



Role of arbuscular mycorrhizal symbiosis in remediation of anthropogenic soil pollution

Laura Yesenia Solís-Ramos¹ · Cristofer Coto-López¹ · Antonio Andrade-Torres²

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Abstract

Agricultural and industrial activity generates high concentrations of organic and inorganic pollutants, many of which are incorporated into the trophic chain, affecting ecosystems. There are several strategies for the remediation of polluted areas; we discuss one of them in the present review that shows the successful evidence of the use of arbuscular mycorrhizal symbiosis in phytoextraction (the removal of contaminants from soil and water sources with mycorrhizal plants), and in the process of phytostabilization (the reduction of the mobility of heavy metals in soil by mycorrhizal roots, absorption onto roots, or precipitation within the root zone). Mechanisms of action of arbuscular mycorrhizal fungi (AMF) including, altered uptake and distribution of heavy metals, improvement in the mineral nutrition and water availability, protection against oxidative stress and increment in the physical stability of the soil by producing glomalin has been discussed with reference to heavy metals (HMs) and persistent oxidative pollutants (POPs). We report plant species associated with species of mycorrhizal fungi as strategy for phytostabilizing heavy metals and reducing biotranslocation to the aerial parts of plants.

Keywords Arbuscular mycorrhizal fungi · Heavy metals · Pollution · Phytoremediation

1 Introduction

Anthropogenic activities (e.g., mining, pesticides, smelting, electroplating, sludge waste, industrial discharge, burning of fossil fuel) have dramatically accelerated the process of environmental contamination by the discharge of hazardous wastes into soil and water (Sodango et al. 2018; Riaz et al. 2020). This situation has caused air and soil pollution, acid precipitation, soil degradation, salinity, increasing UV-B radiation and climate change (Schutzendubel and Polle 2002). Agricultural wastes include a wide range of organic materials (often containing pesticides), animal wastes, and timber by-products (Setyorini et al. 2002). Agricultural soils are a major environmental reservoir for antibiotic residues. Antibiotics are

commonly used in livestock farming and much of it eventually ends up in manure, which is subsequently applied to agricultural land (Cao et al. 2018). Mining and smelting of metalliferous ores combined with combustion of fossil fuels have dramatically increased the global deposition of heavy metals (HMs) over the past two centuries (Agarwal et al. 2017). Cadmium (Cd) is added to agricultural systems through atmospheric deposition, application of sewage sludges and manures, irrigation water, and in fertilizers and soil amendments (Grant and Sheppard 2008). The excessive accumulation of heavy metals in agricultural soils results in a decrease in the soil quality and crop growth (Babadi et al. 2019). The latent for toxicity, carcinogenicity, and bioaccumulation in living systems are also a concern (Tchounwou et al. 2014).

Anthropogenic soil pollution by organic and inorganic compounds is a global problem. Such compounds include HMs, fuels, hazardous waste, explosives, and petroleum products. The most significant inorganic pollutants are HMs that include group of metals and metalloids that have relatively high density and are toxic even at ppb (parts per billions) levels (Csuros and Csuros 2002; Ali and Khan 2017). HMs are considered hazardous due to three reasons: persistence, bioaccumulation, and toxicity (Ali et al. 2019). Bioaccumulation is the process whereby the accumulation of toxic substances in living beings increases in concentration following a rise in the trophic level:

✉ Laura Yesenia Solís-Ramos
laura.solisramos@ucr.ac.cr

¹ Biotecnología de Plantas, Escuela de Biología, Universidad de Costa Rica, P.O. Box 11501-2060 San Pedro, Costa Rica

² Biotecnología y Ecología de Organismos Simbióticos, CAUV-173 Ecología y manejo de la biodiversidad, aandrade@uv.mx, INBIOTECA (Instituto de Biotecnología y Ecología Aplicada), Universidad Veracruzana, Av. de las Culturas Veracruzanos No. 101, Col. E. Zapata, 91090, Xalapa, Veracruz, México

the higher the trophic level is, the stronger the concentration of HMs is as well (Aprile and De Bellis 2020). Regarding their roles in biological systems, HMs are classified as essential and nonessential (Ali et al. 2019). Some examples of metals categorized as essential copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) and non-essential include arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), mercury (Hg), nickel (Ni) and vanadium (V) (Aprile and De Bellis 2020). The metals are toxic at higher concentrations because they induce oxidative stress (reactive oxygen species ROS) through the formation of free radicals, which inhibits most cellular processes at various levels of metabolism (Appenroth 2010; Sytar et al. 2013). HMs are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10 ppm) in various environmental matrices (Tchounwou et al. 2014), but they can also be toxic at relatively low concentrations (Ross 1975). The HMs are persistent in the environment. They accumulate in living organisms and are transferred from one trophic level to another in the food chains (Ali et al. 2019;).

The organic pollutants added as a result of anthropogenic activities, commonly called persistent organic pollutants (POPs), are resistant to environmental degradation process and are affecting health of ecosystems and humans (reviewed by Lenoir et al. 2016a; Oyetibo et al. 2017). POPs can persist in the body fat of humans and animals for decades, and can cause cancer, birth defects, learning disabilities, and immunological, endocrinal, behavioral, neurological, and reproductive problems (Lenoir et al. 2016a). The United States Environmental Protection Agency lists POPs in the soil, such as polycyclic aromatic hydrocarbons (PAHs), as priority pollutants and having carcinogenic and mutagenic properties make them a cause of global concern (Gao et al. 2010).

In order to remove these toxic compounds from polluted soils, different technologies and methods have been developed, most of which include the physical elimination of soil into landfills or extraction through physical or chemical means (Oyetibo et al. 2017). Even though these techniques are fast, their economic and environmental cost and potential detrimental impact on the physical, chemical, and biological properties of the soil make them less desirable and feasible (Glick 2010). As an alternative to these methods, researchers have developed phytoremediation approaches that include the use of plants for the elimination or neutralization of a variety of compounds. Phytobioremediation is the process of using plants and soil microbes for removing and cleaning chemical pollutants from soil, both organic and inorganic pollutants (Dua et al. 2002). Phytoremediation could be classified as: phytoextraction, phytodegradation, rhizodegradation, phytostabilization, and phytovolatilization (Miransari 2011). Phytoextraction and phytostabilization are the most researched processes of phytoremediation. In the process of phytoextraction, plants concentrate the HMs in their aerial parts by removing them from soil, while the process of phytostabilization HMs are not

removed from the environment but immobilizes them in plant roots (Abdelhameed and Metwally 2019) (Fig. 1). Phytostabilization is an alternative strategy that reduces the mobility and bioavailability of heavy metals in soil, thereby preventing their migration into groundwater or entry into the food chain (Chen et al. 2018b).

Plant root-fungal symbioses (mycorrhizas) have recently been projected to have a role in phytoremediation of anthropogenic soil pollution (Dhalaria et al. 2020; Janeeshma and Puthur 2020; Riaz et al. 2020). Mycorrhizas are ubiquitous and comprise two main groups: ectomycorrhizas, formed mainly by forest trees; and, arbuscular mycorrhizas (AM), formed mainly by herbaceous plants. The fungi derive carbon and lipids from the plant and transfer mineral nutrients, mainly phosphorus (P) and nitrogen (N) to the plant (Smith and Read 1997). They also help in alleviation of HM stress in soil, improvement of soil structure, protection of roots from plant pathogens and interaction with other soil microbes (Miransari 2011; Gupta et al. 2019; Gupta and Abbott 2020). The arbuscular mycorrhizal fungi (AMF) belong to the subphylum Glomeromycotina which is composed of approximately 330 fungi species (Schüßler et al. 2001, Spatafora et al. 2016; Tedersoo et al. 2018, Goto and Jobim 2020, Gupta and Abbott 2020; Wijayawardene et al. 2020). These fungi form mutualistic and obligate symbiotic associations with around 80% of vascular plants and particularly important components because, they can significantly increase the efficiency of agroecosystems (Wang and Qiu 2006; Brundrett and Tedersoo 2018; Solís-Ramos and Andrade-Torres 2020). AMF can alter productivity, by acting as biofertilizers, bioprotectors or biodegraders (Xavier and Boyetchko 2002; Gupta et al. 2018; Chen et al. 2018a).

In the present review, we have explored the role of arbuscular mycorrhizal symbiosis in phytoremediation of anthropogenic soil pollution. The review summarizes the current knowledge regarding AMF assisted remediation of HMs and POPs and some of the strategies used by mycorrhizal fungi to cope with stressful environments. Moreover, this review provides the specific information on application of different AMF species along with the mechanism involved in both phytoaccumulation and phytoextraction of these pollutants.

2 Applications of AMF in phytoremediation

Notwithstanding the role of AMF in plant-soil-microbe interactions and plant nutrition, there were fewer studies focusing on the potential of bioremediation. One possible reason is that the initial studies on bioremediation were focused on the use of plant families reported as non-mycorrhizal, such as Brassicaceae and Caryophyllaceae (Abdul 2006). The plant families Chenopodiaceae, Cruciferaeae, Plumbaginaceae, Juncaceae, Juncaginaceae, Amaranthaceae, and some

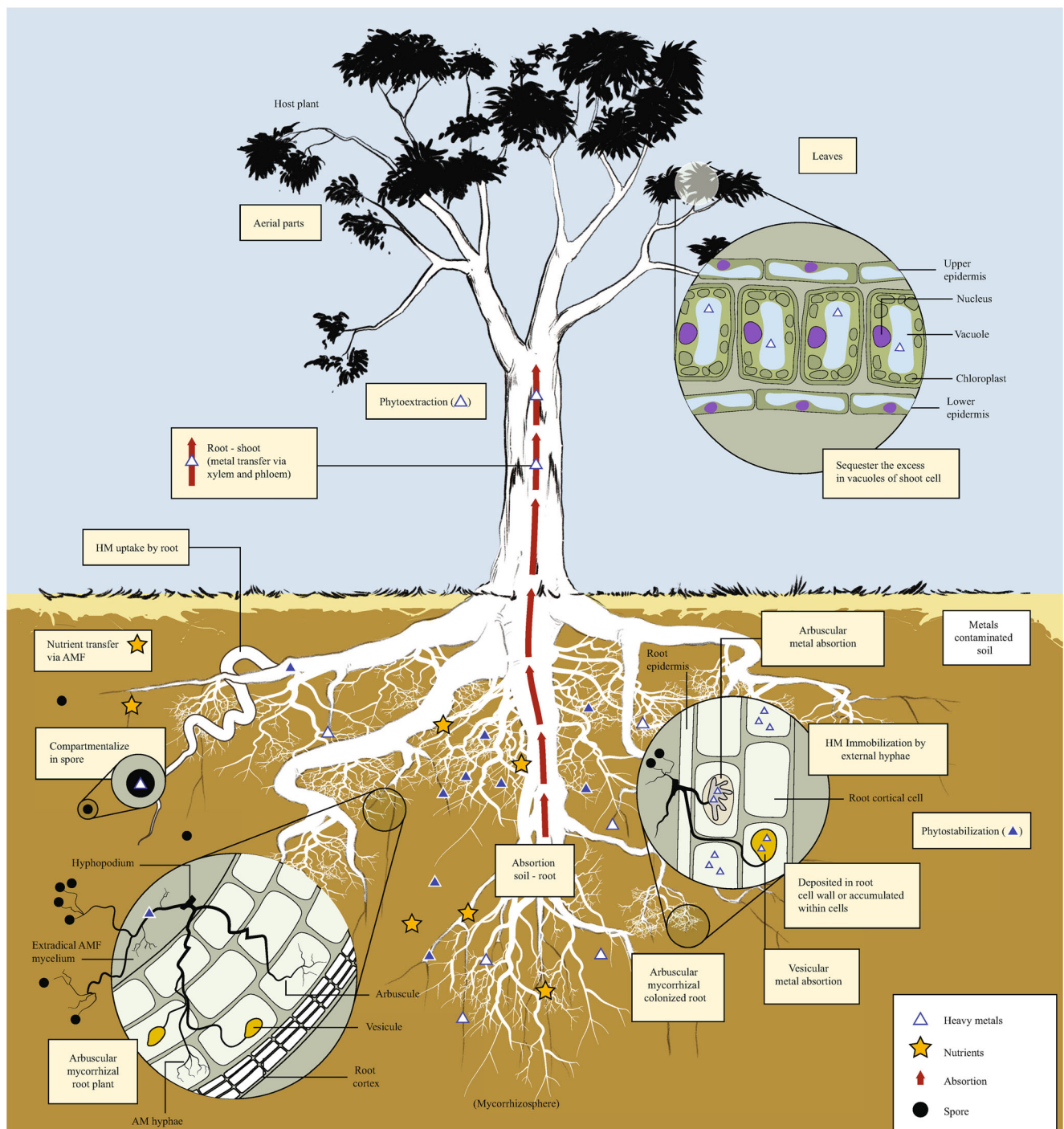


Fig. 1 Scheme summarizing the phytostabilization and phytoextraction strategies for bioremediation of soils contaminated with heavy metals using plants with arbuscular mycorrhizal symbiosis: 1) phytostabilization, absorption of HMs to the roots with AMF, to be

deposited within the cell wall or accumulated within the vacuoles of AMF and or roots. 2) Phytoextraction: HMs are transferred from the roots to the host shoots via xylem and phloem, to be accumulated in the aerial parts (leaf vacuoles)

members of the Fabaceae, do not form symbiosis with AMF (Smith and Read 1997; Brundrett and Tedersoo 2020). However, *Thlaspi praecox* (Brassicaceae) was discovered as metal hyperaccumulating plants species colonized by AMF. The first report of AMF colonization of Zn, Cd and Pb hyperaccumulating *Thlaspi praecox* Wulfen (Brassicaceae)

under greenhouse conditions, which was favored by a high demand of nutrients (for example, during the reproductive period) (Vogel-Mikus et al. 2006). The roots colonization of *Thlaspi praecox* (Brassicaceae) in the polluted soils was characterized by the presence of AMF typical structures of *Glomus* species (Pongrac et al. 2009). Changes in Zn, Cd and Pb

uptake strategies strongly suggest AM colonization may be one of the tolerance strategies of plant establishment of *T. praecox* on polluted sites (Vogel-Mikus et al. 2006).

Research approaches have mostly focused on the diversity and tolerance of AMF in soils polluted with HMs, trying to understand the fundamental basis of the adaptation and tolerance of AMF to HMs in the soil in order to facilitate their soil microorganisms for restoration and bioremediation programs (Leyval et al. 2002). AMF can contribute to phytoremediation in two ways: First, they can either accumulate and sequester toxic metal ions themselves, thus protecting their host from the pollutant (phytoaccumulation) or they can deliver HMs to the host just like essential mineral nutrients such as Cu and Zn, resulting in heavy metal accumulation in the host (phytostabilization) (Chen et al. 2018a). The situation is applied for plant production in polluted sites, with minimal toxic effect on the crop. In the second case, however, harvested plants are destroyed to reduce the heavy metal load of the site.

Arbuscular mycorrhizae exhibit different tolerance levels depending on HM type and concentration. For example, *Acaulospora laevis* is sensitive to Cu and particularly to Cd and *Glomus caledonium* is more tolerant to these two HMs under the same sand culture experimental conditions. This study suggests that *G. caledonium* can be a promising mycorrhizal fungus for the bioremediation of soils polluted with these HMs (Liao et al. 2003). Mycorrhizal fungi are capable of increasing the growth and fitness of plants in soils containing Cd. The addition of AMF to polluted agricultural soils is also a viable option if the fungi decrease or do not increase the amount of Cd accumulated in the parts of plants for human consumption (Hancock et al. 2012). Another study demonstrated that AMF (*Glomus macrocarpum*, *Paraglomus occultum* and *Glomus* sp.) have beneficial effects on plant growth and alleviation of pollutants in *Acacia mangium*, *Sorghum bicolor*, and *Urochloa brizantha* in soils polluted with Zn, Cu, Pb, and Cd, even though there were no differences in HMs concentration between shoots of plants with and without mycorrhizas (Pedroso et al. 2018). Nevertheless, AMF is a complex system and the inconsistent results regarding the effect of AMF on HMs uptake are a consequence of a wide range of factors, such as metal concentration and species (Andrade et al. 2010), competition between metals, physical-chemical soil characteristics, plant-microorganism association type, plant growth conditions, and root density (Lebeau et al. 2008), mycorrhizal fungus species, plant tolerance to contaminants and bioavailability of heavy metals (Yang et al. 2015). It is important to highlight that the results vary between treated and untreated soils (pasteurized/sterilized) (Joner and Leyval 2001).

There has been a diverse influence of the pollutants on these fungi. For example, Whitfield et al. (2004) mentioned that HMs concentration only influences vesicle (lipid storage structures) abundance, which were higher in polluted sites, and probably reflects a difference in the fungal species mix

colonizing the roots, where *Glomus* was the predominant species. However, Del Val et al. (1999) showed that AMF spore number and species richness depend on the level of soil pollution and, host plant species selectively influence AMF population size and diversity. Furthermore, Orłowska et al. (2012) observed a lower amount of mycelium in strains isolated from sites polluted with As, than in those from non-polluted sites inoculated into plants of *Plantago lanceolata*. One study showed that *G. mosseae* had the highest extracellular HMs absorption of Cd and it was higher than Ca and Zn (Joner et al. 2000).

Mycorrhization benefits revegetation processes in polluted areas due to a better establishment of plants in these areas (Pedroso et al. 2018). According to the results by Hassan (2005), cotton plants are good candidates for revegetation and phytostabilization of HMs in polluted soils, since AMF use an exclusion strategy in which the deposition of metals within the mycelium and cortical cells of the roots of AMF prevent the translocation of metals from roots to shoots. The application of amendments allows the increase of P, which, at the same time, can increase biomass as well as growth parameters and, thus, detoxify the potential effects of metals by the dilution, precipitation, or absorption of metals on phosphate granules; in that way, limiting their entrance to root cells. The results of the study of Gu et al. (2017), indicated that AMF inoculation has a species-specific effect: each plant species showed variation in biomass production and metal accumulation. For example, among the plants studied Perennial Ryegrass (*Lolium perenne*), Tall Fescue (*Festuca arundinacea*), Showy Stonecrop (*Hylotelephium spectabile*), and Purple Heart (*Tradescantia pallida*), *H. spectabile* showed the greatest growth response to mycorrhizal inoculation and the lowest concentrations of Pb, Zn, Cu, and Cd in both shoots and roots. A relevant aspect to be considered in the design of bioremediation programs together with the selection of endemic metallophytes and AM fungal strains, is the selection of species that can produce glomalin at high quantities (Cornejo et al. 2017).

The success of AMF for phytoremediation of POPs has showed a wide range of components and their mixtures, such as aliphatic hydrocarbons, fuel oils and other petroleum hydrocarbon mixtures, polycyclic aromatic hydrocarbons (PAHs), explosives, pesticides, and chlorinated organic compounds (Joner and Leyval 2003a). The hyphae and extraradical mycelium of AMF can play an important role in the uptake and translocation of phenanthrene (PHE) and pyrene (PYR) in plants, which suggests their potential use for the remediation of soils polluted with polycyclic aromatic hydrocarbons (PAHs) (Gao et al. 2010; Gao et al. 2011). AMF inoculated into plants significantly contribute to the degradation of petroleum hydrocarbon (Joner and Leyval 2003b; Volante et al. 2005; Verdin et al. 2006; Alarcón et al. 2008; Wu et al. 2009; Hernández-Ortega et al. 2012). There is

evidence that AMF can reduce the presence of aromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylene, BTEX) in artificially polluted soils. It is interesting to see that the effects vary with AMF species and BTEX nature (Volante et al. 2005). One study documented that AMF colonizing and establishing in the rhizosphere of *Eleocharis obtusa* and *Panicum capillare* grown at high petroleum hydrocarbon levels, where twenty-one taxa were identified, encompassing the major families within Glomeromycota. This suggests that AMF can be potentially important microbial candidates in the bioremediation of oil-contaminated soils (De la Providencia et al. 2015). It has also been observed that the application of mycorrhizal fungi combined with surfactants has a potential biotechnological use in the decontamination of soils with organic pollutants (Wu et al. 2008). This was demonstrated by Wu et al. (2008), who found that the colonization of alfalfa roots by AMF (*Glomus etunicatum*) and the application of Triton X-100 favor the accumulation of DDD (1,1-dichloro-2,2-bis (p chlorophenyl) ethane) in the roots and decrease it in the shoots.

3 Mechanism of AMF mediated phytoremediation

The general role of AMF symbiosis in phytoremediation involves several processes including, enhanced uptake through an enhanced microbial activity in soils with low HMs concentrations, metal-binding contributing to plant biomass and tolerance to HMs stress in soils with high HMs concentrations, absorption by extraradical hyphae or spores and chelation in fungal cells or through chelating molecules (Rivera-Becerril et al. 2005; Audet and Charest 2007; Riaz et al. 2020; Dhalaria et al. 2020). AMF can facilitate the movement of HMs to plant roots through various mechanisms, such as: deposition in the cellular wall or fungal vacuoles, sequestration by siderophores that can deposit HMs in root apoplasm or in the soil, metallothioneins or phytochelatins can result in the deposition of HMs in fungal or plant cells, and allocation of HMs from the cytoplasm by metal transporters in the plasmalemma or tonoplast of both symbionts (Miransari 2011).

Some examples are included below to illustrate the mechanisms through which AMF immobilize heavy metals in soil or roots and thus, demonstrate the suitability of AMF for phytostabilization applications (Ambrosini et al. 2015).

1. *AMF influence the uptake and distribution of metals in host plants* - For example, in roots of *Lotus japonicus* inoculated with AMF species *Rhizophagus irregularis*, it was observed that the arbuscules and intercellular hyphae accumulated large amounts of Cd, followed by the vesicles, while plant cells did not. This distribution pattern suggested that after the extraradical hyphae uptake and

translocate Cd to intraradical hyphae, this toxic metal was mainly retained in the fungal structure, particularly in the arbuscules, and did not seem to be delivered to plant cells (Chen et al. 2018b). The tolerance mediated by AMF can occur by metal exclusion mechanisms, where fungal structures, such as the extraradical mycelium, can play an important role (Ambrosini et al. 2015). For example, AMF can immobilize uranium (U) in soil by absorption and, potentially, by the formation of complexes with AMF glycoproteins and intracellular polyphosphates. Even though AMF can transfer U to their hosts and consequently, participate directly in U accumulation by the plants, it is also clear that most of the U translocated by AMF towards their intraradical mycelium remain within AMF structures, thus restricting roots to shoots translocation of U (Dupré de Boulois et al. 2008). In mycorrhizal coffee plants, it has been observed that if the concentration of Cu in the soil is between 50 and 100 mg/kg, the metal is mostly retained in the roots, which acts as a barrier for translocation to the shoots (Andrade et al. 2010).

The AMF promotes the absorption of P through the roots and may cause the formation of less mobile metal-phosphate compounds in plants, reducing the translocation of trace elements from the roots to the shoots. This was observed in the wine plants, in the presence of Cu and inoculated with six AMF species (*Dentiscutata heterogama*, *Gigaspora gigantea*, *Acaulospora morrowiae*, *A. colombiana*, *Rhizophagus clarus*, *R. irregularis*). Where *R. clarus* and *R. irregularis* showed a high colonization in the wine roots and improved the P absorption and roots growth in soils with high levels of Cu (Ambrosini et al. 2015). Different levels of Cd in the soil have an important effect on the behavior of mycorrhiza fungi, and these fungi could increase or decrease the uptake of Cd by plants and regulate accumulation in the plant tissues. In a study related to inoculate sorghum with *Claroideoglomus etunicatum* under stress for Cd, the results revealed the key role of AMF in translocation of Cd in the rhizobox and also, in precise control of Cd concentration of plant tissues (increment or decrease of them depending on Cd composition and Cd availability). The metal is probably stored in roots, in fungal hyphae and mycelium, and its transmission and toxic effects to shoots are largely prevented. AMF action enhance both, plant tolerance and phytostabilization of Cd contaminated soil (Babadi et al. 2019).

HMs can be deposited in root cell walls or accumulated within root cells, forming complexes with organic molecules such as polyphosphates, amino acids, metallothioneins, or phytochelatins (Gupta and Goldsbrough 1991; Andrade et al. 2010). One study showed that AMF isolated from a HM-tolerant plant (*Viola calaminaria*) have a significant effect on HM

accumulation in plant roots in a non-toxic form; apparently, by restricting the transfer of metals to shoots (Tonin et al. 2001). HM can be stored in cellular compartments, including spores and vesicles. Following this storage process, the metabolic rate is reduced and the effect of HMs on plant metabolism is decreased, having a beneficial effect on the plant and AMF growth, for example Cu (Ambrosini et al. 2015). *Rhizophagus irregularis* accumulates Cu in vesicles, improving the tolerance of *Tagetes erecta* L., even when accumulation increases in the roots, which suggests that this system has a potential use as phytostabilizer of Cu in polluted soils (Castillo et al. 2011).

2. *The plants inoculated with AMF reduce the HMs toxicity*- For example, in mycorrhizal coffee plants, it was observed that Cu and Zn in high concentrations cause a decrease in the shoots and roots growth, indicating the high phytotoxicity at these concentration (Andrade et al. 2010). Nevertheless, Cd tolerance with AMF inoculation is ascribed to augmented accumulation of stress metabolites such as sugar, proteins, proline, and glycine betaine, eventually leading to increased growth (Sharma et al. 2016; Janousková et al. 2006; Abdelhameed and Metwally 2019).
3. *The inoculation with AMF significantly increases the antioxidant enzyme activity* - For example, this was evident in trigonella plants (*Trigonella foenumgraecum* L.) inoculated with AMFs (*Glomus monosporum*, *G. clarum*, *Gigaspora nigra* and *Acaulospora laevis*), where the damage to the plant caused by the stress provoked by the metal was reduced due to the increase in the antioxidant enzymes activity (Abdelhameed and Metwally 2019). This was suggested to be a tolerance strategy of mycorrhizal trigonella plants against Cd stress (Abdelhameed and Metwally 2019). This agrees with a study where *Cassia italica* Mill plants under Cd stress, inoculated with the AMF mixture (*Funneliformis mosseae* syn. *Glomus mosseae*, *Rhizophagus intraradices* syn. *Rhizophagus irregularis* and *Claroideoglossum etunicatum* syn. *Glomus etunicatum*), show an increment of the chlorophyll and protein content and additionally, reduced the Cd uptake (Hashem et al. 2016). The inoculated plants with AMF under stress by the metal, reduced the peroxidation of membranes, that may be caused due to the possible role of AMF in phosphate uptake and antioxidant activity. The negative impact mitigation of the stress caused by the metal, due to the increased activity of antioxidants mediate quick scavenging of reactive oxygen species and hence, result in membrane protection, mitigating the negative impact. (Hashem et al. 2016). Mycorrhizal red kidney plants accumulated relatively high metal concentrations (Zn, Cu, Pb and Cd) in shoots more than in their roots. This is attributed to the reduced heavy metal toxicity effects in AMF red kidney plants to antioxidative protection through detoxification of heavy metals, chelation through metal-binding proteins (peptides) and dilution through increased plant growth induced by AMF (*Glomus mosseae*) (Hassan 2005).
4. *The AMF increase the production of GRSP*- Glomalin related soil protein (GRSP) can join some metals. GRSP, an insoluble glycoprotein produced in high quantities by AMF external hyphae, is an important component of the organic matter complex in the soil and plays different roles, like in carbon fixation and cycle, aggregate soil stability, prevent water loss and alleviate in toxic or harsh conditions (Vodnik et al. 2008; Malekzadeh et al. 2016; Gao et al. 2019). This was seen in the study related to inoculated sorghum with *Claroideoglossum etunicatum* under stress for Cd. The results showed that the glomalin production increased, suggesting a role of glomalin in response to soil stresses (Babadi et al. 2019). Also, in *Oenothera picensis* inoculated with *Claroideoglossum claroideum* it was determined the high capability of union of Cu for Bradford-reactive soil protein whose fraction includes the glomalin produced by AMF.

The principal suggested function of GRSP production is to protect the living hyphae and AMF itself, and the effects in the soil are secondary, so, it is a stress induced protein (Cornejo et al. 2008; Ferrol et al. 2009; Malekzadeh et al. 2016; Gao et al. 2019). In the “secondary” roles GRSP can sequester different heavy metals (González-Chávez et al. 2004; Cornejo et al. 2008; Vodnik et al. 2008; Ferrol et al. 2009; Gil-Cardesa et al. 2014; Wu et al. 2014; Singh 2015; Malekzadeh et al. 2016; Ghasemi et al. 2017; Ferreira et al. 2018; Wang et al. 2020a; Wang et al. 2020b) and toxics like phenanthrene (Gao et al. 2017; Chen et al. 2019; Chen et al. 2020), and it contributes to reduce the bioavailability of the toxics. It has different affinities for bonding to HM, depending on factors like metal chemistry and content. It seems that GRSP is more abundant in high concentrations of the toxic (Vodnik et al. 2008; Wu et al. 2014; Malekzadeh et al. 2016; Ferreira et al. 2018; Wang et al. 2020a). The binding mechanisms of the toxics to the GRSP are not well elucidated. González-Chávez et al. (2004) suggests that the binding of Copper was caused by, electrostatic sorption or strong complex formations. Recently, it has been demonstrated that for the bonding of certain heavy metals, ion exchange is the principal mechanism, so functional groups like carbonyl, hydroxyl, amide and carboxyl may participate in this process (Wang et al. 2020a; Wang et al. 2020b).

GRSP is part of the mechanism that AMF could use on alleviation in remediation processes. Elucidating more information of this protein could be considered to maximize

the potential of the applications, because of environmental processes and conditions, and also, the AMF species can affect the production or peak intensity of GRSP (Singh 2012, 2015; Wu et al. 2014; Wang et al. 2020a; Wang et al. 2020b). Diversity of AMF and glomalin content can be considered good indicators of rehabilitation of soils contaminated with Zn, Cu, Pb, and Cd (Leal et al. 2016). GRSP production should be considered in biostabilization of polluted soils since it participates in the sequestration of different PTEs (potentially toxic elements) (González-Chávez et al. 2004; Rilling and Steinberg 2002). AMF protect plants against stress caused by the HMs pollution when it accumulates high concentrations in the radical system and decreases the translocation to the aerial parts (Tonin et al. 2001).

5. *Stimulating the growth of hyperaccumulators* – The plants that have the capacity to tolerate high levels of HMs present in the soil and after that, and accumulate it in their tissues, are known as metallophytes or hyperaccumulators. Tolerance is the capacity of plants or microorganisms to live and adapt to elevated heavy metal concentrations in soil (Dietz et al. 1999). These hyperaccumulators absorb heavy metals, translocate them through tonoplast and accumulate in vacuoles, in that way, they protect cell metabolism from metal toxicity (Maiti et al. 2004). The use of plants, with hyperaccumulating ability or in association with soil microbes including the symbiotic fungi, arbuscular mycorrhiza, are among the most common biological methods of treating heavy metals in soil (Miransari 2011). Once metals enter the hyphae of AMF, they can be immobilized or transferred to the root, and, in the root, they can be sequestered or translocated to the shoot (Leyval et al. 1997).

In a study focused on *Cannabis sativa* (var. Carmagnola) associated with *Glomus mosseae*, in a soil polluted artificially with Cr, Cd and Ni, it was reported a significantly higher concentration of Ni in the plants leaves and stems. So, this association *G. mosseae*-*C. sativa* stimulated the hyperaccumulating plant species, enhancing the root to shoot metal translocation to sequester the exceeding toxic metals in the shoot cell vacuoles by means of molecules such as metallothioneins and phytochelatins (Citterio et al. 2005). *Eucalyptus globulus* is suitable to grow and rehabilitate heavy-metal-polluted soils (Arriagada et al. 2004, 2007). In a study, it was shown that the synergy action of AMF (*Glomus mosseae* or with *Glomus deserticola*), with a saprophyte fungus (*Fusarium concolor* and *Trichoderma koningii*), allowed a higher Cd and Pb growth and absorption in trees stems and leaves of *E. globulus* (Arriagada et al. 2007). The AM fungi seems to contribute to the redistribution of Cd inside the plant. In fact, it was higher accumulation of Cd in the stem that in the leaves of eucalyptus colonized with *G. deserticola*, where the harmful effects on the development of the plant are minimal

(Arriagada et al. 2004). This redistribution of heavy metals in the less metabolically active part of the plant might explain why AMF increased the content of heavy metals and enhanced the growth of eucalyptus (Arriagada et al. 2007) (Table 1).

6. *Mycorrhizal fungi change the structure of the microbial community and the physical and chemical properties of rhizosphere soils* – For example Ogar et al. (2015) and Ma et al. (2019) evaluated the impact of microbial inoculation on phytoremediation. In case of nickel (Ni)-contaminated saline soils using *Helianthus annuus* together with salt resistant plant beneficial bacterium, *Pseudomonas libanensis* TR1 and AMF *Claroideoglossum claroideum* showed bioaugmentation. The results of this study showed that the bioaugmentation using other microbial strains in addition to AMF may be a preferred strategy for improving phytoremediation of metal-polluted saline soils. (Ma et al. 2019).

4 Species of AMF in phytoremediation

Different species of AMF are useful for phytoremediation and their efficiency depend on plant species; however, few AMF are widely used and studied (Table 1). Research has focused mainly on the effects of AMF on HMs, but there are also species that have been used for other kind of pollutants. Studies analyzing species such as *Glomus mosseae*, *G. intraradices*, *Funneliformis mosseae*, or *Rhizophagus irregularis* are the most common. Some of the most studied heavy metals in the presence of AMF are Cd, Pb, Cr, and Ni. In the case of these metals, the symbiosis provides benefits in the alleviation of different plants by using species like *G. mosseae* (Jamal et al. 2002; Janousková et al. 2006; Azcón et al. 2009; Ruscitti et al. 2011; Garg and Aggarwal 2012; Garg and Bhandari 2012), *G. intraradices* (Turnau and Mesjasz-Przybylowicz 2003; Malcová et al. 2003; Janousková et al. 2006; Sudová and Vosátka 2007; Andrade et al. 2008; Ruscitti et al. 2011; Liu et al. 2018; Zhang et al. 2019a), *G. aggregatum* (Singh et al. 2019; Zhang et al. 2019a), *R. fasciculatus* (Singh et al. 2019), *R. intraradices* (Yang et al. 2015; Jiang et al. 2016; Singh et al. 2019), *F. mosseae* (Yang et al. 2015; Singh et al. 2019; Zhan et al. 2019), and *Diversispora spurcum*; this last species has been used for Pb, Cd, and Zn (Zhan et al. 2019). For Pb and Cd, species used have been *G. etunicatum* (Souza et al. 2012; Zhan et al. 2019) and *R. irregularis* (Zhang et al. 2019b;

Table 1 Details of AMF species exploited in phytoremediation and in the presence of inorganic/organic pollutants

AMF	Plants	Pollutant	Mechanisms or remarks	Reference	
<i>Funneliformis mosseae</i>	<i>Glycine max, Lens culinaris</i>	Zn, Ni	Phytoextraction	Jamal et al. (2002)*	
	<i>Nicotiana tabacum</i>	Cd	Phytostabilization	Janousková et al. (2006)*	
	<i>Oryza sativa</i>	Chlorothalonil	Alleviation	Zhang et al. (2006)*	
	<i>Coreopsis drummondii, Pteris vittata, Lolium perenne</i>	Cu	Phytostabilization	Chen et al. (2007)*	
	<i>Trifolium repens</i>	Cd, Mo, Mn, Zn, Cu, Al, As, Ni	Phytostabilization	Azcón et al. (2009)*	
	<i>Medicago sativa</i>	Phenanthrene, pyrene	Alleviation	Gao et al. (2011)*	
	<i>Capsicum annum</i>	Cr	Phytostabilization	Ruscitti et al. (2011)*	
	<i>Lolium multiflorum</i>	decabromodiphenyl ether (BDE-209)	Enhanced debromination	Wang et al. (2011)*	
	<i>Zea mays</i>	Phenanthrene, pyrene	Alleviation	Wu et al. (2020)*	
	<i>Lolium multiflorum</i>	Phenanthrene, pyrene	Enhance dissipation	Yu et al. (2011)*	
	<i>Cajanus cajan</i>	Cd, Pb	Phytostabilization	Garg and Aggarwal (2012)*	
	<i>Cajanus cajan</i>	Cd	Phytoextraction	Garg and Bhandari (2012)*	
	<i>Cucurbita pepo</i>	Aroclor 1242	Enhance dissipation	Qin et al. (2014)*	
	<i>Robinia pseudoacacia</i>	Pb	Phytostabilization	Yang et al. (2015)	
	<i>Canna indica</i>	Atrazine	Alleviation, enhances removal	Dong et al. (2016)	
	<i>Medicago sativa</i>	Atrazine	Alleviation	Fan and Song (2018)	
	<i>Zea mays</i>	Cd, Cr, Ni, Pb	Phytoextraction	Singh et al. (2019)	
	<i>Phragmites australis</i>	TiO ₂ NPs	Alleviation	Xu et al. (2019)	
	<i>Cynodon dactylon</i>	Pb, Zn, Cd	Alleviation, Phytostabilization	Zhan et al. (2019)	
	<i>Zea mays</i>	Simazine	Alleviation	Cheng et al. (2021)	
	<i>Rhizophagus intraradices</i>	<i>Agrostis capillaris, Zea mays</i>	Pb	Alleviation	Malcová et al. (2003)*
		<i>Berkheya coddii</i>	Ni	Phytoextraction	Turnau and Mesjasz-Przybylowicz (2003)*
		<i>Sorghum bicolor</i>	Cu	Phytostabilization	Toler et al. (2005)*
		<i>Nicotiana tabacum</i>	Cd	Phytostabilization	Janousková et al. (2006)*
		<i>Astragalus sinicus</i>	Lanthanum	Phytostabilization	Chen and Zhao (2007)*
		<i>Zea mays</i>	Pb	Phytostabilization	Sudová and Vosátka (2007)*
<i>Helianthus annuus</i>		Cd	Phytostabilization	Andrade et al. (2008)*	
<i>Nicotiana tabacum</i>		Phenol	Alleviation	Ibáñez et al. (2011)*	
<i>Capsicum annum</i>		Cr	Phytostabilization	Ruscitti et al. (2011)*	
<i>Medicago sativa, Festuca arundinacea, Lolium multiflorum, Apium graveolent</i>		Phenanthrene	PAH dissipation	Zhou et al. (2013)*	
<i>Avena sativa</i>		Mixed petroleum	Alleviation and enhanced degradation	Xun et al. (2015)*	
<i>Zea mays</i>		Oxytetracycline (OTC)	Alleviation, enhanced degradation	Cao et al. (2015)	
<i>Robinia pseudoacacia</i>		Pb	Alleviation	Yang et al. (2015)	
<i>Lonicera japonica</i>		Cd	Alleviation	Jiang et al. (2016)	
<i>Zea mays</i>		Cd	Alleviation	Liu et al. (2018)*	
<i>Medicago sativa</i>		Cd	Alleviation	Zhang et al. (2019a)*	
<i>Zea mays</i>		Cd, Cr, Ni, Pb	Phytoextraction	Singh et al. (2019)	
<i>Rhizophagus irregularis</i>		<i>Phragmites australis</i>	Cu	fitorizomediación	Wu et al. (2020)
		<i>Medicago truncatula</i>	Pb	Alleviation	Zhang et al. (2019b)

Table 1 (continued)

AMF	Plants	Pollutant	Mechanisms or remarks	Reference
	<i>Solanum nigrum</i>	Cd	Phytostabilization	Wang et al. (2020)
	<i>Lolium perenne</i>	Fe-CN	Phytostabilization	Sut et al. (2016)
	<i>Trifolium aestivum</i>	Alkane, polycyclic aromatic hydrocarbon (PAH)	Alleviation	Lenoir et al. (2016b)
	<i>Medicago truncatula</i>	Benzo[a]pyrene	Alleviation	Calonne-Salmon et al. (2018)
	<i>Glyceria maxima</i>	Ibuprofen, diclofenac	Alleviation, enhance removal	Hu et al. (2020)
<i>Septoglomus deserticola</i>	<i>Acacia melanoxylum</i> , <i>Cytisus striatus</i> , <i>Allium cepa</i> , <i>Trifolium pratense</i>	hexachlorocyclohexane (HCH)	Alleviation	Sainz et al. (2006)*
	<i>Eucalyptus globulus</i>	As	Phytoextraction	Arriagada et al. (2009)*
	<i>Eucalyptus globulus</i>	Zn	Phytoextraction	Arriagada et al. (2010)*
	<i>Solanum melogena</i> , <i>Sorghum sudanese</i>	Cd, Zn	Phytoextraction	Mohammad and Mittra (2013)*
	<i>Ampelopteris proliferata</i>	Cr	Phytoextraction	Singh et al. (2014)*
<i>Funneliformis caledoniensis</i>	<i>Zea mays</i>	Atrazine	Enhanced degradation	Huang et al. (2007)*
	<i>Medicago sativa</i>	Polychlorinated biphenyls (PCBs)	Enhanced removal	Teng et al. (2010)*
	<i>Lolium perenne</i>	Polychlorinated biphenyls (PCBs)	Phytoextraction	Lu et al. (2014)*
<i>Acaulospora laevis</i>	<i>Festuca arundinacea</i>	PAHs	PAH dissipation	Lu and Lu (2015)*
	<i>Astragalus sinicus</i>	Lanthanum	Phytostabilization	Chen and Zhao (2007)
	<i>Cucurbita pepo</i>	Aroclor 1242	Enhance dissipation	Qin et al. (2014)
<i>Claroideoglossum claroideum</i>	<i>Nicotiana tabacum</i>	Cd	Phytostabilization	Janousková et al. (2006)*
	<i>Eucalyptus globulus</i>	As	Phytoextraction	Arriagada et al. (2009)*
<i>Claroideoglossum etunicatum</i>	<i>Calopogonium mucunoides</i>	Pb	Phytostabilization	Souza et al. (2012)*
	<i>Medicago sativa</i>	Cd	Alleviation, Phytostabilization	Zhan et al. (2019)*
<i>Rhizophagus aggregatus</i>	<i>Zea mays</i>	Cd, Cr, Ni, Pb	Phytoextraction	Singh et al. (2019)*
	<i>Medicago sativa</i>	Cd	Alleviation, Phytostabilization	Zhang et al. (2019a)*
<i>Rhizophagus fasciculatus</i>	<i>Triticum aestivum</i>	Metsulfovax, Bavistin, Thiram, Captan, Aldrin	Alleviation	Chhabra and Jalali (2013)*
	<i>Zea mays</i>	Cd, Cr, Ni, Pb	Phytoextraction	Singh et al. (2019)
<i>Diversispora spurca</i>	<i>Cynodon dactylon</i>	Pb, Zn, Cd	Alleviation, Phytostabilization	Zhan et al. (2019)
<i>Funneliformis constrictus</i>	<i>Zea mays</i>	Diesel	Alleviation	Tang et al. (2009)*
<i>Funneliformis geosporus</i>	<i>Nicotiana tabacum</i>	Cd	Phytostabilization	Janousková et al. (2006)*
<i>Gigaspora margarita</i>	<i>Astragalus sinicus</i>	Lanthanum	Phytostabilization	Chen and Zhao (2007)
<i>Glomus versiforme</i>	<i>Lonicera japonica</i>	Cd	Alleviation, Phytostabilization	Jiang et al. (2016)
<i>Rhizophagus clarus</i>	<i>Costus lucanusianus</i>	Crude oil	Alleviation and enhanced degradation	Nkereuwem et al. (2020)*

*= articles in where the AMF species now have a different name. This table uses the AMF current names according to the index fungorum (<http://www.indexfungorum.org/>). Also, the species appear in order of number of reports

Wang et al. 2020). For the presence of Cd or Cr, AMFs such as *G. deserticola* (Mohammad and Mittra 2013; Singh et al. 2014) have been used, and *G. geosporum*, *G. claroideum* (Janousková et al. 2006), and *G. versiforme* (Jiang et al. 2016) have been used only for Cd.

There are also studies on phytoremediation involving other HMs, such as: Zn, Cu, As, and Lanthanum (La). For these HMs, some investigations have used common species or species that also participate in other common HMs remediation, like the ones mentioned above. *Glomus mosseae* is also used in the presence of Zn and Cu (Jamal et al. 2002; Chen et al. 2007; Azcón et al. 2009). *Rhizophagus irregularis* is used for Cu or La (Toler et al. 2005; Chen and Zhao 2007) and, for La, other less used species are *Acaulospora laevis* or *Gigaspora margarita* (Chen and Zhao 2007). *Rhizophagus irregularis* has also been used for Cu (Wu et al. 2020). In presence of Zn and As, *G. mosseae* (Jamal et al. 2002; Azcón et al. 2009) and *G. deserticola* (Arriagada et al. 2009; Arriagada et al. 2010; Mohammad and Mittra 2013) could have alleviation benefits. For compounds such as TiO₂ nanoparticles or iron–cyanide (Fe–CN), *F. mosseae* (Xu et al. 2019) and *R. irregularis* (Sut et al. 2016), respectively, can be used for alleviation in plants.

Glomus versiforme has a more beneficial role than *Glomus mosseae* in promoting plant growth, nutrient absorption, C: N:P stoichiometric adjustment, and alleviation of rare earth element (REE) and HM toxicity in plants. Corn and sorghum show opposite tendencies in REE uptake in response to AMF colonization. Results suggest that the effect of AMF on REE uptake could be related to plant species, AMF isolate, and REE type and concentration in mine residues. Results indicated that AMF could increase the ability of plants to restore ecosystems polluted with the chemical complex of REE in mine residues or with heavy metals (Guo et al. 2013).

AMF are not only used in HM phytoremediation, since they may have benefits for plant alleviation in the presence of oil or PAHs. For PAHs, Lu and Lu (2015) used *G. caledonium*. For products like Phenanthrene or Pyrene, *G. mosseae* (Gao et al. 2011) has been used, and *G. intraradices* has been used for Phenanthrene (Zhou et al. 2013). In addition, Calonne-Salmon et al. (2018) observed that *R. irregularis* could alleviate the studied host plant in the presence of Benzo[a]pyrene. *Rhizophagus irregularis* alleviated mixed petroleum (Xun et al. 2015) or Phenol (Ibáñez et al. 2011), and *G. clarum* can be used for crude oil (Nkereuwem et al. 2020). There are reports of AM resulting in plant alleviation and enhanced removal of less studied human pollutants used in agriculture or veterinary. For herbicides, Dong et al. (2016) observed that *F. mosseae* may have benefits in the presence of chloro-s-triazine or atrazine. For antibiotics widely used in veterinary, such as Oxytetracycline (OTC), phytoremediation with *R. intraradices* could enhance the process (Cao et al. 2015).

5 Conclusions and perspectives

The pollution caused by anthropogenic activities is a severe global problem. We can apply physical and chemical remediation methods. However, they have a high economic and environmental cost. Phytoremediation is a better option, using plants and microorganisms to remediate contaminated sites. By specifically using AMF cosmopolitan organisms that require a host to complete their life cycle, both, the fungus and the plants receive benefits from this interaction and we obtain better results. Different reports show that use of AMF improves plant tolerance to HMs and POPs pollution, as AMF influence the uptake and distribution of HMs in host plants. They also immobilize the contaminant at the root level, transport it in smaller amounts to the aerial parts (phytostabilization) or efficiently translocate in to the aerial parts of hyperaccumulating plants (phytoextraction).

The application of AMF in the phytoremediation allows: 1) to improve mineral nutrition and water availability, 2) to protect against oxidative stress, 3) to increase soil physical stability, 4) to increase plant tolerance to soil stress, 5) to increase concentration in chlorophyll pigments, amino acids, carbohydrates, total sugars and essential elements such as P and N, 6) that glomalin production protects hyphae and AMF from stress caused by contaminants, 7) that mycorrhizal interaction favors that contaminants can accumulate in AMF structures (spores, extraradical and intraradical hyphae, vesicles, arbuscules), or in the plant (in root cell, shoots leaves or stems), where metabolic activity is reduced and harmful effects to the plant are low.

Depending on the type of contaminant, an appropriate selection of plant species and AMF is required to enhance the phytoremediation process. Selection of a hyperaccumulator plant, but with higher biomass (e.g., forest species) inoculated with AMF species could be considered to aid the phytoextraction process of soil contaminants.

The identification and isolation of indigenous and tolerant AMF strains can have implications for the future of phytoremediation of contaminated soils. Some studies have also documented the application of saprophytic fungi in synergy with the AMF to take advantage of pollution stress tolerance (bioaugmentation). In the case of application of AMF for HMs uptake with plants, better results are obtained by using a consortium of fungi adapted to metal containing soils rather than individual fungal species. However, it is necessary a deeper study and compare the diversity of AMF in HM contaminated and non-contaminated soils when associated with HM tolerant and non tolerant plants. Consequently, we will acquire more knowledge about these symbiotic relationships, which promise to be a safe, clean, sustainable, and economical management strategy that enhances plant growth and facilitates the remediation of heavy metals in contaminated soils (Shahabivand et al. 2012).

Modern biotechnology and gene editing promise a major breakthrough in bioremediation, including the improvement of AMF strains for such complex subjects as HMs. However, there is a need to continue studying indigenous organisms and record natural biodiversity to learn more about the wide range of possibilities that already exist, and to conduct further studies on the application of AMFs in bioremediation strategies. It is also important to continue researching on the basic principles and molecular mechanisms that allow us to understand how contaminants are taken up and how they act at the cellular and tissue level in both AMF and plants, as well as to identify genes involved that are attractive for breeding programs. This will allow us to increase the tolerance and efficiency of plants and fungi to obtain new soil bioremediation strategies.

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Declarations

Ethics approval Authors declare that there is no conflict of interest of any kind.

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