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Manganese Hyperaccumulators and their Hyperaccumulating and Tolerance Mechanisms: A Review of the Current State of Knowledge

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Abstract

Manganese (Mn) is ubiquitous in the environment due to both geological and human activities. It is essential for plants, as for most other living organisms, but can also be toxic when it is present in excess. Some plant species, referred to as Mn hyperaccumulators, can accumulate over 10000µg/g of Mn in their shoot tissues without showing any phytotoxicity. Approximately 24 Mn hyperaccumulators are currently known worldwide. However, ample data is available the Mn hyperaccumulator species and biological significance of Mn hyperaccumulation and tolerance mechanisms. To give new insights, this review highlights the current knowledge of Mn hyperaccumulation and tolerance mechanisms in hyperaccumulators, which include root uptake, xylem loading, transport, sequestration, and detoxification processes. Hyperaccumulators uptake Mn mainly accumulates as Mn²⁺ into the xylem, from which it is then transferred to the shoots. Foliar Mn²⁺ is mainly stored in vacuoles, the endoplasmic reticulum, and the Golgi. It is sequestered by organic ligands and some transporter proteins at a subcellular level in the root and shoot, which can allow the plants to exhibit great tolerance. From the in-depth examine the published literature; the main knowledge gap and future research are highlighted. In addition, Mn hyperaccumulator biomass disposal methods and applications also discussed.

Keywords: Hyperaccumulator; Manganese; Phytomining; Transporters; Root uptake; Manganese sequestration

Introduction

Environmental contamination by Manganese (Mn) has become a serious problem worldwide. Manganese is the fifth most abundant metal in nature [1] and is released by electroplating, the petrochemical and fertilizer industries and mining processes [2]. Mining is a main source of Mn contamination in soils [3]. Worldwide manganese ore production resulted in 17.7 million tonnes (mt) of contaminated metals in 2014, representing a production increase of 5.1% over the previous year. The main producers are South Africa with over 26.4% of the world production, China with 18.0%, Australia with 17.5% and Gabon with 11.3% of the total world production.

Manganese is an important micronutrient for plant growth and plays multiple roles in photosynthesis, nitrogen metabolism, hormone regulation, and the synthesis and breakdown of certain substances [4]. However, Mn accumulation in excess of the normal range of 20-500 mg/kg of the plant dry weight [5] can have negative effects on plants. Excess Mn inhibits seed germination [6] and plant growth [7], causes biomass loss [8], decreases the photosynthetic rate [9], inhibits root development [10] and hinders the adsorption of other nutrients [11]. Furthermore, Mn toxicity affect the human nervous system and cause parkinsonian syndrome [12].

The removal of excess Mn from contaminated sites by physical and chemical methods has been unsuccessful in restoring large areas of land due to the high cost, deterioration of soil quality and

other problems associated with these methods [13,14]. In contrast, phytoremediation is a promising technology for remediating heavy metal-contaminated sites by using hyperaccumulator plants [15,16]. The quality of hyperaccumulator species is judged by the following four characteristics: (1) Hyperaccumulation ability (100 times greater metals accumulation than normal plants); (2) Hypertolerance; (3) Bioaccumulation factor (BF)>1 and (4) Translocation factor (TF)>1 [17]. The first study of a hyperaccumulator plant described the extreme accumulation of nickel by shoots of *Sebertia acuminata* (new name is *Pycnandra acuminata*) in New Caledonia [18]. Approximately 500 plant taxa capable of heavy metal accumulation have been identified worldwide [19], among which approximately 330 plant species are Ni hyperaccumulators, and 20 species are Mn hyperaccumulators [20,21]. In this review examines the current knowledge of Mn hyperaccumulators and their accumulation and tolerance mechanisms, which include root uptake, xylem loading, transport, distribution and detoxification mechanisms. In addition, this review provides information about Mn hyperaccumulator biomass disposal and applications.

Discover of Manganese Hyperaccumulators

Numerous studies have aimed on the identification of Mn hyperaccumulator plants based on tolerance, hyperaccumulation in highly Mn contaminated sites [22-25], hydroponics and pot experiments [22,24,26,27], and microprobe analysis [26,28,29]. A

Mn hyperaccumulator plant is defined as one that accumulates over 10000µg/g of Mn in its shoot tissues [30]. Approximately 24 Mn hyperaccumulator plants are currently known worldwide, including 8 species from Cleastraceae, 4 species from Polygonaceae, 3 species each from Myrtaceae and Proteaceae, and 1 species each from *Amaranthaceae*, *Aralicaceae*, *Apocynaceae*, *Clusiaceae*, *Phytolacceae* and *Theaceae* (Table 1). Jaffre [31-33] discovered the first Mn hyperaccumulator plants with Mn concentrations above 1.0% in the leaves of *Beaupreopsis paniculata*, *Macadamia neurophylla*, *Macadamia angustifolia* (*M. neurophylla* and *M. angustifolia* are now classified as *Virotia* species), *Maytenus furnieri*, *Macadamia sebertiana*, *Garcinia amplexicaulis* and *Eugenia clusoides*, all in New Caledonia. In addition, Brooks et al. [34] analysed 31 species in *Alyxia* from New Caledonia for their Mn content and found that *Alyxia rubricalis* showed the highest Mn uptake (1.15%) in dried leaves. Twenty years later, Bidwell et al. [28] identified the first native Australian Mn hyperaccumulator, *Gossia bidwillii* (formerly *Austromyrtus bidwillii*); Mn concentrations upto 19200µg/g and 26509µg/g were measured in the dried leaves of this rain forest tree. Similarly, Xue et al. [22] identified the first native Chinese Mn hyperaccumulator species (*Phytolacca acinosa*), which accumulate 19300µg/g Mn in shoot tissue. Despite, now *P. acinosa* is identified as *Phytolacca americana* L., *P. acinosa* Roxb based on the morphological characteristics [35]. Yang et al. [26] conducted a potted plant experiment to illustrate the Mn accumulation and tolerance ability of *Schima superba*. The results showed that *S. superba* accumulated 62412.3mg/kg Mn in the stem under 150mmol/l treatment, and when the high Mn supply 40mmol/L. The Mn concentration in shoot exceeded 10000mg/kg, which is suggested concentration for Mn hyperaccumulation. Fernando et al. [29] identified five new Mn hyperaccumulators, namely, *Maytenus cunninghamii*, *Gossia bamagensis*, *Gossia fragrantissima*, *Gossia sankowsiorum*, and *Gossia gonoclada*, in Australia. Between 2010 and 2016, additional new Mn hyperaccumulators, *P. pubescens* [36], *P. perfoliatum* [37], *Celosia argentea* [27], *Eucalyptus grandis* × *Eucalyptus urophylla* [38] and *Polygonum lapathifolium* [39], were discovered in China.

Manganese Phytoavailable and Hyperaccumulation

Rhizosphere mobilization of Manganese

Manganese occurs in the soil in oxidation forms including Mn¹⁺, Mn²⁺, Mn³⁺, Mn⁴⁺, Mn⁶⁺ and Mn⁷⁺. Among them, the most phytoavailable form is Mn²⁺, and the most easily reducible forms are Mn³⁺ and Mn⁴⁺ [40]. Mn phytoavailability is influenced by pH, microorganisms, organic matter content, cation-exchange capacity and interactions with other metals (physiological and metabolic characteristics of the plant) [41]. A low soil pH (acid soil) can increase the redox potential [42] and phytoavailability of Mn [43-45]. At higher soil pH (>8.0), Mn²⁺ can auto-oxidize and will form MnO₂, Mn₂O₃ and Mn₃O₄, which are not available for plants [46,47]. For example, Xu et al. [48] reported that *P. americana* root achieved its maximum Mn adsorption at pH 5-6, while at pH values above 6, Mn adsorption decreased, probably because of the formation of hydroxide complexes. Most of Mn hyperaccumulator plants have been identified in areas with metal-rich and low-pH areas soils. For instance, the *Phytolacca* species were discovered in metal-rich and low-pH (4.8) soil in Xianglan, China, in manganese tailings [22,35]

and in the Roberts Mn mine in Cherokee County, South Carolina, USA [49]. Because, the hyperaccumulator plants rhizospheres have some influence on phytoavailability of heavy metals by release of exudates (carboxylates) and acidification of rhizosphere. For example, Godo and Reisenauer [50] reported that plant roots were released exudates (hydroxycarboxylase), which increased the phytoavailability of Mn in rhizosphere soil. Lambers et al. [45] showed that *P. americana* released protons into the rhizosphere in order to enhance the phosphate solubility, resulting in coincidental mobilization of Mn. Similarly, a comparative study showed that *P. americana* acidifies the rhizosphere soil and enhance Mn solubility more than *Acalypha rhomboidea* (non-accumulating) plant [51].

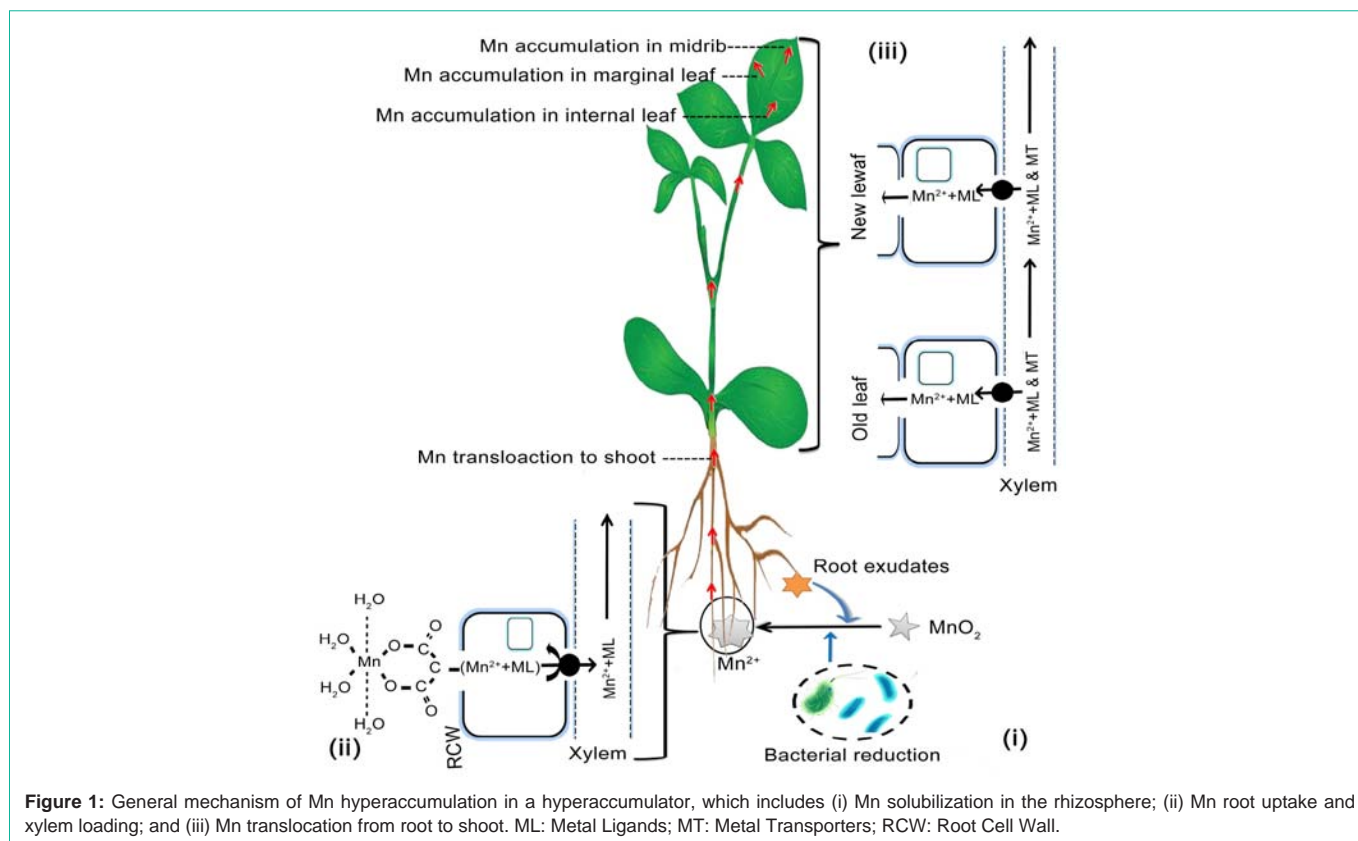
Moreover, rhizosphere microorganisms also have some great potential to reduce MnO₂ into phytoavailable Mn²⁺ by producing certain chelators and siderophores [52] that alter the soil pH and enhance chelation efficacy. For example, bacteria (*Bacillus manganica*) and fungi (*Mycogone*, *Tricholadium*) can reduce Mn⁴⁺ to Mn²⁺, which is the form required by plants. Meanwhile, some rhizosphere bacteria, namely, *Pseudomonas* and *Geobacter*, reduce oxidized Mn⁴⁺ to Mn²⁺, affecting its availability to the plants [41,53,54].

Manganese Uptake and Translocation from Roots-To-Shoots

Uptake and translocation of Mn from roots to shoots are dependent on several steps: (i) Root uptake, (ii) Xylem loading and (iii) Transport and distribution [46,55].

Root uptake

Bioavailable Mn²⁺ uptake by roots is generally exhibited as two phase processes, apoplastic binding phase and symplastic uptake [56]. Apoplastic binding is an initial, non-metabolic and rapid uptake phase [47,57]. In this phase, Mn²⁺ absorbed by root cells, which have a negatively charged cell wall that is strongly attractive to divalent cations such as Ca²⁺, Fe, Zn and K [58,59]. Interactions between Mn²⁺ and other divalent cations have been noted during the root uptake processes. For example, Peng et al. [60] studied the uptake of Mn and its interaction with Cd in *P. americana* and showed that the Cd addition can decreased the Mn uptake in the root and increased it in the stem. The Mn and Cd interaction in plant uptake indicated that *P. americana* might be use the same transport proteins for the uptake of these metal ions. Dou et al. [61] mentioned that the uptake and transport of Mn are closely related to those of Ca in *P. americana* because the ionic radius, charge and coordination chemistry properties of Ca and Mn are similar. Bidwell et al. [28] reported that the Mn hyperaccumulators *Alyxia* sp. and *Austromyrtus bidwillii* accumulate Mn at the expense of Ca and Mg. All of the above studies indicate that Mn transport pathway might be involved in the uptake of Ca, Cd and Mg. In addition, the low Fe/Mn ratio in *P. americana* noticed a high concentration of Mn uptake from soil [60]. Pedas et al. [62] reported that phosphate directly interferes with Mn uptake by causing alterations in membrane transporter. Xu et al. [58] also suggested that P and S probably played important roles in Mn absorption by *P. acinosa* a hyperaccumulator plant. A similar Mn uptake pattern seems to exist in Ni hyperaccumulators. For example, the Ni hyperaccumulators *Alyssum murale* and *Alyssum corsicum* accumulate the greatest Mn concentration upon receiving Ni/Mn treatment, but Ni uptake is inhibited by the addition of Mn. This



result suggests that Ni does not interfere with Mn uptake. Ghaderian et al. [63] showed that the Ni uptake of *Alyssum bracteatum* (a Ni hyperaccumulator) was inversely correlated with Mn, and likewise, Mn uptake was inversely correlated with Ni in the growth medium, suggesting competition between Ni and Mn for uptake at the roots.

Far less is known regarding the molecular mechanism by which Mn binds to root cell wall during the process of uptake. For example, Kopittke et al. [64] showed that Mn binds to a carboxylic group of cowpea root cell wall, which was confirmed by using Mn K-edge XANES spectroscopy. This carboxylic group exists in multiple components of the root cell wall, including pectin, cellulose and lignin [65]. Similarly, Xu et al. [48] used FTIR spectral analysis to show that C-O, =C-O and -OH functional groups are the main binding sites for Mn on the root cell wall of *P. americana*. These study results indicate that Mn²⁺ can bind carboxylic and hydroxylic groups of the root cell wall in both hyperaccumulators and non-hyperaccumulator plants during the uptake process (Figure 1). Further detailed experiments are needed to reveal the specific compounds in the root cell walls that are responsible for Mn binding in hyperaccumulators.

Mn uptake also appears to involve metal transporters. For example, a group of Natural Resistance-Associated Macrophage Protein (NRAMP) transporters including OsNramp5 are involved in Mn uptake on the root surface of rice plants [66,67]; likewise, Cailliatte et al. [68] reported that the AtNramp1 (high-affinity) transporter is involved in Mn uptake by the roots of *Arabidopsis thaliana*. Takahashi and Sugiura [69] showed that transgenic tobacco transformed with tomato root protein (LeGlp1) with a metal binding site at its N-terminus specifically binds Mn with the recombinant

LeGlp1 protein. This binding of Mn to LeGlp1 strongly suggests the involvement of LeGlp1 in Mn uptake from the soil at the root plasma membranes, suggesting in turn that Mn uptake in hyperaccumulators might be associated with Mn-specific transporters such as the NRAMP and ZIP proteins. Liu et al. [27] reported that hyperaccumulative Mn uptake in *Celosia argentea* might be mediated by NRAMP or ZIP transporters.

Xylem loading transport and distribution

Manganese can reach the xylem through symplast pathway and occurs there both as free ions (Mn²⁺) and in complex with organic acids. For example, Williams and Pittman [70] mentioned that in soybean and tomato, approximately 60% of the xylem Mn was in the form of Mn²⁺ and is complexed with citrate or malate. Duo et al. [71] showed that Mn is complexed with oxalic acid in *P. americana* root. Similarly, Mn is associated with citrate in the root of the non-hyperaccumulator cowpea [64]. These results indicate that Mn is present as Mn²⁺ and complexed with organic acids in the root xylem of both hyperaccumulators and non-hyperaccumulators (Figure 2). Far less information is available on membrane transporters in Mn loading in the xylem. For instance, it has been suggested that plasma membrane-localized NRAMP 1&5 [68] and IRT1 [72] transporters are involved in Mn transport in *Arabidopsis*. Likewise, ZmYS1 and HvYSL2 can transport Mn in maize and barley [73,74].

Manganese influx into the leaf symplast occurs across the plasma membrane of the leaf cell [75]. Studies on non-hyperaccumulators have reported that Mn is absorbed by leaf cell membrane transporters such as the IRT family and thereby transported to the cytosol [72]. The higher expression levels of NRAMP proteins and ZIP protein in

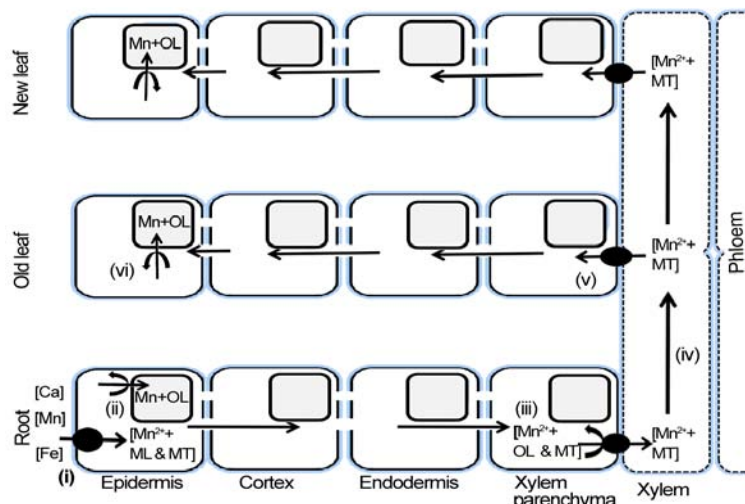


Figure 2: Hypothetical model for Mn tolerance processes in hyperaccumulator plants. (i) Root uptake, (ii) root sequestration, (iii) xylem loading and translocation to shoot, (iv) xylem transport, (v) transport and distribution in leaf, and (vi) foliar sequestration. ML: Metal Ligands; MT: Metal Transporters; OL: Organic Ligands; Mn influx and Mn efflux.

the cambium could transfer higher levels of Mn from the roots to the shoots [76]. In addition, Gustin et al. [77] reported that cation diffusion facilitator/metal tolerance protein (CDF/MTP) could transport Mn^{2+} into subcellular compartments or out of the cytoplasm. The Mn-CDF transporter was first identified in the hyperaccumulator *Stylosanthes hamata* [78]. Xie et al. [38] reported that high concentration of Mn accumulated in stem xylem vessel of *E. grandis* × *E. urophylla*. This result indicated that in Mn hyperaccumulator plants Mn mostly transported by xylem vessel and distributed to stem other parts. These studies suggested that IRT, NRAMP, ZIP and CDF/MTP transporters might be involved in Mn transport from root to the shoot in both hyperaccumulators and non-hyperaccumulators.

After the influx of Mn in leaf symplast, then distributed into various parts of the cells. Xu et al. [58] showed Mn uptake and distribution in the epidermis, cortex, endodermis and vascular bundles of the root cross section of *P. acinosa*. These results showed that Mn content decreased from the epidermis to the cortex and increased from the cortex to the vascular bundles via the endodermis, indicating the translocation of Mn from the cortex to the vascular bundles by an osmotic process). It is concluded that Mn transport exhibits some similarities to active transportation. Furthermore, Mn is transported to photosynthetically active leaves [79], showing that Mn is a most important plant nutrient for photosynthesis. Xie et al. [38] showed that high concentration of Mn distribution in leaf cross section, photosynthetic palisade and spongy tissue of *Eucalyptus grandis* × *E. urophylla*. Similarly, Fernando et al. [80] reported that Mn was evenly distributed in entire leaf cross section and photosynthetic tissues as well as in the palisade and spongy mesophyll of *Gossia*. Menon emphasized mesophyll cells is the major storage site of Mn in hyperaccumulator plants namely *P. americana* and *A. sciadophylloides*. In contrast, high Mn distribution has been observed in non-photosynthetic cells of *M. fournieri* [81,82]. Xu et al. [58] showed that a higher Mn concentration was found in the leaf margin than near the midrib regardless of the total Mn content of individual leaves of *P. acinosa*; consequently, Mn concentrations reduced from the palisade tissue to the spongy tissue in the mesophyll. To date,

no data are available to explain the why hyperaccumulators have different Mn transporters and Mn distribution patterns. Therefore, further detailed experiments are needed for a clear understanding of these processes in Mn hyperaccumulators.

Manganese Tolerance Mechanisms

Hyperaccumulator plants have evolved several tolerance mechanisms to protect their normal growth and developments while under heavy metal ions stress [17]. Generally, Mn hyperaccumulators employ various tolerance mechanisms to detoxify Mn toxicity, such as translocation, sequestration and enhanced the production of antioxidants [44,55].

Root Sequestration Processes

Upon reaching the root apoplast, manganese is complexed by organic acids to alleviate its toxicity [56]. For example, oxalate and malate are favourable for Mn complexation in plant tissues and play a crucial role in Mn hyperaccumulation and detoxification in hyperaccumulator plants [71,83]. The total oxalic acid concentration was found to be higher than the Mn content in the root of *P. americana* (a Mn hyperaccumulator) [71]. This study indicated that oxalic acid could increase Mn tolerance and Mn hyperaccumulation in hyperaccumulators. In contrast, Koppitke et al. [64] estimated that 80% of the Mn^{2+} in roots could bind with citrate in cowpea (a non-hyperaccumulator). Similarly, Fernando et al. [83] showed that Mn is related with citrate rather than oxalate in Mn hyperaccumulators. Therefore, it may be concluded that both oxalate and citrate are involved in Mn sequestration in the root and can enhance Mn tolerance in both hyperaccumulators and non-hyperaccumulator plants (Figure 2).

Foliar Mn Sequestration

Foliar Mn in hyperaccumulators occurs mostly in soluble form (Mn^{2+}), complexed with organic ligands, and sequestered in vacuoles [28,83]. The sequestration of Mn in vacuoles is one of the important detoxification mechanisms in Mn hyperaccumulators

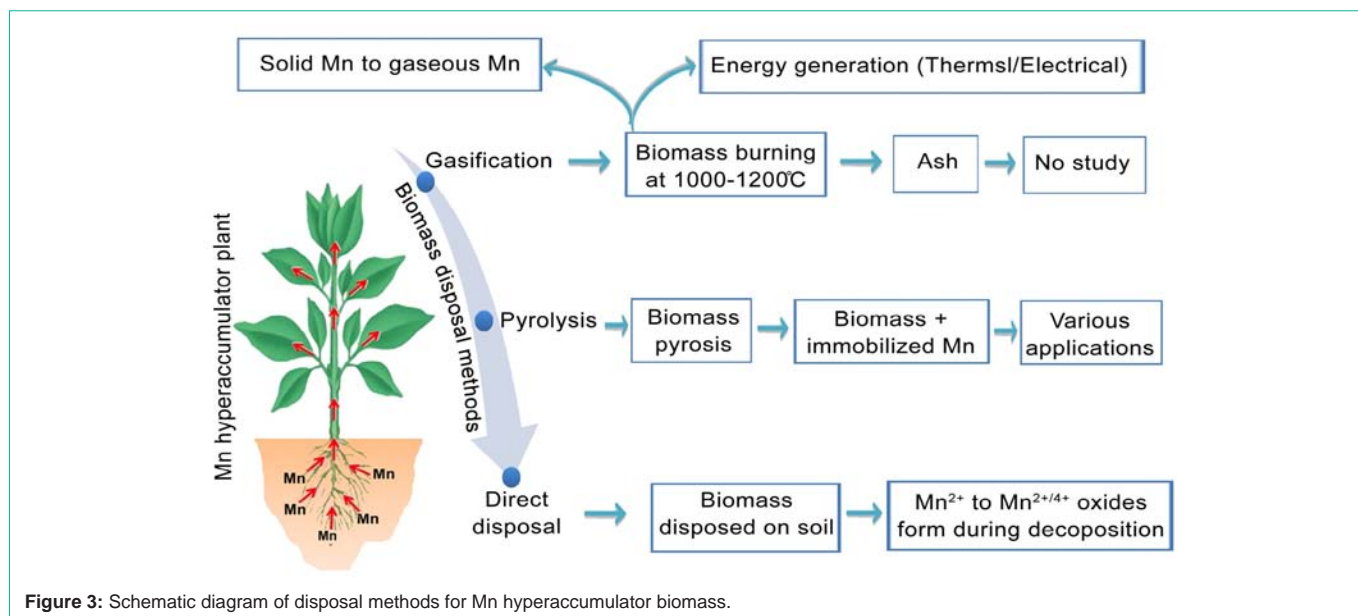


Figure 3: Schematic diagram of disposal methods for Mn hyperaccumulator biomass.

[71,84]. For example, Fernando et al. [82] reported that major Mn sequestration processes occurred primarily in the vacuoles of three Mn hyperaccumulator plants, namely, *Vitotia neurophylla*, *Maytenus founieri* and *Garcinia amplexicaulis*. Similarly, it has been reported that excess Mn accumulates in the leaves of *Gossia bidwillii* (a Mn hyperaccumulator) and is primarily sequestered in the vacuoles of palisade cells and photosynthetic tissues [85]. In contrast, Fernando et al. [86] found high Mn accumulation in non-photosynthetic cells of leaf epidermal tissues of *M. founieri*. Therefore, Mn hyperaccumulators can sequester excess of Mn in both non-photosynthetic tissues and photosynthetic tissues [41]. Further, the sequestration of foliar Mn by organic ligands at the subcellular level confers great tolerance on Mn hyperaccumulators [83,87]. Mn has been reported to be associated with oxalate in the freeze-dried leaves of *P. acinosa* [88]. Similarly, Mn-oxalate complexes were showed in the leaves of *P. americana* [89].

Antioxidative Defense System

Generally, the high concentration of Mn causes Reactive Oxygen Species (ROS) in plants [90] and affects the plant metabolism and growth [9]. Although, hyperaccumulators have effective antioxidative defense system to scavenging the heavy metals induced ROS. The antioxidative defense system comprising antioxidative enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POX), Ascorbate Peroxidase (APX) and nonenzymatic low molecular mass antioxidants. For example, a Mn hyperaccumulator *P. lapathifolium* can maintain high SOD, CAT and APX activities in both root and shoot tissues under the Mn toxicity condition, which implied that *P. lapathifolium* might have highly effective ROS scavenging mechanisms. Gao et al. [91] reported that Mn treatment increase the laccases activity and lignin content, but reduces SPOD activity and phenolics concentrations in apoplastic washing fluid of *P. americana* leaves. These results suggested that lignin formation by laccases and decreased POD activity are involved in the Mn tolerance mechanisms of *P. americana*. Zhao et al. [92] showed that *P. americana* have great ability to protect redox homeostatic disturbance and cellular

damages by enhancing their SOD and CAT activities under the Mn toxicity condition. Furthermore, the excess Mn decreased POX and APX activities these may reduce the ROS generation in *P. americana*. Weng et al. [93] observed that excess Mn increased the SOD, CAT and APX activities in *P. acinosa*. The increased SOD activity could be scavenged O₂⁻ and produced H₂O₂. This H₂O₂ can be scavenged by increased CAT and APX activities. These results suggested that increased SOD, CAT and APX activities could be reduced oxidative stress in *P. acinosa* under the excessive Mn concentration. Previous studies have been reported that a modulation of antioxidative enzymes (SOD, CAT, POX and APX) activities occurs in response to Mn induced oxidative stress. But no information is available on gene expression of these antioxidative enzymes isoforms. Indeed all the antioxidative enzymes have isoenzymes, which may be involved in regulatory mechanisms to heavy metals induced oxidative stress and play role in protecting all the organelles [94]. Therefore, it is most important to understand the role of isoenzymes in regulatory mechanism to excessive Mn toxicity in plants.

Role of Rhizosphere Microorganisms

The hyperaccumulators rhizosphere microorganisms could modify the heavy metal phytoavailability, accumulation and protect the plants from metal toxicity [95]. The rhizosphere of hyperaccumulator plant is consist of many heavy metal resistance endophytic bacteria [59], which significantly increases the uptake of heavy metals and root elongation. Endophytic bacteria producing phytohormones, siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase under the metal contaminated environment and these are promote the plant growth and increase heavy metal induced toxicity tolerance. A study isolated IAA and siderophore producing bacteria in leaves and ACC producing bacteria in root tissues of Mn hyperaccumulator (*P. americana*) under the Mn contaminated soils. These endophytic bacteria enhance the root growth (ranging from 6.4% to 18.3%) and above ground tissues (ranging from 19.3% to 70.2%) and Mn uptake (64%) in penisetum grown under Mn added soil [96]. This study concluded that endophytic bacteria

Table 1: List of manganese hyperaccumulator plants discovered worldwide.

Plant species	Plant family	Location	References
<i>Beauveropsis paniculata</i>	Proteaceae	New Caledonia	Jaffre [33]
<i>Denhamia fourmieri</i>	Celastraceae	New Caledonia	Jaffre [33]
<i>Macadamia neurophylla</i>	Proteaceae	New Caledonia	Jaffre [31]
<i>Eugenia clusioides</i>	Myrtaceae	New Caledonia	Jaffre [32]
<i>Garcinia amplexicaulis</i>	Clusiaceae	New Caledonia	Jaffre [32]
<i>Macadamia angustifolia</i>	Proteaceae	New Caledonia	Jaffre [32]
<i>Maytenus fourmieri</i>	Celastraceae	New Caledonia	Jaffre [32,33]
<i>Macadamia sebertiana</i>	Celastraceae	New Caledonia	Jaffre [32]
<i>Alyxia rubricalis</i>	Apocynaceae	New Caledonia	Jaffre [33] & Brooks et al. [34]
<i>Gossia bidwillii</i>	Myrtaceae	Australia	Bidwell et al. [28]
<i>Phytolacca americana</i>	Phytolaccaceae	China	Xue et al. [22,35]
<i>Polygonum hydropiper</i>	Polygonaceae	China	Wang et al. [23]
<i>Schima superba</i>	Theaceae	China	Yang et al. [26]
<i>Chengiopanax sciadopylloide</i>	Araliaceae	Japan	Mizuno et al. 2008
<i>Maytenus cunninghamii</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia bamagensis</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia fragrantissima</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia sankowsiorum</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia gonoclada</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Polygonum pubescens</i>	Polygonaceae	China	Hua et al. [36]
<i>Polygonum perfoliatum</i>	Polygonaceae	China	Liu et al. [37]
<i>Celosia argentea</i>	Amaranthaceae	China	Liu et al. [27]
<i>Eucalyptus grandis</i> x <i>Eucalyptus urophylla</i>	Myrtaceae	China	Xie et al. [38]
	Myrtaceae	China	Xie et al. [38]
<i>Polygonum lapathifolium</i>	Polygonaceae	China	Kehui et al. [39]

enhanced metal toxicity tolerance ability in plants by producing of ACC deaminase under the toxic metal stressed condition. Glick and Penrose and Glick et al. claimed that endophytic bacteria produced ACC deaminase can metabolize into-ketobutyric acid and ammonia then lowering the ethylene level in plants and promote plant growth under the heavy metal stress. Yuan et al. showed that number of endophytic bacterial community associated with *P. americana* in Mn polluted mining sites [97]. This is indicated that endophytic bacteria have Mn tolerance and play an important role in plant growth. This study has shown that endophytic bacteria are able to improve the Mn stress tolerance mechanisms in *P. americana*. However, less attention has been paid to the root environment of hyperaccumulator and endophytic bacteria, but more studies are needed to ascertain this. On the other hand numerous researchers have highlighted that, Arbuscular Mycorrhizal Fungi (AMF) can enhance the heavy metal tolerance ability in host plants [98]. In addition, AMF can enhance the heavy metal accumulation and biomass levels in hyperaccumulators. Miransari mentioned that, AMF could increase the growth of their host plants by enhance the water and nutrients uptake. Yuan et al. investigated the diversity of AMF species in the rhizosphere and root of *P. americana* grown under the high Mn contaminated mining area.

Conclusions and Scope of Future Research

Manganese hyperaccumulators have evolved highly efficient

physiological mechanisms for the uptake of Mn by their roots, followed by xylem loading, translocation to the shoots and detoxification. The Mn is mainly accumulated as Mn²⁺ and then loaded into the xylem, transferred to the shoot. During the process of Mn²⁺ uptake, Mn²⁺ can bind carboxylic and hydroxylic groups in the root cell wall of hyperaccumulators. Manganese uptake and transport are closely related to the uptake and transport of Ca and Fe. The presence of high levels of oxalate and citrate are involved in Mn sequestration in the roots. Foliar Mn²⁺ is mainly stored in vacuoles, the endoplasmic reticulum and the Golgi. It is sequestered by organic ligands and some transporter proteins at a subcellular level in shoots, which confers great tolerance on the plants. Pyrolysis is a suitable method for the disposal of Mn hyperaccumulator biomass. Phytomining is a novel technology for the recovery of Mn from plant biomass to be used for industrial applications.

This review has focused on the recent progress in understanding the mechanisms of hyperaccumulation and tolerance in hyperaccumulators. On the basis of the recent literature, a picture emerges of Mn uptake, transport and detoxification in hyperaccumulator plants. However, some aspects have not yet been fully understood, particularly at the molecular and genetic level. Moreover, the following research gap needs to be further explored.

- There is very less number of Mn hyperaccumulator plant

species have been identified. Therefore, more plant species need to be explored for Mn hyperaccumulation.

- Detailed research is required for understanding the underlying mechanism of Mn hyperaccumulation and their response to changing Mn levels in the environmental.

- There are no report is available on transporters role in uptake, translocation, distribution and detoxification in Mn hyperaccumulators. Hence, it is necessary for Mn hyperaccumulation.

- In some Mn hyperaccumulators, Mn deposited in non-photosynthetic tissues for the detoxification process, but no data are available to explain why different hyperaccumulators have different Mn distribution patterns. Therefore, further detailed experiments are needed for a clear understanding of these processes in Mn hyperaccumulators.

- More research is required to increase the understanding of the sequestration of Mn in root and leaf cells of Mn hyperaccumulators.

- The excessive Mn cause toxicity to plants by producing ROS, which affects plant growth and metabolism. However, the mechanism of antioxidative defines system in Mn hyperaccumulators are not well known. Some studies have been reported that modulation of antioxidative enzymes (SOD, CAT, POX and APX) activities occur in response to Mn-induced oxidative stress. But no information is available on gene expression of these antioxidative enzymes isoforms.

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