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Review

Arbuscular Mycorrhizal Fungi as Potential Agents in Ameliorating Heavy Metal Stress in Plants

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Abstract: Heavy metal accumulation in plants is a severe environmental problem, rising at an expeditious rate. Heavy metals such as cadmium, arsenic, mercury and lead are known environmental pollutants that exert noxious effects on the morpho-physiological and biological attributes of a plant. Due to their mobile nature, they have become an extended part of the food chain and affect human health. Arbuscular mycorrhizal fungi ameliorate metal toxicity as they intensify the plant's ability to tolerate metal stress. Mycorrhizal fungi have vesicles, which are analogous to fungal vacuoles and accumulate massive amount of heavy metals in them. With the help of a pervasive hyphal network, arbuscular mycorrhizal fungi help in the uptake of water and nutrients, thereby abating the use of chemical fertilizers on the plants. They also promote resistance parameters in the plants, secrete a glycoprotein named glomalin that reduces the metal uptake in plants by forming glycoprotein–metal complexes, and improve the quality of the soil. They also assist plants in phytoremediation by increasing the absorptive area, increase the antioxidant response, chelate heavy metals and stimulate genes for protein synthesis that reduce the damage caused by free radicals. The current manuscript focuses on the uptake of heavy metals, accumulation, and arbuscular mycorrhizal impact in ameliorating heavy metal stress in plants.

Keywords: mycorrhizal fungi; translocation; heavy metals; toxic effects; plant–microbe interactions; amelioration

1. Introduction

A "heavy metal" is any element having metallic properties, with relatively high density, and is harmful at even low concentrations [1]. The most phytotoxic and non-essential heavy metals (HMs) found in the soil are lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg), which are highly mobile and are promptly absorbed by the plants. Besides these, there are other HMs (copper, chromium, zinc, selenium, molybdenum, tin, nickel etc.), which cause environmental contamination and health risks [2–4]. Generally, metal concentration is assessed by the amount of metal in the bedrock and its deposition from the atmosphere. Small amounts of these HMs are already present in the soil and atmosphere, but other things keep on adding these to the environment and include industrial and natural anthropogenic activities, fertilizers, polluted water, sewage sludge, the weathering of soil and minerals [5]. HMs have detrimental impacts on the plant metabolism, soil fertility, terrestrial ecosystems and human health [6]. The HMs get transferred to the human body through soil–plant–food interactions and cause exigent defects [7]. HMs cause harmful impacts on plant growth and productivity by many mechanisms, such as (i) interference with the activity of nutrients, which are essential for plant growth; (ii) effects on plant morphology and physiology; (iii) negative impacts on the soil microbes' growth, including that of symbiotic microbes such as rhizobia; and (iv) effects on soil properties [8,9].

Most of the HMs on the soil surface occur naturally, but the condition becomes worse when these metals are added in surplus amounts in the surrounding atmosphere [10]. High amounts of HMs in plant tissue exert toxicity in plants, disrupt cell membrane permeability, inhibit photosynthesis and mineral uptake, affect plant morpho-physiological and biological processes and ultimately inhibit the growth rate [1,11]. They also result in the production of free radicals such as hydrogen peroxide (H_2O_2), superoxide radical (O^{2-}) and hydroxyl radical ($^{\bullet}OH$), which further react with cellular organelles and cause damage to the cell and DNA, inhibit ATP (adenosine triphosphate) production and cause lipid peroxidation [12,13]. HMs' ability to cause damage relies on the already existing stage of stress exposure, its duration, the concentration of HMs and their bioavailability in plant organs [1,14]. Plants possess indigenous defense mechanisms, i.e., they have antioxidant enzymes that are involved in warding off HM stress. HMs produce ROS (reactive oxygen species), so at their highest concentration, these enzymes do not function. Numerous physical and chemical strategies have been used to ameliorate HM ion concentrations in soil [15,16]. These include soil washing, excavation, acidification, vitrification and land filling. These methods are observed to be expensive and alter the quality of the soil, which makes it unsuitable for the growth of rhizospheric microbial communities. Meanwhile, biological methods (the use of plants and microbes) have been proved to be an innovative solution as they are cost-effective, easy to implement and maintain the natural properties of the environment [9].

In a natural ecosystem, plants continually interact with a large number of microorganisms in the rhizosphere and create mutually beneficial alliances with them. Arbuscular mycorrhizal fungi (AMF)-like beneficial fungi develop a symbiotic association with the plant roots where photosynthetic products prepared by the plants are received by the fungus and, in turn, it helps the plant by enhancing nutrient uptake, protecting them from HM toxicity, enhancing biomass accumulation and improving photosynthesis [17,18]. Approximately 80% of terrestrial plant roots form symbiotic relations with fungi, where fungal hyphae percolate in the cortical cells of plant roots forming arbuscules, vesicles and hyphae [19]. They also render HMs immobile at the cortical region of roots by binding with them, prevent their translocation towards aerial parts of the plant and prevent leaf tissues from damage. AMF are a type of endomycorrhizal fungus (phylum Glomeromycota) that provides nutrients for plant growth, by ameliorating water uptake and controlling stomatal conductance [20]. AMF is considered as one of the best biological tools for enhancing plant growth and shoot biomass as it can detoxify HM-induced stress. AMF reduces HM stress by HM immobilization in fungal structure, precipitation and chelation in the rhizosphere, sequestration in vacuoles and activation of antioxidant mechanisms in plants [16]. The utilization of AMF is an ecofriendly approach and a useful component to attain sustainable productivity by improving soil health and protecting plants against abiotic and biotic stresses [18]. Thus, the main objective of this study is to review the role of AMF in maintaining a sustainable environment in an HM-contaminated ecosystem. It includes the HMs' impact on human health and plants. At the same time, it also highlights various ways in which AMF minimizes the HMs' impact on plants.

2. Heavy Metals (HMs) and Their Impacts

Heavy metals (HMs) such as As, Pb, Hg, Cd, Cr, Ni and Cu are the toxic metals that are listed as the 1st, 2nd, 3rd, 7th, 17th, 58th and 120th most harmful substances by the Agency for Toxic Substances and Disease Registry, respectively, on the basis of their toxic effects on human beings [21]. Different sources of HMs and their impacts on human health are described in Table 1.

Table 1. Health effects on humans of different heavy metals (NS—not specified).

Heavy Metal	Source	Country	Biological Effects	Ref
Arsenic (As)	Drinking water	Hungary	Increase in stillbirths and spontaneous abortion	[22]
	Drinking water	Chile	Increase in stillbirths and infant mortality	[23]
	Drinking water	Bangladesh	Increase in spontaneous abortions, stillbirths and preterm births	[24]
	Drinking water	Taiwan	Reduced birth rate	[25]
	Drinking water	India	Increase in stillbirths	[26]
	Drinking water	Chile	Increased liver cancer mortality for ages 10–19	[27]
	Drinking water	Chile	Increased lung cancer and chronic obstructive pulmonary disease (COPD) deaths for ages 30–39	[28]
	Drinking water	Bangladesh	Chronic cough, breathing problems or blood in the sputum of 39 aged people	[29]
	Drinking water	Mongolia	Skin hyperkeratosis, hyperpigmentation and depigmentation and other skin lesions among men	[30]
	Soil and vegetables	Nigeria	Liver damage, gastro-intestinal effects, lung cancer and skin lesions	[31]
	Rice and edible mushrooms	China	NS	[32]
	Food	Spain	NS	[33]
Cadmium (Cd)	Soil	Thailand	High prevalence of renal dysfunction, bone mineral loss, hypertension and urinary stones	[34]
	NS	Sweden	Tubular and glomerular kidney effects in women	[35]
	Industrial plants	Italy	Delayed onset of puberty in male adolescents and impaired testicular growth	[36]
	Soil and vegetables	Turkey	Gastro-intestinal, renal prostate and ovarian cancer	[37]
	Soil	Nigeria	Damage to central nervous system	[31]
	Rice and edible mushrooms	China	NS	[32]
	Vegetables and Fish	China	NS	[38]
	Food	Spain	NS	[33]
	Smelting	China	Renal dysfunction	[39]
	Rice	Thailand	Kidney, lung and liver problems	[40]
	Food	South west china	Bone damage, kidney injury and cancer	[41]
	Mining areas	South china	Renal effects, particularly in children	[42]
	Drinking water	Iran	NS	[43]
Chromium (Cr)	Ground water	India	Gastrointestinal and dermatological complaints and abnormal hematological function	[44]
	Chromate production plant	USA	Nasal irritation and nasal ulceration	[45]
	Vegetables	Nigeria	Respiratory problems, lung cancer and skin rashes	[31]
	Vegetables	China	NS	[38]
Copper (Cu)	Drinking water	Chile	Nausea, abdominal pain or vomiting	[46]
	Vegetables	Bangladesh	Kidney damage or tumors	[47]
	Vegetables	China	NS	[38]
	Drinking water	Iran	NS	[43]
Mercury (Hg)	Mercury mining sites	China	Postural sway, as well as hand tremor, may be affected by elemental mercury vapor exposure	[48]
	Mercury mines	China	Finger and eyelid tremor, gingivitis, and typical darkline on gums	[49]
	Tapajos river basin	Brazil	Sensory disturbance (especially glove-and-stocking type, which is characteristic of Minamata disease), tremor, failure in two-point discrimination, and slight balancing failure	[50]
	Rice and edible mushrooms	China	NS	[32]
Lead (Pb)	Glass work plant	China	Susceptible autonomic nervous function	[51]
	Battery plant	Turkey	Increased erythrocyte malondialdehyde (MDA) levels, catalase and glucose-6-phosphate dehydrogenase (G6PD) activities, and decreased blood glutathione/glutathione disulfide ratio	[52]
	Automobile plant	India	High blood pressure, less heme biosynthesis	[53]
	Soil	China	Renal dysfunction	[54]
	Vegetables	Nigeria	Neurological, immunological effects	[31]
	Vegetables	China	NS	[38]
	Food	Spain	NS	[33]
	Battery plant	China	Neurological damage	[55]
	Drinking water	Iran	NS	[43]

In the biological processes of organisms, metals play a vital role. Some metals serve as micronutrients and are essential, while others are toxic and have no biological role in organisms.

Moreover, non-essential metal have a bad impact on the environment, thus decreasing the biological activity of the soil. They enter the food chain and affect human health. A worldwide map of heavy metals showing the countries where significant impact has been observed is represented in Figure 1.

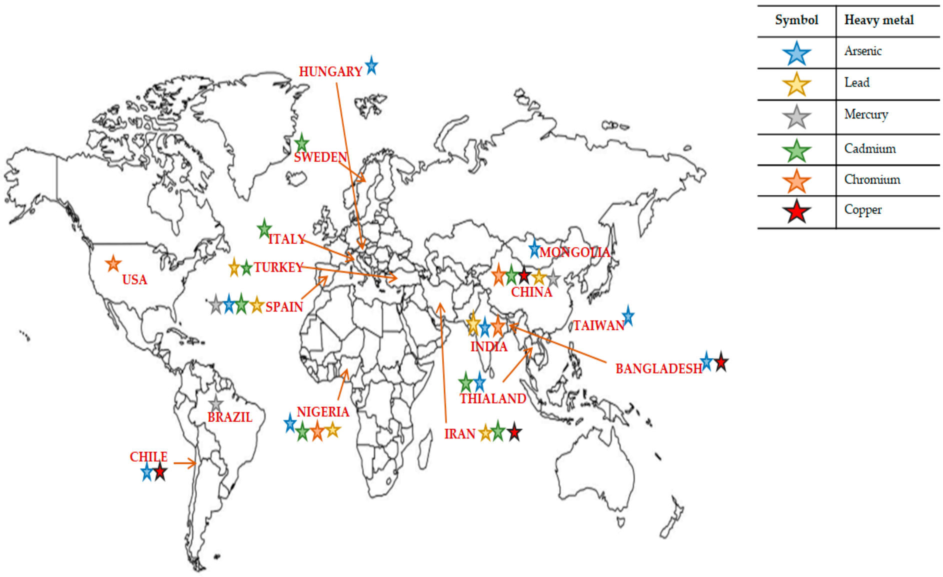

Figure 1. Global map of heavy metals showing countries where significant impact has been observed.

Generally, HMs at their lowest concentrations have the potential to cause intense damage to plants as well as to human beings through the food chain [56]. According to the European Commission, the maximum levels of different HMs permitted in food items are as depicted in Table 2.

Table 2. Maximum level of heavy metals in food components (European Commission, 2006, 2015/1005, June 2015 and National Food Safety Standard (GB 2762-2012)).

Heavy Metals	Food Components	Maximum Levels (mg kg^{-1} wet weight)	Ref.
Lead (Pb)	Leafy vegetables, brassica vegetables and a few fungi like *Pleurotus ostreatus* (oyster mushroom), *Agaricus bisporus* (common mushroom) and *Lentinula edodes* (shiitake mushroom)	0.3	[57] with permission
	Vegetables (excluding fresh herbs and fungi, leafy vegetables and brassica vegetables)	0.1	
	Berries and small fruits	0.2	
	Cereals, pulses and legumes	0.2	
	Fruits (excluding small fruits and berries)	0.1	
Mercury (Hg)	Muscle meat of fish and fishery products	0.50	
Cadmium (Cd)	Fresh herbs, leafy vegetables, celeriac and some fungi like *Pleurotus ostreatus* (oyster mushroom) and *Agaricus bisporus* (common mushroom)	0.2	
	Potatoes and root and stem vegetables (excluding celeriac plants)	0.1	
	Fruits and vegetables (excluding fresh herbs, root and stem vegetables, fungi, potatoes and leafy vegetables)	0.05	
	Soybeans	0.2	
	Cereals (excluding rice, wheat, germ and bran)	0.1	
	Rice, wheat, germ and bran	0.2	
Tin (Sn)	Canned food (except beverages)	200	
	Canned beverages (including vegetable and fruit juices)	100	
	Processed cereal based products (excluding powdered and dried products)	50	
Chromium (Cr)	Fresh vegetables	0.5	[58]
	Grains and its products	1.0	
	Beans	1.0	
	Meat and its products	1.0	
Nickel (Ni)	Oil and its products (mainly hydrogenated vegetable oil)	1.0	

In the case of As, there is no maximum level, but the European Food Safety Authority (EFSA) Panel recommended to reduce the use of inorganic As for dietary exposure. According to EFSA, 0.20–0.30 $\mu g\ kg^{-1}$ is the optimum/maximum level of As that should be present in food.

3. Sources of HMs

The HMs are naturally available in the soil, but their concentrations rise in the environment because of human activities that have an adverse impact on plants and humans. They are mobile between soil–plant systems and are frequently being added to the soil via several agricultural activities like agrochemical usage. Besides the natural weathering process, there are different sources of HMs in the soil like chimney emissions from factories, the excessive supplementation of phosphate fertilizers, additives in pigments and gasoline, pesticides, metal-polluted water and effluent from storage batteries [59–67]. A major source of Hg in the soil is power plants that burn coal for energy generation. The exhaust fumes of automobiles in urban areas contribute to atmospheric pollution. Plants growing along roadsides constitute a large quantity of HMs due to the combustion of gasoline in vehicles. The contamination of soils occurs by HM compounds like lead arsenate ($PbHAsO_4$) and bordeaux mixture ($CuSO_4$), which are used as a pesticides in orchards. The mining and smelting of metalliferous ores with fossil fuel combustion have globally increased the risk associated with HMs in the soil over the past few centuries.

4. Uptake, Translocation and Accumulation of HMs in Plants

Initially, HMs present in the soil are absorbed by the roots and can be translocated to the other parts of the plant. Very often, heavy metals remain in the roots because of the barrier effect. The uptake of HMs by plant roots depends on the bioavailability of the metal ions (i.e., not co-precipitated with oxides or adsorbed to organic matter) in the soil solution and is regulated by soil pH and carbonate contents [68]. The pathways through which HMs enter the roots include H^+ ATPase/pumps, which help in maintaining the negative potential across the epidermal membrane in the roots; HM ions present in the soil make their entry into the plant epidermis either through ion channels or as organic compounds formed after combination with chelates released by plant roots in the soil, thus entering as metal–ligand complexes into the root epidermal layer [69]. Different transporters including members of the HM ATPases (HMA) family, Zrt/Irt protein (ZIP) family and natural resistance-associated macrophage protein (NRAMP) family are also involved in the uptake of HM ions in plants. Generally, root hairs enhance the surface area of roots for HM absorption so that ions migrate quickly inside the apoplast [70–72]. HM ions, once absorbed by the roots, are bound directly to the carboxyl groups of mucilage uronic acid or to the polysaccharides on the rhizodermis and show apoplastic movement from the root epidermis to the cortex region [73,74]. These ions primarily accumulate in root cells because of the obstruction in the apoplastic pathway at the endodermis by casparian strips. Later, after crossing the endodermis, HM ions travel by the symplastic pathway through vascular tissues, i.e., xylem and phloem and are translocated to the above-ground parts of the plant, as shown in Figure 2.

The absorption of HM ions does not remain uniform along the plant roots, as their concentration varies in root apices and other regions of the root. In the case of a HM such as Pb, the highest concentration of this HM was found in the young root cells having thin cell walls [75]. Limited transport of metal occurs from the roots to the above-ground parts of the plant, as during the translocation of HM ions between the roots and shoots, the endodermis acts as a partial barrier [76]. Some plant species transfer a large amount of Pb to their shoots [77]. Pb in the wheat roots remains in the cell wall and can only be isolated as a complex with citric acid [78]. Different Pb complexes were also observed in the stem and leaves of alfalfa [79]. In addition to this, Pb is transferred to the stem and leaves in *Sesbania drummondii* [80]. Pb transportation is limited in the different parts of the plant due to the immobilization of insoluble Pb salts as precipitates in intercellular spaces, fixation in the cell wall by negatively charged pectins, and accumulation in the vacuoles of cortical and rhizodermal cells and the plasma membranes of cells [81–87]. The minimum availability of heavy metals at higher trophic levels can be possible by their minimum uptake. Hyperaccumulator plants (e.g., *Pteris vittata*, *P. cretica*, *P. biaurita*, *Sesbania drummondii*, *Helianthus annuus*, *Sedum alfredii*, *Atriplex halimus*, *Amanita muscaria* and *Polygonum aviculare*) are required for soil remediation as these plants are capable of growing in high metal concentrations, absorbing them through their roots and translocating them with minimal or no toxicity to the aerial parts of the plant. The amount of HM ions that is taken up from the soil by the plants is measured by the transfer factor [88,89].

$$Transfer\ Factor = \frac{Concentration\ of\ Heavy\ metal\ ions\ in\ shoot}{Concentration\ of\ Heavy\ metal\ ions\ in\ root}$$

A higher TF (Transfer Factor) value is observed in non-mycorrhizal fenugreek plants than in mycorrhizal ones [90]. Plants are classified on the basis of the transfer factor as its values are different in both hyperaccumulators (TF > 1) and non-hyperaccumulators (TF < 1) [91].

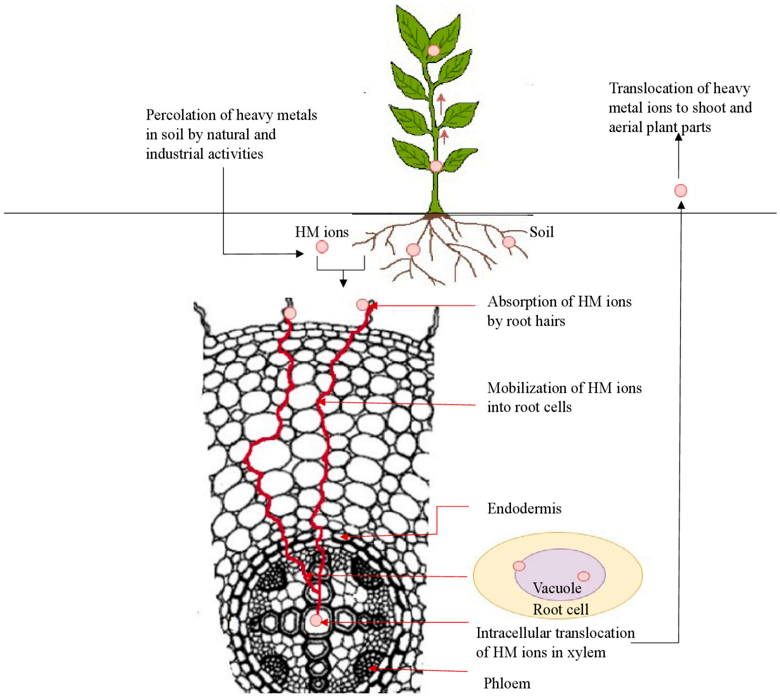

Figure 2. Uptake, translocation and accumulation of heavy metal (HM) in plants.

5. Impact of HMs on Plants in the Absence of AMF

Soil contaminated by toxic metals causes acute and chronic impacts on plant growth and productivity. Extreme concentrations of HMs affect the morphology of plants, which results in a decreased transpiration rate and damage to the root system [92,93]. Generally, HMs interact with the sulfhydryl group (–SH) of enzymes, which agitates chloroplast functioning by restricting the activities of enzymes related to chlorophyll biosynthesis, the aggregation of protein complexes involved in photosystems (PS), and carbon dioxide (CO_2) fixation [94]. Metal stress decreases chlorophyll synthesis, which is an imperative pigment in photosynthesis, by reducing the supply of essential elements like magnesium, zinc and iron, eventually affecting the plant–nutrient relationship [90]. It has been reported that the level of chlorophyll decreased when the seedlings of *Phaseolus vulgaris* were treated with Cd [95–97]. Photosynthetic plants, upon exposure to HMs, experience harmful effects in respiration and adenosine triphosphate (ATP) content. With the increasing metal stress, the ratio of chlorophyll a/chlorophyll b decreases, which implies a shift in the ratio of PSII/PSI. The experimental results indicate that Cd and Pb stress decreases the photosynthetic pigments (chlorophyll and carotenoids content) in the host plant in the absence of AMF [98,99].

HMs interfere with protease and amylase enzymes, which prevents germination and the development of seedlings [100]. Pb exposure causes inhibition of the germination of seeds as reported in *Oryza sativa*, *Pinus halepensis* and *Hordeum vulgare*, whereas mercury also affects seed germination as described in the case of *Enhalus Acoroides* and *Vigna radiata* [81,100–103]. It is recorded that a high concentration of Cd affects the growth of lettuce plants [104]. Many changes take place in mitochondria as they lose cristae and undergo swelling, nuclei becomes deep-colored, plasma membranes get injured, and vacuolization takes place in dictyosomes and endoplasmic reticulum after 42–72 h of Pb exposure, particularly in *Allium sativum*. HMs also interact with the cytoplasmic proteins of the plant and decrease protein synthesis, which leads to physiological changes in the plant [85]. A reduction in protein pool also results from the different actions of Pb such as the oxidation of proteins, the stimulation of protein activity, an increase in ribonuclease activity and the modification of gene expression [105–109]. Some reports indicate that in the absence of AMF, Cd stress decreases chlorophyll content and growth and increases the accumulation of H_2O_2 in *Solanum lycopersicum* [110].

HMs reduce essential mineral uptake and the plasticity of the plant cell wall, which further influences the turgor pressure of the cell. In *Helianthus annus*, a reduction in mineral uptake was observed when the plant was exposed to cadmium stress [111]. Metal toxicity leads to the production of free radicals like ROS that are formed during cellular metabolic processes, and the overproduction of these species is considered as oxidative stress. Free radicals react with cell organelles, membranes, biomolecules like lipids, proteins and nucleic acids (e.g., DNA, RNA); reduce the normal functions of cells; and induce abnormality. Free radicals cause DNA damage and the inhibition of ATP and lipid peroxidation, thereby generating oxidative stress [112,113]. They also damage electron transport chains in chloroplasts and mitochondria, which leads to the disruption of proteins by oxidative reactions or proteolytic activity. A high concentration of toxic metals causes the membrane to rupture and results in cell death. Abscisic acid is a phytohormone responsible for the opening and closing of stomata [114]. HM ions cause the accumulation of abscisic acid in aerial plant parts and roots, which leads to the closing of stomata, limits gaseous exchange with the atmosphere and reduces the transpiration rate [115,116]. Various impacts of heavy metals on plants are shown in Figure 3.

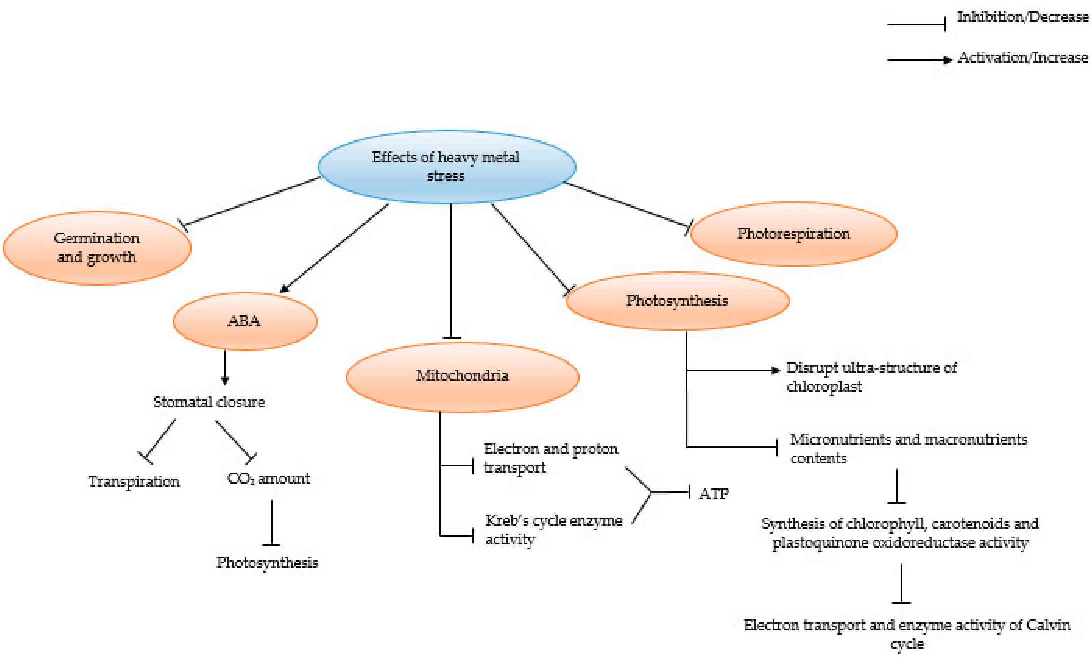

Figure 3. Effects of heavy metals on plants [100,117].

6. Role of AMF in HM Detoxification in Plants

Once HMs have entered the soil, they have long-term effects on plants. AMF are an essential bioagent, as they can significantly enhance the efficiency of the ecosystem by producing fungal structures like arbuscules and aid in the exchange of inorganic compounds and minerals required for the growth of plants, thereby providing considerable strength to plants. They also act as a biological filter for HMs and help in mitigating them. They help the plant to grow actively under stressful conditions by assisting the plant through increased water uptake, photosynthetic rate and nutrition [118]. Generally, HMs get immobilized in the fungal hyphae living in symbiotic association with plants, which reduce their availability to plants by retaining the HMs in the cell wall, vacuole or cytoplasm by chelation, thus decreasing metal toxicity in the plants [119,120]. Various researchers have signified the beneficial impact of AMF on the growth and productivity of plants [121]. Several different strategies have been ratified for how AMF influence the uptake, translocation and accumulation of HMs and enhance plant tolerance to HM stress. These strategies include various steps viz. the retention of HMs in the mycorrhizal roots and external hyphae, the stimulation of nutrient absorption, the sequestration of HMs in vacuoles, the binding of HMs on the fungal cell wall, the protection of the reaction center and the rectification of gas exchange capacity, the increasing of the antioxidant response of plants, the chelation of HMs in the cytosol of fungi and the induction of glomalin by AMF, and AMF-mediated phytoremediation.

6.1. Retention of HMs in Mycorrhizal Roots and External Hyphae of AMF

AMF colonization with higher plant roots enables the plant to retain HMs in their roots and external mycelium. AMF shows a mechanism to ameliorate HM toxicity in plants by retaining HMs in mycorrhizal structures such as the fungal mycelium and vesicles, where high amounts of HMs are concentrated, which further prevents their mobilization to aerial plant tissues as shown in Figure 4 [122].

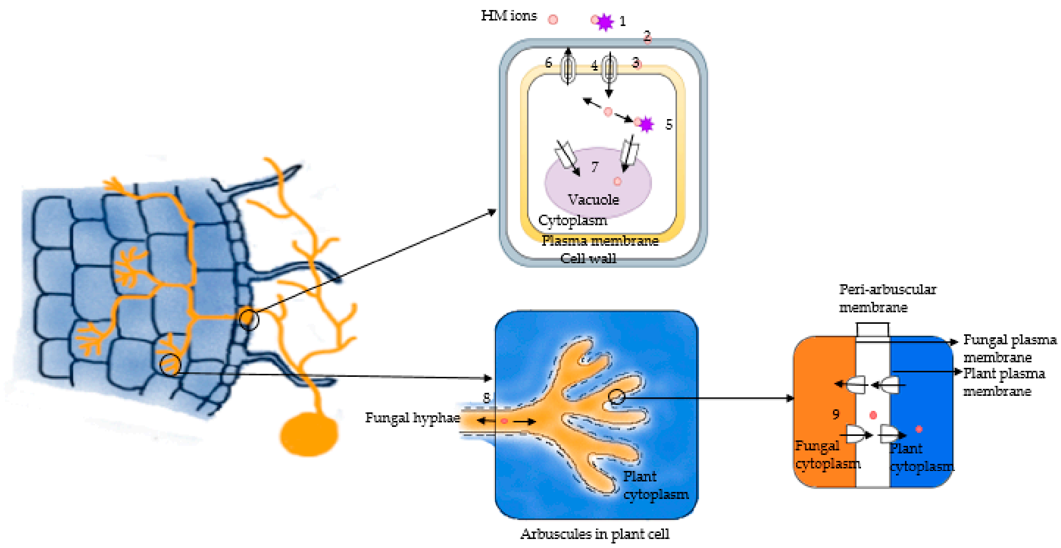

Figure 4. Mechanism of heavy metal (HM) detoxification in plants by arbuscular mycorrhizal symbiosis. (1) Chelating agents (phytochelatins and organic acids from the plant and glomalins from the fungus) bind with HM ions in the soil. (2–4) Transport of metal ions across the cell wall and plasma membrane (acting as selective barrier and with the help of transporters) of plants and fungi. (5) Chelation of HM ions in the cytosol. (6) Export of HMs via active or passive transport from plant or fungal cells. (7) Sequestration of HM ions in the vacuoles of plant and fungal cells. (8) Transport of HM ions in the fungal hyphae. (9) Export of HM ions from the fungus to plant cells via transporters in arbuscules. Modified from [123] with permission.

The fungal vesicles in mycorrhizal plants are analogous to the plant vacuoles, which are implicated in accumulating toxic compounds. The external mycelium of roots has a predominant effect on the immobilization of metals in the stressed soil because of its wider surface area, which makes them more efficient than the plant roots for absorbing metals. This retention mechanism by AMF decreases the translocation of HMs and protects leaf tissues from injury by retaining them in roots, therefore contributing to phytostabilization. In mycorrhizal plants, the HM tolerance potential is correlated with the yield of mycelial biomass and fungal growth, as metals remain accommodated in the fungal mycelium. Moreover, the vesicles in the fungi also increase with the increase in the concentration of HMs [99]. Pakchoi shoots were observed with significantly decreased concentrations of Pb upon the addition of mycorrhizal fungi, e.g., *Rhizophagus intraradices*, *Glomus versiforme* and *Funneliformis mosseae* [124]. The AMF colonization of *Aster tripolium* roots enabled the fungi to retain Cd in the mycelium and promoted plant tolerance to Cd [125]. The AMF inoculation of *Melastoma malabathricum* plants led to an increase in the absorption area of the roots in the soil and resulted in better plant growth [126].

6.2. AMF Promotes Nutrient Absorption in Plants

The AMF stimulate plant growth even in stressed conditions by modifying the soil structure. Generally, nutrients are absorbed from the soil by fungal hyphae and directed to the fungus–plant interface, where bidirectional exchange take place [127]. Under the influence of AMF, some enzyme characteristics are altered viz. the activity, quantity and number of enzymes in the soil, particularly the activity of acid phosphatase, which improves growth and nutrient uptake in the host plants [128]. A phosphate transporter is present in mycorrhizal fungi for the direct transport of inorganic phosphate (Pi) from the soil to the plants [129]. On the extraradial mycelium of *Glomus mosseae*, *G. intraradices* and *G. versiforme*, several genes encoding transporter proteins such as *GmosPT*, *GiPT* and *GvPT* have been identified that are involved in the uptake of phosphate [130–132]. AMF raise the concentration of phosphorus and other essential mineral nutrients in plants with enhancement in the uptake from soil, by extending beyond the limits of the plant roots and increasing the absorption area [133]. Higher contents of nitrogen, potassium and phosphorus have been observed in host plants subjected to HM stress in the presence of AMF [134–136].

6.3. AMF Sequester HMs in Vacuoles

Once HMs reach their target site, the mechanism of metal sequestration by polyphosphate granules in fungal vacuoles is activated [90,137]. Generally, sequestration limits the harmful effects of HMs, as the main compartment of HM storage in fungal and plant cells is the vacuole. Various reports have suggested that H^+ pumps in the vacuole, especially vacuolar proton ATPase (V-ATPase) and vacuolar proton-pyrophosphatase (V-PPase), aid in the uptake of HMs in vacuoles. Symbiosis increases the phosphorus content in the host plant, which provides metabolic energy as adenosine triphosphate (ATP) for the sequestration of metals within the vacuoles of the cells, thus restricting them to a smaller area and preventing their translocation to the other parts of the plant, as shown in Figure 4 [138–142]. Sequestration allows the concentration of a large amount of HMs in the above-ground parts without any phytotoxic effects on the plants. The vacuoles of leaf cells are the sequestration sites for non-essential and essential HMs [143]. The regulation of metal transport across the membrane is a decisive element in the control of metal homeostasis.

6.4. AMF Assist in HM Binding on the Fungal Cell Walls

The fungal cell wall also helps in the adsorption of HM ions. The fungal cell wall is made up of chitin and polysaccharides that are used as a barricade for HM ions and other solutes to get into the cells, thereby regulating their adsorption. Various functional groups such as imidazole carboxyl groups, amino groups and free hydroxyl groups provide binding sites for HMs and form negatively charged structures that adsorb most of the metals in the soil and block their movement in plants [144].

In addition, AMF store metals in the spores [145,146]. Several authors have reported that with the increase in metal concentrations, the cell walls of fungi act as a first line of defense as they obstruct the entry of HMs [11,138]. AMF induces the biosynthesis of cell wall in the roots of the host plant, which promotes metal reduction because with the increase in the thickness of cell wall, the area for metal absorption also increases [147]. At high concentrations of metal ions, a few of them pass through the cell wall, get deposited in the cell membrane of the fungus or are transported to the fungal cytoplasm, thereby reducing the amount of metal ions in the plants.

6.5. AMF Protects the PSII Reaction Center and Rectifies the Gas Exchange Capacity

A high amount of HMs in the soil is translocated to other plant parts and affects the enzyme activity alliance with chlorophyll biosynthesis, which further affects the photosynthesis process, mainly the photosystem II reaction center. The inoculation of AMF increases the chlorophyll content, especially chlorophyll a, which is responsible for photosynthesis in the host plant. Several findings indicate higher chlorophyll synthesis in plants inoculated with AMF that results in improved photosynthesis [110,134]. It has also been studied that AMF promote the enhancement of chlorophyll biosynthesis by increasing the de novo synthesis of proteins, which has a direct impact on the uptake of essential elements such as Mg and Fe, which constitute an imperative part of the chlorophyll molecule [111]. AMF symbiosis ameliorates the photosynthetic capacity and phytochemical efficiency of the host plants. It also improves the gas exchange capacity by stomatal opening and increasing the transcription fluxes.

6.6. Heavy Metals Enhance the Antioxidant Responses of Plants

Plants have antioxidative defense systems to protect themselves against heavy metal stress. These defense systems of plants include various enzymatic ROS scavengers such as glutathione peroxidase (GPX), catalase (CAT), superoxidase dismutase (SOD), glutathione reductase (GR), peroxidase (POD) and ascorbate peroxidase (APX), which protect against disruption and dysfunction in plant cells [90,148]. These antioxidant enzymes are highly active during metal stress. Generally, SOD detoxifies superoxide radicals (O^{2-}), thereby averting stress-induced cellular damage. SOD activity generates H_2O_2, which is transformed into water by APX or CAT [111,128]. Studies performed by various researchers have demonstrated that AMF have the ability to promote the synthesis of antioxidants and increase their activity under metal stress. For instance, Garg et al. (2013) reported that AMF, under oxidative stress, upregulate antioxidant enzyme activity and reduce ROS production in the host plant *Cajanus cajan* and aid in mitigating metal stress [149]. It has also been observed that the level of the antioxidant enzyme activities of APX, GR, CAT and SOD increases under cadmium stress [150]. Yang et al. (2015) demonstrated that AMF symbiosis with *Robinia pseudoacacia* improves ROS-scavenging capabilities and enhances the enzymatic activities under different concentrations of Pb stress [99]. It has also been reported that AMF activate and stimulate the antioxidant system in Cd-stressed soil in *Trigonella* plants [151].

6.7. AMF-Assisted HM Chelation

A unique amelioration strategy adopted by AMF is the chelation of HMs with ligands having high affinity for metal ions in the cytosol. The production of chelating compounds occurs if the concentration of toxic metals exceeds the threshold value inside the cells. Chelation helps in warding off HMs by rendering them less available in the cytoplasm and also by decreasing their solubility and reactivity. Various chelating agents are present in AMF and plants such as metallothioneins (MTs), organic acids, phytochelatins (PCs) and amino acids, which play a crucial role in the tolerance to HMs in plants by chelating these substances [2,152]. Generally, HMs enter the cytoplasm of plant cells through the Zrt-Irt proteins (ZIP) belonging to the family of metal transporters. The HM tolerance mechanism relates to the synthesis of thiol peptides called phytochelatins, which form complexes with HMs. AMF are capable of enhancing the synthesis of phytochelatins [153,154]. These peptides are synthesized enzymatically with glutathione (GSH) as a substrate, in the presence of the enzyme phytochelatin synthase (PCS), which is activated by the existence of HMs [155]. Phytochelatin (PC) is involved in metal–thiol binding. The metal chelator (HM-PC) complexes are destined to reach cell vacuoles by moving through cytoplasm and then tonoplasts [138]. The excess metal ions in plants and fungi are stored in cell vacuoles, and phytochelatin–metal complexes are pumped into the vacuoles, whereby the complexed metals are made inert by the P1B-ATPase transporter (ABC-P1B) and ATP-binding-cassette, as shown in Figure 5 [156].

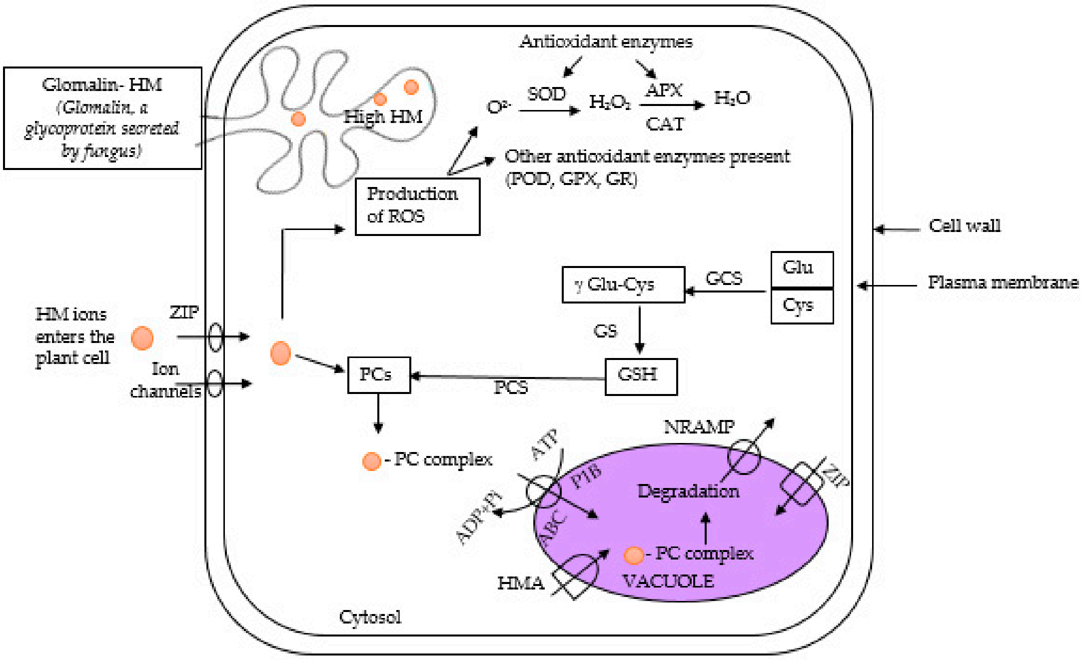

Figure 5. Mitigation of heavy metal (HM) stress by chelating agents. The figure shows the uptake of HM that is mediated via zinc and iron like protein (ZIP) transporter and other ion channels; HM ions, when entering the cytosol, activate the enzyme phytochelatin synthase (PCS) and catalyze the formation of phytochelatins (PCs) from glutathione (GSH), which is synthesized from glutamate (Glu) and cysteine (Cys) by the transfer of γ glutamyl-cysteinyl (γ Glu-Cys) conjugates onto GSH via glutathione synthetase (GS); PCs binds with the cytosolic HM ions and form heavy metal–phytochelatin (HM-PC) complexes, which enter the vacuole via ATP-binding-cassette (ABC), P1B ATPase (P1B) and other transporters such as heavy metal ATPases (HMA), natural resistance-associated macrophage protein (NRAMP) and ZIP transporters. HM ions also lead to the production of reactive oxygen species (ROS) such as superoxide radical (O^{2-}), which is dismutated by superoxide dismutase (SOD) and produces hydrogen peroxide (H_2O_2), which is further broken down into water (H_2O) by ascorbate peroxidase (APX) and catalase (CAT). Peroxidase (POD); glutathione peroxidase (GPX); glutathione reductase (GR).

Zhang et al. reported that mycorrhizal inoculation in *Zea mays* decreases Cd toxicity and mobility by transforming Cd into an inactive form, which might be ascribed to the increased content of PC and GSH [147]. As AMF increases the PCs biosynthesis so more PC-Cd complexes are formed and transferred to the vacuole, thereby decreasing their negative effect [157]. Reports indicate that inoculation with *F. mosseae* enhances the level of GSH in *Nicotiana tabacum*, further reducing the Cd and As content in the leaves and roots of the plant [158].

Another group, i.e., metallothioneins (MTs), is also involved in the sequestration of HMs. MTs are a group of proteins having a low molecular weight, high metal content and non-enzymatic, cysteine-rich, long polypeptide chains that consist of a large number of amino acids. They have a common sequence pattern, i.e., Cys-X-Cys where X is an amino acid other than cysteine. These are the metal ligands that bind metal ions in a metal thiolate in clusters in the cytosol or sub-cellular compartments for the accumulation and sequestration of HMs in plant vacuoles, as shown in Figure 4. Metallothioneins also have the competence to bind to HMs through the thiol group (-SH) of cysteine residues and help in protection against oxidative stress [159]. They are biosynthesized in mycorrhizal plants and AMF by certain hormones and HMs. Hasegawa et al. reported an increased Cd tolerance in cauliflower upon the insertion of *CUP1* genes from yeast that, in response, enhanced its accumulation by 16-fold in plant tissues [160]. Some authors have found that various classes of MT genes are activated by HMs [161,162].

6.8. Glomalin-Induced Soil Metal Complexes

Glomalins are the glycoproteins produced by AMF and exist as homologs of heat shock protein 60 (hsp 60) that play a role in the immobilization of HMs [163]. Glomalin-related soil protein (GRSP) also exists as an alkaline soluble protein material with AMF. These are stable compounds impervious to heat degradation, generated mostly in the inner layer of the cell walls of mycorrhiza, and are released into the soil during hyphal turnover or after the death of the fungus [164]. Besides Glomeromycota, no other fungal group generates these glycoproteins in significant quantity [165]. The production of glomalin depends upon the level of metal ions present in the soil [165,166]. The symbiotic association between plants and the glomalin producers AMF enhances atmospheric CO_2, which leads to an increase in the production of glomalin. When glomalin is released in the soil, it performs several functions such as improving the stability of soil aggregates, reducing soil erosion, enhances soil quality and forming protein–metal complexes, as shown in Figure 4, which decrease the content of metal ions in the soil. These proteins further aid in the wall binding and influence the plants and AMF to withstand metal stress. The presence of certain functional groups in the glomalin protein structure assists in binding with HMs. Glomalins help in retaining the water content in the soil by adjusting the frequency of water transport between plants and the soil, and ultimately increases the development of the plant. Different studies carried out by Chern et al. (2007) and Gonzalez-Chavez et al. (2009) explained the involvement of glomalin in reducing the toxicity of HMs [167,168].

6.9. AMF-Assisted Phytoremediation of HMs

Phytoremediation is a technique in which plant species known as hyper-accumulators are used for growing in HM-contaminated soil. Metal ions are absorbed through plant roots and translocated in plants with no or minimal toxicity. Plants used in the phytoremediation of As are *Pteris vittata*, *P. cretica* and *P. biaurita* [169]. Hyper-accumulator plants for Pb are *Sesbania drummondii* and *Helianthus annuus*, and, for Cd, *Sedum alfredii, Sesbania drummondii* and *Atriplex halimus. Amanita muscaria* and *Polygonum aviculare* are the known hyperaccumulator plants that can accumulate a large amount of Hg from the soil [170]. The efficiency of phytoremediation is increased by inoculating phytoremediator plants with the AMF (as they are considered as a biological tool for improving phytoremediation). AMF are the components of the soil microbiome that play a vital role in the mobilization and immobilization of metal ions and alter their availability to plants. Their contribution to the process of phytoremediation is assistance in the phytostabilization and phytoextraction steps [171,172]. Phytostabilization includes the immobilization of HMs in the roots or soil, thus decreasing their bioavailability and mobility, while phytoextraction identifies the HMs for uptake and transports them to above-ground parts of the plant [173]. Mycorrhizal colonization in plant roots allows the stabilization and retention of HMs in the plant roots and rhizospheric region. Several factors of AMF play a role in this process such as glomalin production, which helps in binding the soil–metal complexes, and extended hyphal growth, which increases the absorptive area of the plant roots. In AMF-colonized plants, the effectiveness of phytostabilization and phytoextraction also depends on the extent of colonization of AMF, the AMF species, growth, the biomass yield and the ability of hyperaccumulator plants [174].

7. Impact of AMF-Induced Genes on Metal Toxicity

Heavy metal efflux is a strategy used by AMF to protect plants from metal toxicity [137,175]. Several transcriptional genes from fungal cells are involved in HM efflux and get activated upon metal exposure. *GmarMT1*, a cDna-encoding metallothionein-like functional polypeptide was identified from the germinating spores of *Gigaspora margarita* to provide tolerance against Cu and Cd [176]. Furthermore, it has also been reported that exposure to HMs upregulates the expression of *GmarMT1* in the symbiotic mycelium. *GintABC1* was isolated as a putative ABC transporter from the extraradial mycelium of *Glomus intraradices,* involved in Cu and Cd mitigation [177]. Several genes are also involved in the maintenance of cellular homeostasis against HMs; those include *GmarMT1*, *GintABC1*, *GrosMT1* and *RintZnT1* [178]. *GmarMT1* codes for MTs reported in G. *margarita* (BEG34), maintains the redox potential of the fungus and safeguards against oxidative stress. *GintABC1* aids in the detoxification of zinc and copper [179]. A zinc transporter, *RintZnT1*, isolated from *Rhizophagus intraradices* is involved in the vacuolar sequestration of Zn [180]. In *Gigaspora rosea*, the expression of *GrosMT1* was reported [181]. Benabdellah et al. (2009) indicated *GintGRX1* as a multifunctional enzyme and the first glomeromycotan glutaredoxin that responds to oxidative stress [182]. *RintPDX1* was isolated from *Rhizophagus intraradices* and is involved in the reduction of Cu and hydrogen peroxide [183]. The upregulation of various transcriptional factors activating zinc transporter and glutathione S-transferase was observed in the extra- and intra-mycelia of the AMF *Glomus intraradices* in response to metal stress [184].

Altogether, these results indicate that the molecular regulation of genes plays an important role in HM accumulation and detoxification in fungal cells, which further prevents their translocation toward the host plant.

8. Conclusions and Future Prospects

In the twenty-first century, one of the biggest challenges of humanity is to feed the growing population of the world without an increase in the present environmental problems. However, due to the advancement of industrialization and human-induced activities, HM toxicity in soil is increasing rapidly, which further affects morpho-physiological processes in plants. There are few metals that are beneficial, while some impart negative effects on the plant and also on human health through the food chain.

Under HM stress, an excessive amount of ROS is produced that reacts with cellular organelles and results in oxidative damage to plant cellular structures. In response, plants activate several antioxidant enzymes, which scavenge the generated ROS to protect the cells from damage. Moreover, several soil engineers and environmentalists have approved different strategies to overcome the challenges and ameliorate the adverse impacts of HMs on plants. Among these approaches, the use of AMF is considered as one of the most promising and effective strategies.

Several changes such as the enhancement of the antioxidant response and modification in root morphology are seen in plants due to the influence of AMF, and their symbiotic relations improve the growth of the plant by enhancing nutrient acquisition. They also act as a biofertilizer for plants and biosorbant for HMs and enhance plant performance via specific intrinsic molecular mechanisms. They help in improving soil aggregation by secreting glomalin, which forms protein–metal complexes, thereby restricting the entry of HMs into the plant. AMF also adsorb a large amount of HMs onto the fungal walls and also sequester them in fungal vacuoles (biological barriers). They also help in enhancing the phytoremediation of HMs and play a key role in the mobilization and immobilization of HMs. However, the signaling pathways and molecular mechanisms that are linked to the formation of successful mycorrhizal symbiosis need better understanding. Genomic knowledge regarding AMF is poor and needs to be unraveled.

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