169:361–368
75. Pál M, Horváth E, Janda T, Páldi E, Szalai G (2006) Physiological changes and defense mechanisms induced by cadmium stress in maize. J Plant Nutr Soil Sci 169:239–246
76. Ros R, Morales A, Segura J, Picazo I (1992) In vivo and in vitro effects of nickel and cadmium on the plasmalemma ATPase from rice (Oryza sativa L.) shoots and roots. Plant Sci 83:1–6
77. Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, Saumitou-Laprade P, Verbruggen N (2007) A major quantitative trait locus for cadmium tolerance in Arabidopsis halleri colocalizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiol 144:1052–1065
78. Satoh-Nagasawa N, Mori M, Nakazawa N, Kawamoto T, Nagato Y, Sakurai K, Takahashi H, Watanabe A, Akagi H (2012) Mutations in rice (Oryza sativa) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. Plant Cell Physiol 53:213–224
79. Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI (2000) Cadmium and iron transport by members of a plant metal transporter family in Arabidopsis with homology to Nramp genes. Proc Natl Acad Sci U S A 97:4991–4996
80. Sasaki A, Yamaji N, Yokosho K, Ma JF (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24:2155–2167
81. López-Millán A-F, Sagardoy R, Solanas M, Abadía A, Abadía J (2009) Cadmium toxicity in tomato (Lycopersicon esculentum) plants grown in hydroponics. Environ Exp Bot 65:376–385
82. Mizuno T, Usui K, Horie K, Nosaka S, Mizuno N, Obata H (2005) Cloning of three ZIP/Nramp transporter genes from a Ni hyperaccumulator plant Thlaspi japonicum and their Ni^{2+}-transport abilities. Plant Physiol Biochem 43:793–801
83. Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun DJ, Salt DE (2004) The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator Thlaspi goesingense acts to enhance efflux of Zn at the plasma membrane when expressed in Saccharomyces cerevisiae. Plant J 39:237–251
84. Zhao F, Jiang R, Dunham S, McGrath S (2006) Cadmium uptake, translocation and tolerance in the hyperaccumulator Arabidopsis halleri. New Phytol 172:646–654
85. Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34
86. Kim DY, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50:207–218
87. Song W-Y, Park J, Mendoza-Cózatl DG, Suter-Grotemeyer M, Shim D, Hörtensteiner S, Geisler M, Weder B, Rea PA, Rentsch D (2010) Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci U S A 107:21187–21192
88. Park J, Song WY, Ko D, Eom Y, Hansen TH, Schiller M, Lee TG, Martinoia E, Lee Y (2012) The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. Plant J 69:278–288