Metal Hyperaccumulator Plants and Their Role in Phytoremediation

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Abstract

Several hundred plant species are documented as metal hyperaccumulators, and a majority of them are restricted to metalliferous soils and are known as obligate hyperaccumulators. However, some other plant species are widely spread in metalliferous and non-metalliferous soils, and hyperaccumulate metals when occurring in metalliferous habitats. These plant species are listed as facultative hyperaccumulators. This phenomenon of metal hyperaccumulation has profound implications in the field of phytoremediation.

Metal hyperaccumulator plants have developed a number of regulatory mechanisms, including heavy metal absorption, transportation, chelation, and detoxification, for their survival in the metal-contaminated environment. Several metalloproteins or metallochaperone-like proteins containing conserved heavy metal-associated (HMA) domains are involved in metal binding and transport. P1B-metal transporting ATPases are of particular interest for their role in metal transport at the cellular and subcellular levels in accumulator plants. The genomic data of accumulator plants in *the Brassicaceae* have shown many upregulated and downregulated genes in accumulator plants when encountering heavy metal stress. Nucleotide and protein sequences from different websites such as http://www.ncbi.nih.gov, http://www.tigr.org, http://www.brassica.info, etc. that encode heavy metal ATPases and transporter protein homologs were collected.

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The functional and evolutionary similarities in the genes and proteins induced by heavy metals among different accumulator and non-accumulator species were analyzed. In the present communication, we have overviewed these findings and highlighted the role of transporter proteins in metal homeostasis in hyperaccumulator plants.

Keywords

Phytoremediation · Hyperaccumulator plants · Metal transporters · Heavy metal ATPases

1.1 Introduction

Heavy metal pollution is a serious global challenge that needs urgent attention. The high amount of heavy metals, especially toxic metals, reduces plant growth and negatively affects the physiological and metabolic processes, including the inhibition in respiration and photosynthesis, which could lead to plant death (Garbisu and Alkorta 2001; Schmidt 2003; Schwartz et al. 2003). In addition, metal contamination in the soil has a negative impact on the soil microbial population, and it alters the composition and structure of the soil (Giller et al. 1998; Kozdroj and van Elsas 2001; Kurek and Bollag 2004). In the USA and China major problem of land contamination by heavy metals have been reported and represent a great challenge for agriculturist and environmentalists (McKeehan and Kan 2000; Liu et al. 2007). Small industrial units are pouring their untreated effluents into surface drains that extend through agricultural fields in India, Pakistan, and Bangladesh, causing significant soil and water pollution (Lone et al. 2008). The plants absorb contaminants through the root system and transport them up in the shoots. Heavy metals such as Cu, Zn, Mn, Fe are essential micronutrients for plant growth but are potentially phytotoxic to plants when found in high amounts in the soil. As Cd, Pb, Cr, Ni, and Hg have been identified in polluted soils and water and most of these metal/metalloids are non-essential to plant growth and toxic to the plant both at a cellular and subcellular level (Memon et al. 2001). The toxicity of these metals alters or inhibits numerous metabolic processes at the cellular level, such as inhibiting enzymes required for cell functioning and disrupting the membrane integrity. The toxic amount of the metal increases the production of reactive oxygen species (ROS) (Pagliano et al. 2006). It induces oxidative stress, deteriorates membrane integrity, and damages the DNA (Quartacci et al. 2001). However, some unique plant species can grow and flourish on both the natural metalliferous soils and as well as on heavy metal polluted soils because of anthropogenic activities.

The European Union launched a comprehensive heavy metal survey program to estimate the heavy metal content of the topsoil of European Union Countries named LUCAST Top Soil Survey of the European Union (Tóth et al. 2013). This survey has opened up new possibilities to get detailed information on the soil cover in Europe, including the heavy metal content data of these soils (Tóth et al. 2016). This survey

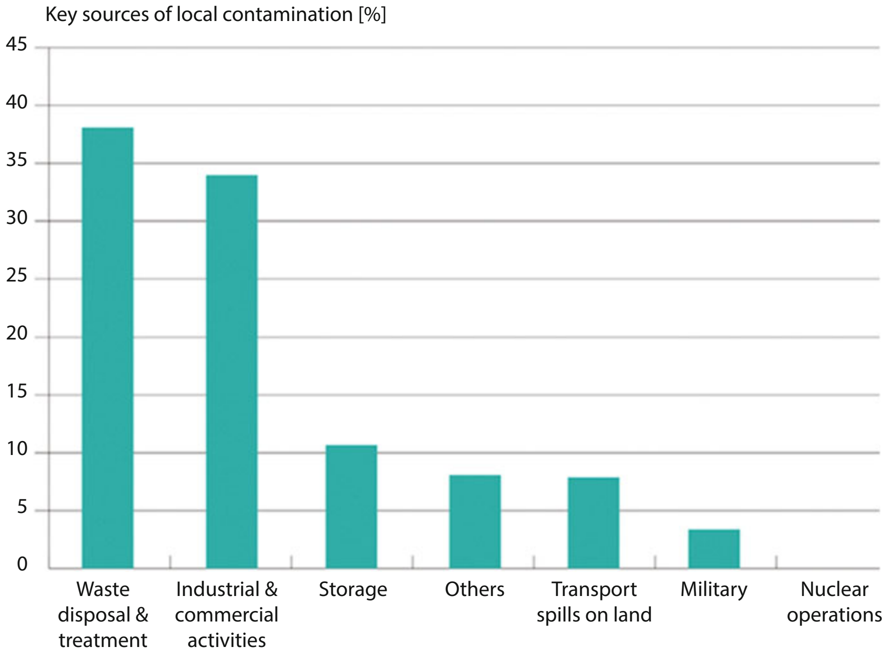

Fig. 1.1 Key sources of contamination reported in 2011 (Van Liedekerke et al. 2014)

is useful to identify the potential heavy metal-contaminated sites and will allow the environmentalists to monitor, control, and clean these contaminated sites for reuse.

The European research study called "Progress in the management of Contaminated Sites in Europe" reported about 2.5 million potentially contaminated sites, of which about 14% (340,000 sites) are estimated to be contaminated (Van Liedekerke et al. 2014). Among EU countries, Belgium, Finland, and Lithuania reported having the highest number of contaminated sites. The major sources of contamination that have the highest impact on soil and water pollution across Europe are shown in Fig. 1.1 (Van Liedekerke et al. 2014). The key contributing factors for soil and water pollution seem to be waste disposal and treatment and industrial and commercial activities (Fig. 1.1).

The most common contaminant in soils and groundwater across Europe is shown in Fig. 1.2. It is noticed that heavy metals are the major contaminants present in water and soils in Europe.

The estimated cost of managing contaminated areas in Europe is around €6.5 billion per year. It corresponds to an average annual national expenditure on managing contaminated sites in on average about €10 per capita (Van Liedekerke et al. 2014). Because of the high cost of the conventional management techniques, there is an urgent need to find out cheaper and more efficient remediation technologies that can be successfully applied to remediate polluted soil and water across Europe and the rest of the world. One of the most efficient biological approaches to contaminated

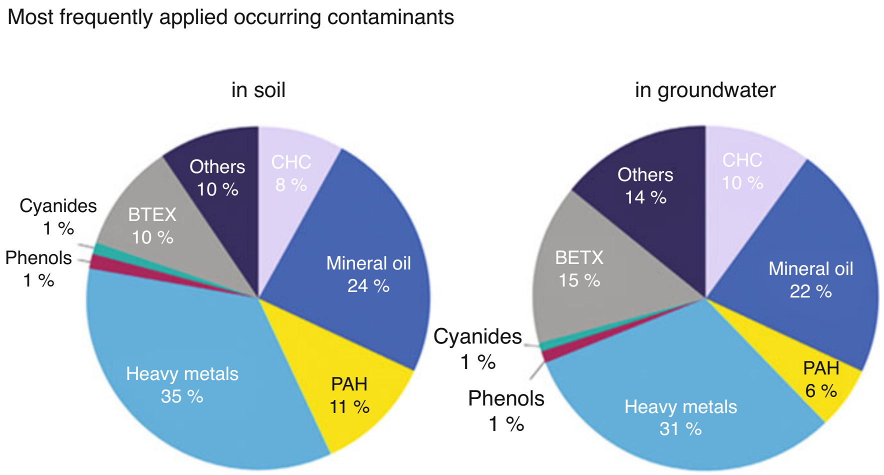

Fig. 1.2 The most frequently occurring contaminants in soil and groundwater are: BTEX—Aromatic Hydrocarbons; CHC—Chlorinated Hydrocarbons; PAH—Polycyclic Aromatic Hydrocarbons

soil and water remediation is phytoremediation. It is considered a new and promising technology for the reclamation of polluted sites and is less costly than other conventional methods like physicochemical approaches, etc. (Garbisu and Alkorta 2001; McGrath et al. 2001; Raskin et al. 1997).

1.2 Advancing Phytoremediation Potential to Clean up the Environmental Pollution

Phytoremediation is a biological process where plants extract, remove, stabilize, or degrade the pollutants from the soil and waters (Salt et al. 1998). Some specific plants can extract, immobilize or metabolize and accumulate organic and inorganic contaminants and remediate the polluted areas for reuse for either agriculture or social (recreational parks, gardens, etc.) purposes. It is considered economical and environmentally friendly biotechnology where plants and microorganisms are used to remove contaminants from polluted soils and industrial waste.

Currently, several physicochemical approaches are being used to clean up contaminated soils. However, physicochemical approaches are generally costly, used on a small scale because they have toxic effects when used on a large scale. Therefore, phytoremediation as a safe biological approach represents a perspective alternative and efficient solution for sustainable environmental cleanup (Salt et al. 1998; Peer et al. 2005; Golubev et al. 2011). Primarily, phytoremediation technology aims to remove or degrade or immobilize environmental pollutants, especially anthropogenic origin, to restore the contaminated sites for reuse in agriculture, forestry, and other public and private applications. Six different phytoremediation methods are briefly listed here; all are commonly used in the phytoremediation of metals and other organic contaminants from soil and water. They include phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, and phytodegradation (Salt et al. 1998; Peer et al. 2005; Thakare et al. 2021; Sarma et al. 2021).

Phytoextraction technology is generally focused on the use of plants to extract and remove metals from soil and water and has been extensively developed by several academic and industrial groups in several countries. The major criterion of this technology is to extract and accumulate metals from the polluted sites and accumulate them in the aerial part of the plant, which can be removed to dispose of or burnt to recover metals (Chaney et al. 2018). We will be mainly discussing this technology in our review paper. Recently Chaney's group has introduced a new term, "agromining," which is possibly derived from this technology and encapsulates the entire series of processes involved in producing metals for commercial or industrial use (van der Ent et al. 2015; Chaney et al. 2018). Rhizofiltration uses plant roots or rhizomes for extracting metals from wastewaters. Phytostabilization is a technique that uses plant roots to absorb contaminants from the soil and make them harmless by preventing them from leaching. In Phytovolatilization, plants take up the elements like Se, As, and Hg and translocate and volatilize pollutants from their foliage. Phytodegradation technology uses plants and related microorganisms to degrade and remove organic contaminants from the soil and water (Salt et al. 1998; Garbisu and Alkorta 2001; Peer et al. 2005).

Phytoremediation offers many advantages over the other conventional physical and chemical methods like precipitation with lime, ion exchange, and precipitation with bio-sulfide, biosorption, etc., which are costly and difficult to handle at a large scale (Khalid et al. 2017). Phytoremediation efforts are mostly focused on using plants in combination with root rhizosphere microorganisms to eliminate toxic heavy metals from soils and water and speeding up the degradation of organic and inorganic contaminants (Silver and Phung 2005; Gerhardt et al. 2017; Sonowal et al. 2022). The advantages that phytoremediation offers are the low cost, minimization of the chemical and biological volume to be disposed of, high efficiency in detoxifying very dilute effluents, and the reuse of the collected heavy metals from contaminated areas. There are several factors that could be considered in developing effective and successful phytoremediation technology. One of the most important factors is identifying or developing (through molecular breeding) an ideal plant/or plant species for effective phytoextraction of toxic metals from the polluted soils or the environment (Suman et al. 2018). Other factors include the use of modern agronomical practices, optimizing crop and soil management practices, and developing cutting edge-technologies for extracting metals efficiently from biomass (Zhuang et al. 2007; Kidd et al. 2015). To develop a suitable plant for phytoextraction following parameters should be considered: rapid metal entry into root tissues needs to be accompanied by efficient metal transport into the shoots. Metal uptake efficiency primarily depends on the bioavailability of the metal in soil (Lu et al. 2018). Bioavailability of heavy metals is the primary factor for effective phytoextraction and describes the degree of availability of the pollutants which plant can take from the soil and sediment. However, metal bioavailability is a complex process and is dependent on many other factors related to the soil structure and chemical composition (McGrath and Zhao 2003). Rhizospheric microbes and root exudates such as siderophores and organic acids can alter the bioavailability of heavy metals in the soil (Thijs et al. 2017). Several elements in the soil and plant roots can mobilize the metals from the soil and enhance the metal uptake through the plant roots. For instance, initial metal uptake can be achieved by mobilizing the metal bound to soil particles through the secretion of organic exudates like mugenic and aveic acids from roots which cause the acidification of the soil and the chelation of metals (Muszyńska et al. 2015). After uptake, the metal is translocated from roots to shoots through xylem tissues. To enter the metal in xylem tissues, it must cross the endodermis through the transporters or channels in the membrane. Once the metal is loaded into the xylem (possibly through metal ATPases and other transporters), it is transported into the leaves and then can be stored in different cells, depending on the chemical form of the metal, since it can be converted into less toxic forms through different chemical mechanisms (conversion or complexation) (Peer et al. 2005).

There are many advantages in using phytoremediation technology for removing contaminants from the environment compared to traditional technologies. First of all, it is cheap and cost-effective, around 50% to 90% cheaper than other conventional chemical or engineering options (Salt et al. 1998; Peer et al. 2005). Secondly, it is easy to dispose of the plants, and it will cause limited disturbance to the landscape (Batty and Dolan 2013). The metals can be extracted easily from biomass to prevent the resulting plant material as hazardous waste. There are some disadvantages of using this technology; for example, it takes a longer time to remediate the soil for reuse. This can be addressed using plant species with a short growth cycle and high biomass (Pollard 2016; Suman et al. 2018).

1.3 Use of Hyperaccumulator Plants for Phytoremediation of Metals from the Polluted Soils

Plants can degrade organic and inorganic contaminants, mainly with the help of root rhizosphere microorganisms (Lone et al. 2008). The metal hyperaccumulator plants grow on metalliferous soils and accumulate extraordinarily high amounts of heavy metals in the aboveground parts, far above the levels found in most plant species, without suffering phytotoxic effects (van der Ent et al. 2013). Hyperaccumulators have three essential characteristics which are lacking in non-hyperaccumulator species; an increase in heavy metal uptake rate, high root-to-shoot translocation, and a greater ability to detoxify and sequester heavy metals in shoots. On the molecular level hyperaccumulator plants have different gene expression and regulation patterns than non-accumulator plants (Goolsby and Mason 2016). The hyperaccumulator plants efficiently absorb and translocate metals from the roots to the shoots and sequester them in the cell wall and vacuole (Memon and Schroder 2009; Memon 2016). Accumulator plants constitutively overexpress the genes encoding membrane transporter proteins, such as ZIPs, HMAs, MATE, YSL, and MTPs for metal transport in the cell (Rascio and Navari-Izzo 2011; Memon and Schroder 2009; Memon 2016). Hyperaccumulator plant species are an important economic source for removing the contaminants from the soil, and the metals can be harvested from the growing plants for marketing (Chaney et al. 2018).

Hyperaccumulator plants actively absorb and take up large amounts of one or more heavy metals from the soil and efficiently translocate to the shoot and accumulated in the aboveground parts of the plant, especially with the leaves, at concentrations 100–1000 fold higher than those found in non-hyperaccumulator species without showing any toxicity symptoms (Reeves and Brooks 1983; Bhargava et al. 2012). Although a distinct feature, hyperaccumulation also relies on hyper tolerance, a distinct feature of the hyperaccumulator plants essential for these plants to avoid heavy metal toxicity. Goolsby and Mason (2015) have highlighted several key issues related to defining the hyperaccumulation trait and proposed a more objective definition of hyper accumulation than the definition previously proposed by van der Ent et al. (2013). This redefined definition reflects both the genetic and physiological mechanisms underlying hyperaccumulation and the evolutionary aspects of this phenomenon. They suggested that hyperaccumulation and tolerance should be considered two distinct continuous traits mediated by genetically and physiologically distinct mechanisms. The plant phenotypes span a wide range of combinations of both traits producing four general categories: tolerant accumulator (traditional hyperaccumulators; e.g., *Astragalus bisculcatus* for Se), non-tolerant accumulator (excluded from the naturalistic definition of hyperaccumulation; e.g., *Thlaspi goesingense* for Zn), non-tolerant non-accumulator, and tolerant non-accumulator (Goolsby and Mason 2015). The two last categories are typically collapsed together as non-hyperaccumulators (for example, *Arabidopsis thaliana* for Cd and *Silene vulgaris* for Cu).

The heavy metal accumulation ability of the plant varies significantly and is dependent on the type of the species and cultivars within the species. The different mechanisms of ion uptake are operating in each species, based on their genetic, morphological, physiological, and anatomical characteristics. To date, there are more than 700 plant species known worldwide to accumulate metals in large amounts, and these accumulator species are of interest for their potential use in the phytoremediation of metal-contaminated soils (Reeves et al. 2018). For example, *Noccaea caerulescens* (*Thlaspi)* and *Arabidopsis hallari* are characterized as hyperaccumulator plants of Zn/Cd. Several crops *Brasssica* spp. such as *B. nigra* L., *B. juncea* L. Czern, *B. napus* L., and *B. rapa* L. exhibit enhanced accumulation of Cu, Zn, and Cd (Ebbs et al. 1997). A list of hyperaccumulator plants is given in Table 1.1. In this table, several plant species belong to different families accumulate metal both in roots and/or shoots. Hyperaccumulator plants have got a considerable interest in exploiting their accumulation traits for practical use, in particular, to develop cheap and clean technologies for phytoremediation of heavy metal from contaminated soils or for phytomining valuable metals from mineralized sites (Chaney et al. 2018).

However, there are many factors that could be considered for efficient phytoremediation and also for beneficial agromining, such as plant tolerance to pollutants, agronomic characteristics of the plant species, climatic conditions (rainfall, temperature), soil physicochemical properties, and the recent technologies

Table 1.1 A list of hyperaccumulator plants. Metal shoot/root ratio and the plant tissues where metal is highly accumulated is given (Memon, Kusur, and Memon unpublished data)

Plant name	Metal	TF (C_s/C_r)	Tissue	References
Arabidopsis halleri	Cd	0.23	Root	Bert et al. (2003)
Arabis paniculata		1.45	Root, shoot	Tang et al. (2009)
Arabis gemmifera		6.13	Shoot	Kubota and Takenaka (2003)
Thlaspi caerulescens		–	Shoot	Baker et al. (1994)
T. goesingense		0.5	Root	Lombi et al. (2000)
N. praecox (T. praecox)		–	Shoot (5960 ppm)	Vogel-Mikuš et al. (2008)
Sedum alfredii		1.05	Root,shoot	Xiong et al. (2004)
Tamarix smyrnensis		1.36	Root,shoot	Manousaki et al. (2008)
Rorippa globosa		2.21	Shoot	Sun et al. (2011)
Arabis gemmifera	Zn	6.48	Shoot	Kubota and Takenaka (2003)
A. paniculata		1.98	Shoot	Tang et al. (2009)
T. goesingense		–	Shoot	Baker et al. (1994)
Thlaspi caerulescens		–	Shoot	Reeves and Brooks (1983)
Arabidopsis halleri		0.16	Root	Küpper et al. (2000)
Sedum alfredii		0.43	Root	Sun et al. (2005)
Salix viminalis		–	Shoot	Schmidt (2003)
Brassica napus		4.02	Shoot	Brunetti et al. (2011)
Aeolanthus biformifolius	Cu	–	Shoot (13,700 ppm)	Brooks et al. (1978)
Crassula helmsii		–	Shoot (9200 ppm)	Küpper et al. (2009)
Elsholtzia splendens		0.033	Root	Weng et al. (2005)
Sorghum sudanense L.		3.41	Shoot	Wei et al. (2008)
Chrysanthemum coronarium L.		7.58	Shoot	Wei et al. (2008)
Brassica napus		2.13	Shoot	Brunetti et al. (2011)
Spartina argentinensis	Cr	5.1	Shoot	Redondo-Gómez (2013)
Brassica juncea		0.56	Root	Seth et al. (2012)
Brassica napus		5.04	Shoot	Brunetti et al. (2011)
Elodea canadensis		0.05	Root	Ranieri et al. (2013)
Arabis gemmifera		0.15	Root	Kubota and Takenaka (2003)
Hemidesmus indicus	Pb	0.66	Root	Sekhar et al. (2005)
Brassica oleracea		0.54	Root	Zhu et al. (2004)
B. campestris		0.62	Root	
Arabis paniculata		1.96	Shoot	Zeng et al. (2009)
Brassica juncea		0.2	Root	Seth et al. (2012)
T. caerulescens		–	Shoot (0.66 ppm)	Baker et al. (1994)

(continued)

Table 1.1 (continued)

Plant name	Metal	TF (C_s/C_r)	Tissue	References
Sedum alfredii		0.003	Root	Sun et al. (2005)
Brassica napus		5.04	Shoot	Brunetti et al. (2011)
Sesbania drummondii		1.1	Root, shoot	Ruley et al. (2006)

TF, translocation factor = (Cs, concentration of metal in shoots/ Cr, concentration of metal in roots)

available for the recovery of metals from the harvested plant biomass. The naturally occurring heavy metal accumulator plants are good candidates for phytoextraction (Table 1.1) because they take metal from the soil in two or three orders of magnitude than non-accumulator plants growing on natural uncontaminated soils. Table 1.1 shows the TF (translocation factor) value of metals in plants. Several accumulator plant species had translocation factor (TF) of metals more than one, suggesting that plants remove the metals from the soil by phytoextraction and translocate them to shoots (Brunetti et al. 2011; Kubota and Takenaka 2003). On the contrary, non-accumulator plants have TF less than one and cannot accumulate metal in shoots.

It appears that both chemical and biological approaches are still not wholly efficient and need more efforts for their effective use in the future (Kidd et al. 2015). Some plants may accumulate one metal, whereas others can accumulate two or more metals at a time, which could be beneficial for phytoremediation and phytomining (see Table 1.1) (Chaney et al. 2007).

Although the annual biomass yield is an essential trait for phytoremediation, the ability to hyperaccumulates and hypertolerate metals is of greater importance than high biomass (Chaney et al. 1997). Hyperaccumulator plants absorb and transport many valuable metals from the contaminated soil and accumulate them in their shoots. These marketable metals could be recovered from the plant biomass for use in the metal industries (Brooks et al. 1998; Chaney et al. 2018). Commercial technologies have been developed for Ni phytomining using Alyssum Ni hyperaccumulator species (Broadhurst et al. 2004). However, other high price metals (Au, Tl, Co, and U) can be extracted using hyperaccumulator plants from the soil or mine tailing containing concentrations of the metals at a level uneconomic for conventional extraction techniques.

1.3.1 Selection of Plant Species for Phytoextraction

As mentioned above, one of the requirements for plants to be used in phytoremediation of soil is to take up the heavy metals from the contaminated soils efficiently. In other words, if a plant species accumulate and concentrate metals in their shoots at levels greater than those in the soil is an excellent candidate for remediation of the polluted soils. The plants that grow in their natural habitats and accumulate 100 µg/g for Cd, Se, and Tl; 300 µg/g for Co, Cu, and Cr; 1000 µg/g for Ni, Pb, and As; 3000 µg/g for Zn; and 10,000 µg/g for Mn in their dried foliage are proposed to be hyperaccumulators (Rascio and Navari-Izzo 2011; van der Ent et al. 2013). To find out the hyperaccumulator plants and their accumulation capacity and specificity to the metal accumulation, a global database (www.hyperaccumulators.org) was created in 2015 and is administered and maintained by the Center for Mined Land Rehabilitation, University of Queensland, Brisbane, Australia. The data about all known metal and metalloid accumulator plants are deposited, continuously updated, and is free to use (Reeves et al. 2017). This database currently contains more than 700 different metal hyperaccumulator species, and most of the plant species are Ni accumulators (523 spp.). Some plant species accumulate Cu (53 species.), Co (42 species), Mn (42 species), Se (41 species), Zn (20 species), Pb (8 species), Cd (7 species), and As (5 species). A very few plant species are accumulators of rare elements (Reeves et al. 2017). The most strongly represented hyperaccumulator plant species are in the Brassicaceae (83 species) and Phyllanthaceae (59 species) families.

1.3.2 Hyperaccumulator Plant Species in Brassicaceae

Brassicaceae comprises approximately 338 genera and 3700 species. In the Brassicaceae family, the Brassica genus contains about 100 species, including essential oilseed crops (for example, *Brassica napus*, *B. juncea*) and many common vegetable plants (Ozturk et al. 2012; Warwick and Black 1991). Among Brassica*,* *B. rapa* has the smallest genome, at ca. 529 Mb, and *B. napus* have the largest one, at ca 1132 Mb (Lysak et al. 2005; Nagaharu 1935). The genome of both species is sequenced, and the data is available in the public domain http://www.brassicagenome.net/databases.php; https://www.ncbi.nlm.nih.gov/genome/?term=brassica%20napus (Memon 2016; Liu et al. 2016). Around 80–90% homology between the exons of putative orthologous genes in Arabidopsis and Brassica is reported (Ozturk et al. 2012). Due to that, the annotated Arabidopsis genome sequence can be exploited for the comparative analysis of *Arabidopsis* and *Brassica* genomes. The plant species in Brassica (e.g., *B. juncea; B. napus; B. nigra*) produce high biomass and accumulate and tolerate high metals (including Cd, Cu, Ni, Pb, U, Zn) in their tissues (Anjum et al. 2012a, b; Kumar et al. 1995). *B. juncea* is considered a suitable candidate for phytoremediation of multiple heavy metals from the soil. It is highly metal tolerant and comparatively accumulates more metals in its shoots than other Brassica species Zn, Cd, and Pb. For example, this species accumulates a high amount of Cd in their shoots (1450 µg Cd/g dry wt), three times more than reported in *B. napus* (555 µg/g dry wt). In addition, it absorbs a huge amount of other metals such as Pb (28% reduction) and Se (reduced between 13 and 48%) (Szczygłowska et al. 2014). It also accumulates more Zn from the soil than *Noccaea caerulescens*, a known hyperaccumulator of zinc. It appears that *B. juncea* produces ten times more biomass than *N. caerulescens* (Anjum et al. 2012a, b; Szczygłowska et al. 2014). *B .nigra* Diyarbakir ecotype (Southern Anatolia), a diploid, is known as Cu accumulator (Memon and Zahirovic 2014) and accumulated around 20,000 μg g^{-1} DW Cu in their shoots (Ozturk et al. 2012; Cevher-Keskin et al. 2019). Because of the high Cu accumulation capacity of *B. nigra,* this plant could become a suitable candidate for phytoremediation of Cu-polluted soils (Cevher-Keskin et al. 2019; Dalyan et al. 2017; Kumar et al. 2012; Memon and Zahirovic 2014).

1.4 Subcellular Localization of Metals in Hyperaccumulator Plants

To understand the mechanism of metal hyperaccumulator, the detailed physiological knowledge of metal absorption by roots, translocation to the shoots, and the subcellular localization of the metals in the leaves are of great importance (Memon and Schroder 2009; Tangahu et al. 2011). Microarray analysis with Cu accumulator *B. nigra* Diyarbakar ecotype showed several hundredfold increases in metal transport ATPases and other genes related to metal transport and accumulation in plants treated with 500 μM Cu (Memon and Zahirovic 2014). Several other genes related to signal transduction, metabolism, and transport facilitation were highly expressed with high Cu. For example, the genes involved in the glutathione pathway (γ-ECS, PC, etc.) were also highly expressed in root and shoot tissues (Memon and Zahirovic 2014; Merakli and Memon, unpublished data). Because of its high growth both at low and high Cu, this plant was classified as a facultative metallophyte (Memon 2016).

It is interesting to know the mechanisms responsible for making these metals in an innocuous form in the plant cell. One of the primary mechanisms for detoxification in the plant cell is storing and depositing the metals in the vacuolar compartment (Memon et al. 2001; Reeves et al. 2018; Tangahu et al. 2011). Different organic acid chelators such as malate, citrate, histidine, and nicotinamide play a role in translocating metal cations through the xylem (Salt et al. 1995; Stephan et al. 1996; von Wirén et al. 1999). To maintain the metal homeostasis in the cell, hyperaccumulator plants efficiently absorb metal from the soil and transport it to shoots and sequestered them in the subcellular compartments (e.g., cell wall, vacuole, etc.) or secreted in the trichomes (Hanikenne and Nouet 2011; Memon and Schroder 2009; Memon and Yatazawa 1982; Ovečka and Takáč 2014). Previously, we carried out an electroprobe X-ray microprobe analysis to understand the subcellular localization of Mn in the leaves of Mn accumulator plant *Acanthopanas sciadophylloides* and tea. The micro-distribution pattern of Mn showed that a large portion of Mn was located in the cell wall and vacuolar compartment of epidermal cells (Fig. 1.3), and it was almost absent from the cytoplasm (Memon et al. 1981; Memon and Yatazawa 1984). One of the detoxification mechanisms proposed was Mn^{2+} complex with malate in the cytoplasm and then transported to the vacuole where it is dissociated from malate and forms a stable complex with oxalate. Under this condition, malate functions as a "transport vehicle" through the cytoplasm and oxalate as the "terminal acceptor" in the vacuole (Memon and Schroder 2009;

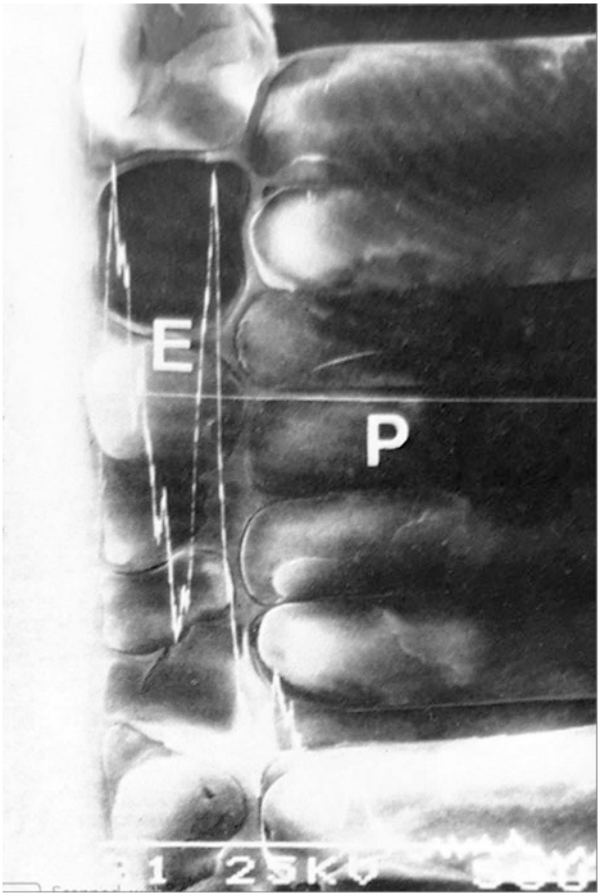

Fig. 1.3 Secondary electron images line scan profile of a leaf section of a tea plant with Mn (Ka radiation) peaks. It shows the localization of Mn at the subcellular level in the epidermis. *E* epidermis, *P* palisade parenchyma cells (Memon et al. 1981)

Memon and Yatazawa 1984). There are several other mechanisms involved in metal detoxification, e.g., production of superoxide dismutase, peroxidase, catalase, glutathione reductase, and nonenzymatic antioxidants (e.g., flavonoids, reduced glutathione, ascorbic acid), which play a significant role in neutralizing the free radicals caused by ROS and minimize the plant cell damage (Küpper et al. 1999; van de Mortel et al. 2006; Li et al. 2015).

Metal accumulation and compartmentalization patterns differ depending on plant species and element type. According to Küpper et al. (2000), *A. hallari* accumulates more Zn and Cd in the mesophyll cells than in the epidermis, but *N. caerulescens* accumulates six times more Zn and Cd in epidermis cells than in mesophyll cells. *B. juncea* (a metal tolerant and accumulator plant), on the other hand, accumulates 40 times more Cd in trichomes compared to leaves (Dalyan et al. 2017; Küpper et al. 1999). *Alyssum lesbiacum* also accumulates a significant amount of Zn and Ni in leaf trichomes (Reeves et al. 2018).

Various alternative detoxifying and accumulation mechanisms have been proposed (Hanikenne and Nouet 2011; Isaure et al. 2015; Memon 2016; Rascio and Navari-Izzo 2011) in which metals can be bound and sequestered by phytochelatins, metallothioneins, metalloenzymes, and metal-activated enzymes. Recent advancements in the next-generation sequencing technologies have opened up new possibilities to understand the metal detoxification mechanisms in plants at the cellular and molecular level (Verbruggen et al. 2013).

1.5 Metal Transporters and Their Function in the Plant Cell

Several genes and proteins related to metal absorption and transport have been identified and characterized in several accumulator plants. These metal transporters are subdivided into six main groups, including natural resistance macrophage protein (NRAMP), ZRT-like protein (ZIP), cation diffusion facilitator (CDF), Yellow-stripe-like (YSL), and heavy metal P1B-type ATPases (HMAs) (Guerinot 2000; Memon 2016; Merlot et al. 2018). Table 1.2 shows the genomic structure and protein length of different metal transporters, including metal ATPases, NRAMPs, and ZIP proteins identified from different plant species. To maintain the metal homeostasis in the cell, a metal accumulator plant can activate several transporters, which can function either in excluding metal at the root or sequestering them at the subcellular level in the vacuole, chloroplast, and some other cellular compartments. Analysis of the *A. thaliana* genome has shown the genes of several metal transporter families, including 15 members of zinc and iron transporters (ZIP), eight members of Cation Diffusion Facilitator (CDF), six members of copper transporters (CTR), six members of NRAMP homologous, and eight members of Cu, Zn/Cd transporting ATPases (Mäser et al. 2001; Merlot et al. 2018) (http://www.cbs.umn.edu/arabidopsis/). The role of some other transporter families, such as vacuolar cation proton exchanger (CAX) and ABC transporters in metal homeostasis, have been elucidated (Colangelo and Guerinot 2006; Hall and Williams 2003; Memon 2016; Memon and Schroder 2009; Sarma et al. 2018). Li et al. have identified 55 AtHMPs and 46 OsHMPs in dicot Arabidopsis and monocot rice, respectively (Li et al. 2020). These proteins are named metalloproteins or metallochaperone-like proteins containing heavy metal-associated (HMA) domains comprising a conserved HMA domain with around 30 amino acid residues. Several other proteins that transport or detoxify heavy metals have this conserved domain. Two cysteine residues in this domain bind with copper, zinc, cadmium, cobalt, and other heavy metals (Li et al. 2020). These HMA domain-containing proteins fall into four groups; HPPs (heavy metal-associated plant proteins), HIPPs (heavy metal-associated isoprenylated plant proteins), ATX1-like copper transport proteins, and heavy metal ATPases (HMAs) (Memon 2016).

Among the genes of transporter families described above, P1B-type ATPases, an ion pump, which utilizes the energy resulting from ATP hydrolysis to carry membrane transport of multiple metal ions in the subcellular level, is of particular importance. These ATPases maintain the homeostasis of the heavy metals in the

Table 1.2 Genomic structure, cDNA, and protein length of different transporters of different plant species (Memon, Kusur, and Memon unpublished data)

Plant name	Gene name	Genomic DNA base pairs (bp)[a]	cDNA base pairs (bp)	Exon	Intron	Protein length amino acids (aa)
A. thaliana	HMA1*	4776	2460	13	12	819
M. truncolata		9415	2490			829
B. napus		4359	2331			776
G. max		14,420	2454			817
S. tuberosum		9994	2454			817
A. lyrata		3448	2421	11	10	806
B. rapa		4207	2457	13	12	818
Z. mays	HMA2	6917	3726	10	9	1241
O. sativa		7771	3204			1067
B. napus		8845	2661	15	14	886
B. rapa		6062	2715	9	8	904
O. lucimarinus		2328		1	1	776
G. max		8194	1683	10	9	560
A. lyrata	HMA3	3369	2274	10	9	757
C. sativus		6642	2667			888
Z. mays		3484	2959	6	5	893
B. oleracea		4012	2292	8	7	763
B. rapa		8243	3864	10	9	1287
B. napus		3396	2291	9	8	763
G. max	HMA4	12,008	2865	8	7	954
A. lyrata		7886	3828	10	9	1275
S. oleracea		9964	2901			966
B. napus		8158	3585			1194
B. oleracea		7550	3588			1195
B. rapa		7723	3573			1190
M. truncolata		9737	2991	5	4	996
A. thaliana	HMA5	3657	2988	6	5	995
B. napus		3604	2922	4	3	973
B. oleracea		5077	2922			973
B. rapa		3542	2934			977
A. thaliana	HMA6	7322				
C. sativa		7368	2856	19	18	951
A. thaliana	HMA7	7773		10	9	
C. sativa		5401	3021			1006
N. tabacum		4525	2667	3	9	888
G. max	HMA8	8496	2711	17	16	903
C. sativa		5735	2655			884
A. lyrata	NRAMP1	3274	1581	12	11	526
B. napus		5770	1599	13	12	532
B. oleracea		3319	1599	11	10	532

(continued)

Table 1.2 (continued)

Plant name	Gene name	Genomic DNA base pairs (bp)[a]	cDNA base pairs (bp)	Exon	Intron	Protein length amino acids (aa)
B. rapa		3344	1599			532
A. thaliana	NRAMP2	2703	1593	4	3	530
A. lyrata		2708	1599			532
B. napus		1969	1077	5	4	358
B. rapa		2755	1599			532
A. thaliana	NRAMP3	2630	1530	4	3	509
A. lyrata		2539	1524			507
B. napus		3735	1542			513
O. sativa		2367	1536	14	13	511
A. thaliana	NRAMP4	2632	1539	3	2	512
B. napus		1741	1539	6	5	512
B. oleracea		2465	1536	3	2	511
B. rapa		2350	1539			512
A. thaliana	NRAMP5	2321	1593	4	3	530
A. lyrata		2322	1590			529
B. napus		2590	1596			531
B. oleracea		2577	1596			531
A. thaliana	NRAMP6	4441	1584	13	12	527
B. napus		3338	867	7	8	288
B. oleracea		3220	1561	13	12	520
B. rapa		7237	1512			503
A. thaliana	ZIP1	1551	1068	2	1	355
G. max		3147	1065	3	2	354
A. thaliana	ZIP2	1696	1062	2	1	353
O. sativa		4301	1101	3	2	366
N. attenuata		1251	996			331
A. thaliana	ZIP3	2861	1020	3	2	339
O. sativa		259	1095			364
H. annaus	ZIP4	2524	1254	5	4	417
G. hirsutum		2531	1256	4	3	422
O. sativa	ZIP5	4301	1101	3	2	366
N. attenuata		3458	1032			343
A. lyrata	ZIP6	1639	1008	2	1	335
*M. truncula*ta		612	306	1		101
C. sativus		612	306			101
A. thaliana	ZIP7	1613	1098	3	2	365
O. sativa		3353	1155	4	3	384
A. thaliana	ZIP8	1728		5	4	
O. sativa		3239	1173	3	2	390
A. lyrata	ZIP9	2516	1170	4	3	389
Q. suber			1059	1	1	187

(continued)

Table 1.2 (continued)

Plant name	Gene name	Genomic DNA base pairs (bp)[a]	cDNA base pairs (bp)	Exon	Intron	Protein length amino acids (aa)
A. thaliana	ZIP10	1804	1095	3	2	364
O. sativa		2621	1215	5	4	404
A. thaliana	ZIP11	1051	981	2	1	326
H. annaus		2932	981			326
A. thaliana	ZIP12	1758	1068	2	2	355
O. brachyantha		11,024	1776	20	19	591

HMA Heavy metal ATPase, *NRAMP* Natural resistance-associated macrophage protein, *ZIP* Zinc-regulated, iron-regulated transporter-like proteins

[a] Partial sequences (bp) are given for some of the genomic DNAs

cell and are present in prokaryotic and eukaryotic cells, including bacteria, plants, and mammals.

1.6 Function of Heavy Metal ATPases (HMAs) in Plants

There are three main pumps (ATPases) present in plant cells. The first Fo-F1 type ATPase is present in chloroplast and mitochondrial membrane and is involved in ATP synthesis. V-type ATPases are present in the tonoplast membrane and generate the H^+ gradient required for transport across the tonoplast membrane. The third one P-type ATPases are present in the plasma membrane and other organelle membranes and are involved in the active pumping of charged substrates across the cell membranes and form a phosphorylated intermediate during the reaction cycle (Palmgren and Nissen 2011). The P-type ATPases are classified into five major families (P1-P5) and divided into several subgroups (Axelsen and Palmgren 2001). Heavy metal ATPases (P1B ATPases) are a subclade of P1-ATPase and transport heavy metals such as Cu, Zn, Cd, Pb, and Co and are the main pumps required in metal detoxification and metal homeostasis in the cell (Østerberg and Palmgren 2018). P1B-ATPases contain six to eight transmembrane domains (TMs), an HP locus, and a CPx/SPC motif (Williams and Mills 2005), required for metal binding and transport. The majority of these ATPases possess conserved regions such as DKTGT, GDGxNDxP, PxxK, and S/TGE in their sequence necessary for their proper function (Williams and Mills 2005). Based on their substrate specificity, these ATPases are subdivided into two groups, Cu/Ag (Cu^+-ATPases) and Zn/Cd/Co/Pb transporters (Zn^{2+}-ATPases) (Axelsen and Palmgren 2001).

The plant genome contains many copies of P1B-ATPases, especially *Arabidopsis thaliana* has eight, rice has nine, and soybean has 25 genes in their genome (Fang et al. 2016; Williams and Mills 2005). Table 1.2 shows the genomic size and structure, cDNA, and protein length of different metal ATPases identified in the genome in various plant species. HMA2, HMA3, and HMA4 have high sequence homology among them and transport Zn and Cd. HMA2 and HMA4 are the plasma membrane transporters in pericycle cells and are involved in root-to-shoot transport of Zn/Cd. HMA3 is located in the tonoplast and has a detoxification function through vacuolar sequestration of Zn/Cd (Hanikenne et al. 2008; Hussain et al. 2004; Liu et al. 2017; Morel et al. 2009; Wong and Cobbett 2009). Table 1.2 shows that HMA4 protein has longer amino acid sequences than other metal transporters and has an essential function in Zn/Cd hypertolerance and hyperaccumulation in accumulator plants like *Arabidopsis hallari* and *Noccaea caerulescens*. Three copies of HMA4 have been identified in *A. hallari* and are highly conserved in coding sequences but diverge in promoter sequences (Nouet et al. 2015). Their complementation experiment with the *A. thaliana, hma2hma4* mutant (severe Zn-deficiency phenotype) showed that all three copies restored root-to-shoot translocation of Zn. Each copy had a different impact on the metal homeostasis in the *A. thaliana.* This observation indicates a functional difference among the three *A. halleri HMA4* copies, possibly due to the differences in expression levels rather than in expression profile (Nouet et al. 2015).

The C-terminus of the HMA4, one of the well-known ATPase transporter located in the plasma membrane, binds Zn, has considerably divergent amino acid motifs between *A. thaliana* (non- accumulator) and *A. hallari* (accumulator). The di-Cys motif in this region has a high affinity for Zn binding in accumulator plants (Lekeux et al. 2018). Similarly, BjHMA4 transporter protein in *B. juncea* showed a repeat region BjHMA4R in the C-terminus not far from the last transmembrane domain in the cytosol (Wang et al. 2019). It binds Cd^{2+} and improves Cd tolerance and accumulation in *B. juncea.* AtHMA1, a chloroplast membrane protein, transports Cu and Zn into and out of the chloroplast, respectively (Zhao et al. 2018). SpHMA1 in *S. plumbizincicola* leaves a chloroplast Cd exporter and protects photosynthesis by inhibiting the Cd accumulation in the chloroplast (Zhao et al. 2018). The RNA interference of chloroplast SpHMA1 and CRISPR/Cas9-induced HMA1 mutant lines significantly increased Cd accumulation in the chloroplasts than wild-type *Sedum plumbizincicola*. AtHMA5 is localized in the plasma membrane and contributes to the detoxification of excess Cu in roots by increasing Cu translocation from roots to shoots (Kobayashi et al. 2008). On the contrary, AtHMA6 (PAA1) and AtHMA8 (PAA2) are located in chloroplast envelope and thylakoids and transport Cu into the chloroplast (Abdel-Ghany et al. 2005; Shikanai et al. 2003). 20 *HMA* genes (*GmHMA1* to *GmHMA20*) in soybean are phylogenetically divided into 6 clusters (Fang et al. 2016). Six GmHMAs (5, 19,13,16,14, and 18) were classified as Zn^{2+} ATPases, while the remaining HMAs were identified as Cu^{+}-ATPases (Fang et al. 2016). 17 *HMA* genes in *Populus trichocarpa* were shown to be differentially regulated by high metal stress (Li et al. 2015).

Genomic analysis of metal accumulator species *A. hallari, N. caerulecence, B. juncea, B. napus, and B. nigra* have identified the specific role of several metal transporters, including metal ATPases in metal accumulation and tolerances in plants (see Table 1.2) (Cevher-Keskin et al. 2019; Dalyan et al. 2017; Memon 2016). HMA genes are identified both in model plants like *A. thaliana*, rice and in other crop plants like *B. napus, B. rapa, B. juncea, Glycine max,* and *P. trichocarpa* (see Table 1.3). The role of HMA1 to HMA4 in Cu, Zn, and Cd transport in the model plants has been extensively studied and well-characterized at the gene and protein level. Functional studies of these transporters in yeast have given helpful information related to the function of these transporter proteins in the eukaryotic cells (Fang et al. 2016; Wang et al. 2019).

In the last decade, many plant transporter genes involved in metal uptake and translocation are characterized. However, identification and functional analysis of many other transporter genes are still awaiting identification. More studies on the expression and function of these transporter genes at the cellular and subcellular levels coordinated with the structural analysis of the transporter proteins will reveal the fundamental role of these transporters in the detoxication mechanism in accumulator plants. Two different approaches could be taken related to the expression of transporter genes in the accumulator and non-accumulator plants. For accumulator plants, the overexpression of metal uptake and translocation transporters would increase the translocation of toxic metals to aerial parts, which would target phytoremediation. For non-accumulator edible crop plants, the low uptake transporters could be engineered or overexpressed to minimize the transport of toxic cation in edible crops.

1.7 Conclusion

The recent developments in phytoremediation have been summarized, and the role of obligate and facultative accumulator plant species in metal accumulation and detoxification has been discussed. X-ray microprobe analyzer data with frozen leaf tissues of accumulator plant shows the subcellular localization of metals in the cell, especially their localization in the cell wall and storage vacuole, and keeps the toxic amount of metal away cytoplasm. The recent genomic analysis of one diploid Brassica rapa and another tetraploid (amphidiploid) *Brassica napus* have shown the differential gene expression of metal transporters when encountering low and high metal concentrations in the soil. Recent progress in the genetic and molecular analysis of the metal transporters has elucidated the molecular mechanism of metal absorption, accumulation, and detoxification in hyperaccumulator plants and their role in phytoremediation. Phytoremediation is an environmentally friendly and green technology that holds great potential for environmental cleanup. In the future, it will become an established technology for removing hazardous pollutants from the environment. It will guarantee a greener and cleaner planet for all of us in the coming years.

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