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(Review Article)

Arbuscular mycorrhizal fungi as a potential tool for bioremediation of heavy metals in contaminated soil

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Abstract

Human activities have introduced large amounts of heavy metals into natural ecosystems in recent years. As a result, the accumulation of heavy metals and metalloids in plants, animals, and humans, which may have caused some health problems. Chemical and physical methods can remove the heavy metal in contaminated soil, but both are very expensive and ineffective. Arbuscular Mycorrhizal Fungi (AMF) are mutualistic symbionts in most plant roots. Furthermore, AMF are the essential mycorrhizae for phytoremediation, and the extensive hyphal network of them can increase the uptake of micro and macronutrients, water and heavy metals from the soil. However, AMF hyphae colonized in plant roots have an ability for compartmentalizing heavy metals inside plant roots. Furthermore, AMF hyphae are capable of secreting a glycoprotein, named glomalin, which can bind heavy metals and subsequently remove heavy metals absorbed by the plants from contaminated soil. Glomalin can develop the properties and structure of the soil, which helps to enhance soil fertility. This paper presents the role of AMF in the ecosystems and as potential tools for bioremediation of heavy metals in the soil.

Keywords: Heavy metals; Bioremediation; Phytoremediation; Arbuscular mycorrhizal fungi; Glomalin

1. Introduction

Contamination of soils, water, and air with hazardous heavy metals and toxic chemicals is one of the main problems in the world today [1]. In recent decades, primarily due to heavy industrialization, urbanization, and inadequate discarding of wastes, the concentration of heavy metals in soil has increased. The anthropogenic activities, responsible for heavy metals contamination of soils are mining, waste from urban centers, accidental chemical spills, and the usage of agro-chemicals, fertilizers, and sewage sludge at high dosages for prolonged periods [2, 3]. Accumulation of metals and metalloids in ecosystem pose a risk to food safety issues and potential health risks. Contaminants can also cause a harmful effect on the soil ecosystems [4].

Polluted area remediation can be defined as a set of practices to mitigate or resolve the effects of contaminants [5]. Among the techniques for environmental recovery, bioremediation and phytoremediation are strategies that used bacteria, fungi, plants, and biological-based processes [6, 7, 8]. Bioremediation is the removal of contaminants using natural biological activity. However, it will not always be the best due to the wide range of pollutants, the time involved is relatively long, and the residual contaminant levels achievable may not always be appropriate. Therefore, finding most effective microorganisms to be functioned as bioremediators are important in cleanup of the contaminated soil [9].

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Phytoremediation, as a sustainable and low -cost technology based on the elimination of heavy metals from the environment by plants, is becoming a mostly important objective in plant research. Effective phytoremediation can be achieved by using arbuscular mycorrhizal fungi (AMF), which form symbiotic relationships with most land plants in the world [10].

2. Heavy metal contamination of soil

Both natural and human-made activities are responsible of entering heavy metal into the ecosystems, which can adversely affect plants and organisms [11]. The prime source of heavy metals in soils is the parent material from which they are created. Normally, basaltic ingenious rocks are rich in heavy metals such as Copper (Cu), Cadmium (Cd), Nickel (Ni), and Cobalt (Co) whereas shales contain large amounts of Lead (Pb), Copper (Cu), Zinc (Zn), Manganese (Mn), and Cadmium (Cd). Heavy metals in rocks can be released to the soil environment through natural processes such as meteoric, biogenic, terrestrial, volcanic processes, erosion, leaching, and surface winds [12, 13].

Long-term irrigation and application of inorganic fertilizers, animal manure, and pesticides in agriculture tends to increase the concentrations of heavy metals in the environment [14]. The fertilizers and the pesticides used in agriculture usually contain significant amounts of heavy metals such as Copper (Cu), Arsenic (As), Cobalt (Co), Chromium (Cr), Molybdenum (Mo), Strontium (Sr), Titanium (Ti), Vanadium (V), Manganous (Mn), Iron (Fe), Nickel (Ni), Zinc (Zn), Cadmium (Cd), Lead (Pb), Mercury (Hg), Barium (Ba) and Scandium (Sc) [15,16]. Metals are non-degradable and therefore, persist for long periods in aquatic as well as terrestrial environments. They may be transported through the soil to reach groundwater or taken up by plants, including agricultural crops [17, 18].

The existence of heavy metals in the environment can be caused a number of adverse effects. Such impacts affect all components of the environment such as hydrosphere, lithosphere, biosphere, and atmosphere. Heavy metal pollution in the soil is one of the main ecological problems globally [14]. Heavy metals have been found in farmlands, water resources, and plants as they bio accumulate in various food chains, resulting in high-risk ecological and human health problems [19].

The content of heavy metals enriched by the plant exceeds the threshold of tolerance, and it even leads to the death of the plant. Long-time exposure to heavy metals is the cause of adverse health effects in humans, affecting the nervous system, kidney, liver, and respiratory functions. For example, mercury usage is widespread in industrial processes and various products [20]. Monomethyl mercury and dimethyl mercury are highly toxic, causing neurotoxicological disorders. Also, Pb poisoning causes problems with the effect on the gastrointestinal tract, affects kidneys, and acute or chronic damage to the animal nervous system [21, 22].

In addition, a number of environmental risk factors have been recognized globally as probable causes of Chronic Kidney Disease (CKD), namely exposure to heavy metals (As, Cd, Pb, Hg, and Cr), agrochemicals, and nephrotoxic substances [23, 24]. The addition of fertilizers to soils and the application of agrochemicals to crops has become common practice in agriculture [25]. The main purposes are the improvement of the nutrient supply in soil by adding fertilizers and protecting crops from diseases by applying pesticides. Long-term practice of these two activities may result in soil degradation as the foreign contaminants are accumulated over time [26].

3. Bioremediation process of heavy metal removal

Many methods and strategies have been adopted to solve the problem of soil contamination. In general, remediation technologies can be categorized into two main strategies: in situ remediation and ex-situ remediation [27]. In situ remediation is treating the pollutant in the original place without moving the contaminated soil itself. In contrast, ex situ remediation involves excavating and removing the polluted soil elsewhere for treatment [28]. In situ remediation offers a number of potential technical, economic, and environmental advantages [29].

Some conventional remediation technologies are based on biological, physical, and chemical methods, which may be used in conjunction with one another to reduce contamination to a safe and acceptable level [30]. Physical remediation is the method of reverse or ending damage to the soil using physical technologies, including soil replacement, isolation, containment method, and thermal treatment [31]. Chemical remediation is a technique in which chemical reagents, reactions, and principles are used to eliminate contaminants. The main remediation methods include solidification/stabilization, vitrification, soil flushing, soil washing, and electrokinetics etc. [32].

Bioremediation is a state-of-the-art technique used to restore heavy metal contaminated soil ecosystems to a certain degree [33]. The technique uses the biological mechanisms inherent in plants and microorganisms to remove, destroy, or immobilize hazardous contaminants from polluted environments [34].

Phytoremediation should be considered to be effective for remediating heavy metal contamination because of its cost-effectiveness, high advantages, and long-term applicability [35]. Phytoremediation is a technology that uses various types of plants to remove, transfer, stabilize, and destroy heavy metal contaminants in the soil and groundwater. Phytoremediation should be considered for remediation of contaminated sites because of its cost-effectiveness, aesthetic advantages, and long-term applicability [36, 37].

In developing countries, phytoremediation is one of the recent technologies used in the removal of heavy metals. The mechanism of phytoremediation is plants and their associated microorganism to remove heavy metals [38]. Phytoremediation encompasses five processes of metal removal from soil or water. These processes include rhizofilteration, phytostabilization, phytoextraction, phytovolatilization, and phytodegradation. Rhizofilteration uses plants to absorb, concentrate, and precipitate organic and inorganic pollutants from aqueous sources [39, 40].

For the process of phytoremediation, there are helpful plant species that have been identified. Depending on the researches in Carvalho et al., 2001, four aquatic plants; Cattail (*Typha domingensis*), duckweed (*Lemna obscura*), hydrilla (*Hydrilla verticillata*) and swamp lilly (*Crinum americanum*) have been reported to accumulate Selenium (Se) [41]. Water hyacinth *(Eichhornia crassipes*), common duckweed (*Lemna minor*), sharp dock (*Polygonum amphibium* L.), water lettuce (*Pistia stratiotes*), water dropwort (*Oenathe javanica*), calamus (*Lepironia articulate*), pennywort (*Hydrocotyle umbellate* L.) and water velvet (*Azolla pinnata*) have also been reported to remediate Cd, Cr, Co, Ni and Pb [42]. Besides, that several other water plants which show a significant ability to accumulate multiple metals are water fern (*Salvinia molesta*), giant duckweed (*Spirodela polyrhiza*), water hyssop (*Bacopa monnieri*), fern (*Azolla filiculoides*) and tape grass (*Vallisneria americana*) [43].

3.1. Arbuscular mycorrhizal fungi

Fertilizers are instant nutrient suppliers used to improve plant growth in agriculture. Most countries utilize chemical fertilizers, and only a few countries practice organic farming [44]. Organic fertilizers and biofertilizers received significant interest among farmers as they are eco-friendly. Arbuscular mycorrhizal fungi (AMF) are widespread soilborne fungi and form symbiotic and mutualistic interaction with the roots of most plant species [45]. Approximately 95% of the world's plant species are mycorrhizal and possibly benefit from AMF [46]. Arbuscular mycorrhizal fungi were also shown to improve plant growth even in adverse environmental conditions against biotic and abiotic stresses. The use of AMF is increasing in agriculture, forestry, and environmental reclamation, to improve crop yield and soil health [47, 48].

Arbuscular mycorrhizal fungi play a central role in many microbiological and ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter, and plant health and nutrition [49]. Arbuscular mycorrhizal fungi can aid plant development by increasing plant contact with relatively immobile minerals, such as improving soil texture by binding soil particles into stable aggregates that resist wind and water erosion and binding heavy metals into root tissues limits their translocation into shoots [47, 48, 50]. Furthermore, it was reported that AMF improve nutrient uptake by increasing the nutrient absorption soil volume of plant roots [51]. This approach can be beneficial for host plants growing in unfavorable soil conditions like nutrient-deficient soils or contaminated areas [52].

Phosphorus (P) is the most critical plant nutrient in relatively large quantities. It plays a vital function in all biological roles in energy transmission by forming energy-rich phosphate esters and an essential component of macromolecules such as nucleotides, phospholipids, and sugar phosphates [53]. The most significant benefits of mycorrhizae are the increase in P absorption by the plant. This P absorption contains three sub-processes; (a) phosphorus absorption from the soil by AMF hyphae, (b) translocation along the hyphae from external to internal mycelia, (c) the transfer of phosphate to cortical root cells [54].

Nitrogen (N) is required for the structure of amino acids, purines, pyrimidines and, is thus, indirectly involved in protein and nucleic acid synthesis. Arbuscular mycorrhizal fungi colonized plants have increased the amount of N in their shoots [54]. Arbuscular mycorrhizal fungi hyphae can extract nitrogen from the soil and transport it to plants. They contain enzymes that collapse organic nitrogen and contain nitrogen reductase, which alters nitrogen production in the soil [55]. Arbuscular mycorrhizal fungi hyphae increase nitrogen transmission in communities since the network of AMF mycelia links different plant species rising nearby and helps join the pool of available nutrients for these plants [56].

While several mycorrhizal fungi can contact inorganic forms of N and P, some litter-inhabiting mycorrhizal fungi make proteases and distribute soluble amino acids through hyphal networks into the roots [57]. When considering micronutrients, the extra metrical hyphae of AMF absorb, and transport K, Ca, SO_4^{-2}, and AMF colonization changes the concentration and quantities of K in shoots. Arbuscular mycorrhizal plants collect vast amounts of micronutrients (Co, Zn, Cu) under low soil nutrient accessibility [58].

Arbuscular mycorrhizal fungi also play a significant part in the plant water economy. The AMF improve the hydraulic conductivity of the roots and expand water absorbed by the plants or otherwise adjust the plant physiology to decrease the stress response to drought [59]. The permeability of cell membrane to water may also be changed by mycorrhizal colonization through the enhanced P nutrition, and colonization by AMF can expand the drought resistance of plants [60, 61].

Furthermore, AMF support the binding of soil particles and influence soil aggregation and soil conservation [62]. Arbuscular mycorrhizal fungi are also recognized to improve soil fertility, as they produce glomalin upon accumulation in soil. The formation of micro – aggregates by glomalin aids in soil stabilization [63]. Arbuscular mycorrhizal fungi have an excessive possibility in the recovery of disturbed lands, and these can be used in the recovery of wastelands [64]. Furthermore, AMF improve plant survival in stressful situations by increasing nutrient absorption. Arbuscular mycorrhizal fungi make the most resistant to adverse situations formed by unfavorable conditions related to soil or climate [65, 66].

Although, mycorrhizal plants have a greater tolerance to toxic metals, root pathogens, stresses such as drought, salinity, high soil temperature, adverse pH, and transplant shock [67], the involvement of AMF on heavy metals uptake from contaminated sites is a significant phenomenon. They can colonize plant roots in metal contaminated soil and water. Furthermore, AMF can increase the efficiency of the phytoremediation potential of plants through several mechanisms. Therefore, we can apply them as inoculums to heavy metal contaminated sites to improve the phytoremediation potentiality of plants [68].

Some techniques for the mass production of AMF used for commercial production are available now. The potential of AMF as biofertilizers and bio-protectors to improve plant productivity has been broadly known but not entirely exploited because of the obligate biotrophic nature of arbuscular mycorrhizal fungi and further reasons such as the unstable act of mycorrhizal fungi in plant production systems [69, 70]. Since arbuscular mycorrhizal fungi are more and more considered in agriculture, horticulture, and forestry programs, and environmental reclamations, as biofertilizers and bio-protectors, it is required to screen and choose the effective AMF spices [71].

Also, selecting the suitable host plant species and the standardization of several parameters are essential for optimizing the AMF inoculum. There is a requirement to develop low-cost technologies to intensify these eco-friendly and economically important microorganisms [72]. The fungicide can directly change the functions of AMF. Chemical fungicides have been found to be harmful to AMF [73]. Also, AMF can generate several mechanisms for protection extended by the host plants against the pathogen attack. Plants are the focus of invasions by several pathogenic organisms of fungi, bacteria, viruses, and nematodes. Mycorrhizal plants regularly face minor injury from infection than non –mycorrhizal plants [74, 75].

The quantity of root colonization and spores of plants can be changed in different locations and disturbances to reduce soil [76]. Also, AMF ecotypes can change from long-term variation due to their properties [77]. Therefore, indigenous soil AMF can change soil environments and stimulate plant growth better than non-indigenous isolates [78]. Isolation of indigenous stress-adapted AMF could be a probable biotechnological tool for inoculating plants in disturbed ecosystems [79].

Flue-gas-desulphurization sludge soil degradation produces deviations in the diversity and population abundance of arbuscular mycorrhizal fungal [80]. Therefore, such elimination of AMF can lead to problems with the host plant survival and establishment [50]. Furthermore, if AMF are abundant in terrestrial ecosystems, mechanical or chemical disturbance of the soil can substantially decrease effectiveness of AMF populations [81].

3.2. Role of AMF in the phytoremediation of metal contaminated soil

Metal tolerance of AMF can be explained by several approaches, such as spore numbers, root colonization and the abundance of ectomycorrhizal fruiting bodies [82, 83]. Microbial communities are often found to recover after initial contamination by heavy metal [84]. This adaptation has been divided into two factors [85]. The first one is a slight decrease in metal availability due to immobilization reactions occurring in the rhizosphere. The second factor is a gradual variation in microbial community structure, based on changes in phospholipid fatty acid profiles, resulting in more tolerant organisms [86].

Arbuscular mycorrhizal fungi enhance the ability to sequester and accumulate heavy metals in their biomass and the host plant's roots [51, 87]. Arbuscular mycorrhizal fungi can effectively colonize within the roots of some hyper-accumulator plant species and exhibit heavy metal tolerance mechanism and accumulation [88]. In addition, AMF can tolerate a broader range of heavy metal concentration and other adverse conditions in soil [89, 90].

Some researchers have shown that AMF allow the metal accumulation in plant roots [91] and avoid its translocation to the shoot; thus, AMF promotes phytostabilization in soil [92, 93, 94]. Because the heavy metals were binding to the cell walls of the fungal hyphae in roots and not release to the shoot. So, they can act as a filtration barrier against the transfer of heavy metals to plant shoots. In contrast, other researchers have shown that AMF increases phytoextraction, causing a rise in metal translocation to the shoots [94]. Arbuscular mycorrhizal fungi can alleviate heavy metal threats by secreting several compounds, which influence in metal precipitation in polyphosphate granules present in the soil, adsorbing metals to fungal cell walls, and chelation of heavy metals inside the fungus [88].

Organic acids and glomalin release from plants and fungi correspondingly play a significant role in immobilizing heavy metals in the soil. Arbuscular mycorrhizal fungi colonized plants release organic acids, which increase heavy metal sequestration and sorption and organic acids precipitated as polyphosphate granules chelate and immobilized heavy metal in the soil [88].

Soil management applications decrease mycorrhiza's sporulation and colonization ability by disrupting the extra radical mycelium network [95]. The disruption of the hyphal network decreases its surface area [96]. In order to prevent stress condition in an environment, AMF grow further extensive mycelium [97]. It was reported that the AMF isolates *G. intraradices* Br1 consistently conferred heavy metal tolerance on various plants, tomato, maize or *Medicago truncatula*, in various heavy metal contaminated soil under optimum fertilization was reported by Hildebrandt et al. (1999) [98].

The thickness and general morphological characteristics of the cell wall are essential for retaining trace elements by AMF. Thick hyphae contain fewer trace elements due to their small surface area than isolates with thin hyphae. The trace elements include in tissues of AMF and the retention capacity is higher for Cu and Zn [99]. In contrast, the retention of Cd and Pb is lower in tissues of AMF. The retention rate is decreased according to the following order, Cu>Zn>>Cd>Pb [100]. The protection and enhanced capability to uptake minerals result in more excellent biomass production, an essential criterion for a successful remediation. In this way, AMF can help plants to adapt and survive in contaminated habitats [101].

A steady accumulation of Cu in the vacuoles of spores and an increase of Cd in the hyphae of *Glomus intraradices* can be observed when the fungus is exposed to the excess of metals [102]. Free amino acids, hydroxyl, carboxyl, and other groups are containing the cell wall of fungi representing binding sites for the adsorption of certain heavy metals [103].

Lasat (2002), experimental results of AMF relations on metal root uptake appears to be metal and plant-specific [104]. Greater root length densities and presumably more hyphae enable plants to explore a larger soil volume, thus increasing access to cations (metals) not existing to non-mycorrhizal plants [105].

3.3. Characteristics of glomalin and functions

Arbuscular mycorrhizal fungi produce the iron-containing glycol-proteinaceous substance glomalin, accumulating in soils to concentrations of quite a few mg per cm^3 of soil. Glomalin pools respond quickly to ecosystem perturbations such as elevated atmospheric CO_2 concentrations, warming, and various agricultural management practices [106,107].

Glomalin from hyphae of AMF and protein from soils show similarity in N-linked oligosaccharides. Glomalin is linked with soil carbon storage via its effect on soil aggregate stabilization, and it also presents a potentially significant soil C pool [108]. The protein is quite recalcitrant, being extracted by autoclaving. The majority of research on glomalin has been dedicated to its importance in the soil system because glomalin concentrations are consistently significantly associated with soil aggregation [109,110].

Glomalin was revealed using specialized extraction protocols for soil that revealed quantities of up to several mg of proteins per 1 g of soil. Glomalin is produced by hyphae of all members of AMF genera, but not by other groups of soil fungi tested so far [111]. It is regularly assumed that proteins do not include a key component of soil organic matter, carbon or nitrogen, since the ubiquity of proteases in the soil. Plant-derived proteins are rapidly degraded after they come into interaction with soil proteases [112].

Many studies have described the contributions of glomalin to phytoremediation while examining the roles of glomalin in heavy metal sequestration of polluted soils. Stated the probability of glomalin in reducing capability and toxicity of "potentially toxic elements" such as Cu, Cd, and Pb. Besides, it is sometimes reported that glomalin-related soil proteins (GRSP) bind to about 28% Cu and 6% of Zn in soil extremely polluted with these heavy metals [113,114].

It looks as if the higher concentration of the pollutants, the higher capability of GRSP to bind to them and make the pollutants unavailable. These studies prove the substantial aids of AMF to phytoremediation through glomalin production [115]. There are connections between GRSP production and heavy metal concentrations in the soil. They clarified that toxicity-induced stress by heavy metals might enhance glomalin production by AMF [108].

Glomalin is regularly determined by two procedures, the Bradford dye-binding protein assay and enzyme-linked immunosorbent assay (ELISA). Because many laboratories lack the necessary equipment to perform the ELISA assay, various studies quantify GRSP using the colorimetric Bradford method, which measures total protein [111]. Severe assessments of using the Bradford assay to quantify GRSP have shown cross-reactivity with non-AM fungi proteins added before autoclaving and with other non-proteinaceous resources added to the GRSP extract. This can result in an overvaluation of GRSP stocks [113].

An ELISA (enzyme-linked immunosorbent assay) with monoclonal antibody MAb32B11 developed against crushed spores of Glomus intraradical for the quantity of glomalin in soil and plant roots. Despite the reported links between ELISA and Bradford values, the Bradford method is for total protein assay and thus less accurate for glomalin. The Bradford total protein assay involves Coomassie dye that binds to practically all proteins [111].

Both microorganisms and plant roots secrete approximately proteinaceous constituents, such as amino acids, co-extracted with glomalin and noticed by the Bradford assay. These findings suggest that Bradford active soil protein (BRSP) is not entirely associated with AMF and requires further research to be confirmed [116]. Similarly, some tests have confirmed that the ELISA technique is more suitable and specific for GRSP quantification. According to the additional findings, the Bradford method and ELISA assay may help quantify glomalin pools when organic matter concentrations are low, such as in washed, autoclaved soil, or other controlled experimental situations [117].

Easily extracted glomalin (EEG) and total extracted glomalin (TEG) protocols are used to extract glomalin from the soil, which Wright and Upadhyaya established [116]. However, later extraction procedures were modified slightly by some other researchers. Easily extracted glomalin was extracted from 1 g of soil with 8 ml of 20 mM citrate, pH 7.0 at 121.8 ^{0}C for 30 min. TEG was obtained by repeated extraction from 1 g of soil with 8 ml of 50 mM citrate, pH 8.0 at 121.8 ^{0}C for 60 min after each autoclaving cycle. Then the supernatant was removed by centrifugation at 5000 rpm for 20 min and stored [116,117].

Some other methods can attempt to quantify the amount of glomalin in soil extracts. Synchrotron-based X-ray absorption near-edge structure (XANES) spectroscopy is a successful soil analysis that can be used to identify the composition of the GRSP extracts [118]. Also, the Pyrolysis field-ionization mass spectrometer (Py-FIMS) provides a molecular-scale m/z fingerprint of complex samples and has been applied to the characterization of soil and quantify the glomalin it soils extraction [119,120]. Furthermore, MS-based proteomics is used to identify target proteins based on the matching sequence of peptide sequences [121].

4. Conclusion

A wide range of toxic pollutants including heavy metals, are disposed of daily to the soil and water. Removing these toxic heavy metals from the ecosystem needs advance remediation techniques. Phytoremediation is one of the low-cost remediation techniques which are used by microorganisms to remove heavy metals. Arbuscular mycorrhizal fungi play essential roles in the ecosystem, considering plant growth, soil fertility and bioremediation of contaminated soil. Glomalin is a glycoprotein that is secreted by Arbuscular mycorrhizal fungi. It can regulate the absorption of heavy metal into the plant. Furthermore, many methods, Bradford assay, column chromatography, and molecular techniques can be used to extract glomalin from the soil and determine the soil glomalin levels.

Compliance with ethical standards

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Disclosure of conflict of interest

None to be declared.

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