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Ami Lokhandwala
Natubhai V Patel College of Pure and Applied Sciences Vallabh Vidyanagar, Gujarat, India

Pratibha Parihar
Natubhai V Patel College of Pure and Applied Sciences Vallabh Vidyanagar, Gujarat, India

Madhumati Bora
Natubhai V Patel College of Pure and Applied Sciences Vallabh Vidyanagar, Gujarat, India

Correspondence
Madhumati Bora
Natubhai V Patel College of Pure and Applied Sciences Vallabh Vidyanagar, Gujarat, India

Mycorrhizal contacts can get better adaptability for host plant under metal stress

Ami Lokhandwala, Pratibha Parihar and Madhumati Bora

Abstract

Massive accumulation of heavy metals in both land and terrestrial ecosystems has become a severe fret globally via improper disposal of municipal solid waste and industrial discharge, offensive use of agrochemicals, pig manure, coal, and wood ashes etc. Heavy metals are bioaccumulated and biomagnified at unparalleled level; where they intermingle with various essential nutrients leading to undue rise of reactive oxygen species resulting in to serious anomalies in living organisms. Phytoremediation though a slow and inexpensive technique for heavy metal exclusion but is an in-situ and much focused process. Arbuscular mycorrhizal fungi have been reported to assuage heavy metal stress of plants. Alleviation of heavy metals from contaminated soil by plants is either through phytoextraction or by phytostabilization. In Arbuscular Mycorrhizal Fungi colonized plants phytostabilization includes immobilization of heavy metals in fungal vesicles or hyphae. Release of organic acids, production of glomalin protein, metallothionine protein and secretion of plant growth promoting substances by mycorrhiza add to the remediation process by helping plants to eliminate heavy metals from polluted soil. This review is aimed to explore the role of mycorrhiza mediated phytostabilization in heavy metal remediation.

Keywords: Heavy metals, phytostabilization, arbuscular mycorrhiza
Abbreviations: Heavy metals -HMs, Arbuscular Mycorrhizal Fungi- AMF

1. Introduction

1.1 Heavy metal toxicity to plants

One of the major abiotic stresses detrimental to crop yield and productivity is heavy metal toxicity. Heavy metals (HMs) like arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) belong to group of non biodegradable, persistent inorganic chemical constituents with atomic mass over 20 units and the density higher than 5 $g \cdot cm^{-3}$. They are cytotoxic, genotoxic, and mutagenic to all living organisms. In terrestrial ecosystems, soil serves as the largest sink for HM deposition [1]. The input of HMs in soil can be natural or anthropogenic. In terrestrial plants the root and the rhizomes are general the only organs that are in contact with HMs. Essential and non-essential HMs get transported in plants via xylem and phloem transporter systems [2]. Some of these metals like Zn, Cu, Manganese (Mn), Ni and Cobalt (Co) in trace amounts serve as essential nutrients necessary for plant growth, while others such as Cd, Pb and Hg have no known biological function. However, excess of these same HMs can lead to the reduction and inhibition of growth in plants [3]. Chlorosis in young plants, root cells damage, weakened photosynthesis, distorted enzymatic functions, stunted growth, plant death, arrest in enzyme activities like nitrogenase, nitrate reductase, nitrite reductase, glutamine synthatase and glutamate hydrogenase are some of the toxic effects observed due to excessive concentrations of Cr [4, 5]. It has been also reported that As can replace Mg of chlorophyll molecule and block photosynthesis, it can also block various metabolic processes in cell by interacting with sulphydryl group of proteins, can replace phosphate in ATP, cause ultrastructural disorders, increase amount of plastoglobuli, and cause decline in net stomatal conductance, cellular CO_2 concentration and transpiration rate [6, 7, 8]. Furthermore, it has also been reported that As and Hg interact with the sulphydryl group and form S-Hg-S bridges affecting seed germination and embryo growth [9]. Exposure to higher concentration of Cd showed restricted growth, leaf area and suboptimal production of photosynthetic pigment in plants [10]. Pb can result in modification of chloroplast structure since S- and N- ligands of protein have higher affinity for Pb [11]. It also leads to plastoquinone restriction, impairment in carotenoids synthesis pathway, interference in electron transport chain, altered membrane permeability, insufficient supply of CO_2 and inactivation of numerous enzymes [11, 12]. Excess HMs deposited in soil can have detrimental effects on human beings through various absorption pathways of ingestion, dermal contact, inhalation, and oral intake [13, 14, 15, 16, 17].

2. Phytoremediation: Pros and Cons

Plants are able to battle against stresses like HMs by producing reactive oxygen species (ROS) which decide the destiny of functioning of cells. ROS have been classified into two groups on the basis of biological significance of HMs: redox active and inactive elements [18]. Redox active elements have direct participation in redox reactions which leads to production of superoxide radicals, hydrogen peroxide and most deadly hydroxyl radicals via combined Haber-Weiss and Fenton reaction [19]. Redox inactive elements disrupt electron transport chain and accelerate ROS production inside the cells [18, 20]. To cause efflux of HMs, plant use certain detoxification mechanisms like antioxidant defense system, cellular homeostasis and certain metal uptake and transportation genes [8, 21, 22]. Plants chelate HMs extracellularly by production of root exudates and/or restrict the uptake of HMs inside the root cells by adsorbing them to the root cell walls. The mega chelators - Phytochelatins and metallothioneins bind to HMs and the resulting chelates are exported from the cytoplasm across the tonoplast and then are seized inside the vacuole [23]. Other organelles are also involved in storage of HM bound chelates; for example Fe is bound to ferritin and stored inside chloroplasts [24].

Remediation of HMs involving conventional techniques is not only expensive but poses a threat to humans and our environment; as such techniques leave behind non-biodegradable products and also harm the soil microflora from that particular site. These techniques, instead of solving the problem shift it to future generations [25]. On the other hand, plants offer inexpensive and sustainable on-site approaches for HM remediation from contaminated soil, by the technique called phytoremediation. The concept of phytoremediation was first proposed in 1983 [26] and then was developed through the study of plant species ability to remove pollutants from environmental components. Phytoextraction, phytostabilisation, phytovolatilization and rhizofiltration are four processes recognized under phytoremediation [27]. Though phytoremediation technique has exceptional advantages like unique translocation, bioaccumulation and contaminant degradation capabilities, prevention of dramatic landscape disruption; but it has certain constraints like it is a time-consuming process, intermediates formed during the cleansing process may be cytotoxic to plants [28] and as cleaning of phytoremediated sites takes several years it poses acute risks for human health and other ecological receptors [29]. There comes the role of rhizospheric soil organisms like Arbuscular Mycorrhizal Fungi (AMF) who contribute in tolerance towards HM-contamination in plants. Presence of AMF can increase the scope and decrease the time consumption of HMs eradications by plant.

3. Mycorrhiza mediated remediation (MIR): Phytostabilization

Basic principles in detoxification mechanisms by plant with or without mycorrhizae involves binding, accumulation and transportation of HMs; but presence of mycorrhiza offers extra profit through presymbiotic hyphal extension, metabolization and sometimes immobilization of these HMs [30]. Comprehensive translocation process of HMs in plants with and without with AMF are depicted in figure 1 and explained below:

1. Secretion of chelating agents like organic acids/histidine by plants and glomalin by AMF: Organic acids like citric acid, malic acid or total organic acids play role in metal chelation [31, 32, 33]. Histidine is reported for hyperaccumulation of heavy metals especially Ni [34]. Glomalin; an insoluble glycoprotein is the fungal counter-part which acts as chelating agent [35, 36].
2. HM-chelation by root exudates and/or binding of HMs to the rhizodermal cell walls: plant roots secrete some enzymes which play key role in transformation and chemical speciation of heavy metals, facilitating their uptake by plants [37, 38, 39]. Acidification/alkalinisation, modification of the redox potential, exudation of metal chelants and organic ligands are some root activites which influence HMs solubility [40, 41, 42]. Most of the HMs demonstrated bind to cell wall components like chitin, cellulose, cellulose derivatives and melanins [43].
3. Plasma membrane as selective barrier: It has been reported that plasma membrane act as first selective barrier for transport of essential and non-essential heavy metals like Ni, Aluminium (Al) and Cd; which brings out various changes in cell membrane like changes in transmembrane electrical potential difference, membrane permeability and also leads to structural changes [44, 45, 46].
4. Active and passive transport through plant/fungal plasma membrane: Translocation of HMs in plant occurs via two different routes: active and passive. Passive uptake is driven by concentration gradient while active uptake is substrate specific and energy driven [47, 48]. Fungus also take up the heavy metals through their membranes via passive transport i.e., bioaccumulation and/or via active transport i.e., bioabsorption [49].
5. Chelation of HMs in cytosol and their export: Thiol compounds, glutathione, organic acids, metallothioneins, phytochelatins are certain chelating agents present in plant and mycorrhiza which chelate HMs in cytosol and are exported [23, 50, 51]. High nitrogen and sulphur associated with polyphosphate granules in mycorrhiza is thought to be involved in HM- thiol binding [43]. The metal-chelator complexes thus formed travels across the cytoplasm, tonoplast and sequester in vacoule [23]. ATP or by the electrochemical gradient generated by H+-ATPase or by V-ATPase and pyrophosphatase (PPase) energize the protein systems present in cytosol and tonoplast respectively [52, 53]. Other plant organelles like chloroplast and endoplasmic recticulum also act as storage organelles [24, 54]. Protein of Cation diffusion facilator (CDF) family present in chloroplast might be invovled in accumulation of HM [24, 55].
6. Transport of HMs through mycorrhizal hyphae: Formation of extramatrical mycelium is important for acquisition of HMs. It has been reported that AMF shows a unique response of increased presymbiotic hyphal extension in *Glomus intraradices* when exposed to Cd and Pb [56]. Presymbiotic hyphae of *Glomus intraradices* formed presymbiotic spores, whose initiation was more affected by HMs than was of presymbiotic hyphal extension. They also reported that patterns exhibited by *Glomus intraradices* spore germination, presymbiotic hyphal extension, symbiotic extra radical mycelium expansion, and sporulation under elevated metal concentrations indicate that AMF can accumulate high amounts of HMs in it. *Glomus mosseae* hyphae have also been reported to play role in sequestration of Cu, Zn, Cd and Ni [57]. Later, the HMs are exported in the arbuscules and imported back to plant cells [25].

4. Mechanism of heavy metal tolerance by AMF

The essence of heavy metal tolerance lies in understanding the effect of contaminated soils on AMF and its establishment. AMF is resistant to HMs only when colonized with plants; abandoned spores and pre-symbiotic hyphae are sensitive to HMs [58]. It has been reported that spores extracted from HM-contaminated soil are more tolerant than spores from uncontaminated soils [59, 60]. Tolerance towards the HMs is not passed from one generation to next; so such type of elevated tolerance of spores in polluted soils is due to phenotypic plasticity rather than genetic changes [61]. Further in this context, it has also been reported that tolerance levels of AMF of HMs varies with different species [25]. Phytostabilization is one of the most potential mechanisms via which AMF shows HMs tolerance.

Phytostabilization involves reduction of off-site contamination by decreasing the mobility of contaminants by precipitation within the soil, adsorption or uptake by roots or accumulation within roots [25, 62]. Plant roots play a vital role in this process, and so plant species having profuse root system are better HMs stabilizers. AMF enable plants to accumulate the HMs beyond the rhizosphere. Precipitation of polyphosphate granules in soil, adsorption to fungal cell walls and chelation of metals inside the fungus are some of the strategies applied for enhancing the phytostabilization [23, 63]. Glomalin is an insoluble iron containing glyco-proteinaceous compound released by AMF which can extract Cu, Cd and Pd from polluted soil [30, 64]. AMF provide certain plasticity for stabilization of aggregates via release of Glomalin Related Soil Protein (GRSP) and are also known as "flexible string bags" due to tensile strength of mycorrhizal hyphae [65]. A positive relationship between extractable glomalin production and soil aggregation ability has been reported [66, 67]. It had been postulated that environmental stress may enhance the glomalin production in mycorrhiza [64]. In this context, the role of glomalin in phytoremediation has also been recognized on the basis of their observations that one gram glomalin extracted from polluted soils accumulates 4.3 mg Cu, 0.08 mg Cd and 1.12mg Pb [68]. In host plants like *Poncirus trifoliata* [69] and *Posidonia oceanica* sea grass [70] it has been reported that macroaggregate stability in root/hyphae chamber is due to mycorrhizal colonization in which GRSP played a primary role.

Decrease in HMs concentration from contaminated soil result from rapid adsorption on AM mycelial cell wall which provides larger surface area for HMs binding and, its cation exchange capacity is comparable to other fungi [71]. The enhanced Cd tolerance in *Cajanus Cajan* (L.) Mill [72] colonized with *Glomus mosseae* is bestowed by AMF partly due to its influence in arresting NaCl-induced Cd uptake by root tissues. However it has also been reported that Cd uptake varies with mycorrhizal species wherein *Glomus constrictum* enhances Cd phytostabilization while *Glomus mosseae* decreases Cd uptake in maize [73]. On the other hand *Glomus mosseae* accumulates higher amount of Pd relative to Cd and Co in barley [74]. Mycorrhiza colonized plants also showed accumulation of Zn from soil but at its lower concentrations only [75]. It has also been reported that mycorrhizal symbiosis enhances Cr uptake from soil in plants like sunflower, dandelion and flax [76, 77, 78]. It is interesting to note that mycorrhiza has also shown potential in uptake of As. Species like *Glomus mosseae*, *Glomus versiforme*, *Acaulospora scrobiculata, Funneliformis geosporum*, *Rhizophagus intraradices* when colonized with host plants showed increased uptake of As and Arsenite as compared to control plants [79, 80, 81, 82]. Apart from hyphae, vesicles also share their part in detoxification mechanisms for HMs [25]. Moreover it was also found that HMs get accumulated in parenchyma cells coinciding with fungal structures [83]. AMF also brings changes on genetic level; which is also attributed to HM-loss from plant tissue called as "dilution-effect" [84, 85]. AMF creates a balanced environment for plants which ultimately helps to cope up the plants with elevated levels of HMs. This buffering effect is brought by alterations in polyamine metabolisms or by altering the expression of genes involved in oxidative stress or lipid peroxidation mechanisms [86, 87, 88]. Thus phytostabilization with various other altered mechanisms at protein and gene levels enhances the HM sequestering capacity of plants.

5. Conclusion
Available reports illustrate the role of AMF as an enhancer in phytostabilization process; but mechanisms underneath seems complex and it varies with different AMF species and with HM pollutants. It is therefore difficult to provide a generalized scheme of mechanism, but one can exploit the phytostabilization capacity of AMF using specific plant and fungal genotypes. However, this perception of its mechanism and tolerance has provided a standard which can be worked out to prepare better designs of research plans with practical industrial and agronomic applications.

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