

Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils

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Arbuscular mycorrhizal (AM) associations are integral, functioning parts of plant roots and are widely recognized as enhancing plant growth on severely disturbed sites, including those contaminated with heavy metals. They are reported to be present on the roots of plants growing on heavy metal contaminated soils and play an important role in metal tolerance and accumulation. Isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems. This review highlights the potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils.

ARBUSCULAR mycorrhizal (AM) fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots¹. About 95% of the world's plant species belong to characteristically mycorrhizal families² and potentially benefit from AM fungus-mediated mineral nutrition³ due to the fundamental role played by these glomalean fungi in biogeochemical element cycling³. AM symbiosis occurs in almost all habitats and climates⁴, including disturbed soils^{5,6} and those derived from mine activities⁷⁻⁹.

AM fungi can contribute to plant growth, particularly in disturbed or heavy metal contaminated sites, by increasing plant access to relatively immobile minerals such as P^{10,11}, improving soil texture by binding soil particles into stable aggregates that resist wind and water erosion^{12,13}, and by binding heavy metals into roots that restricts their translocation into shoot tissues^{14,15}. Furthermore, the fungi can accelerate the revegetation of severely degraded lands such as coal mines or waste sites containing high levels of heavy metals^{16,17}. AM fungi form an integral component of successfully revegetated flue-gas-desulphurization sludge ponds¹⁸.

Soil degradation produces changes in the diversity and abundance of AM fungal populations¹⁹. This is critical because of the role of mycorrhizal fungi in plant establishment and survival²⁰. Such elimination of AM fungi populations can lead to problems with plant establishment and survival²¹. Even if AM fungi are ubiquitous in terrestrial ecosystems, mechanical or chemical distur-

bance of the soil can substantially reduce AM fungal population vigour and functioning²². The number of spores and root colonization of plants occurring at sites are often reduced by soil disturbance²³. However, AM fungal isolates adapted to local soil conditions can stimulate plant growth better than non-indigenous isolates. Indigenous AM fungal ecotypes result from long-term adaptation to soils with extreme properties²². Therefore, isolation of indigenous stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in disturbed ecosystems²⁴. This review highlights the potential of AM fungi for inoculation of plants as a prerequisite for successful restoration of heavy metal contaminated soils.

Heavy metal pollution and importance of AM fungi

Ecosystems have been contaminated with heavy metals due to various human and natural activities. The sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, sewage sludge amendments, the use of pigments and batteries. These metals are commonly called heavy metals, although this term strictly refers to metallic elements with a specific mass higher than 5 g cm⁻³, able to form sulphides²⁵. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni and Co²⁶, while others have no known biological function, such as Cd, Pb and Hg. Migration of these contaminants into non-contaminated areas as dust or leachates through the soil and spreading of heavy metal containing sewage sludge are examples of events that contribute towards contamination of our ecosystems.

It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be immobilized²⁷. Traditionally, remediation of heavy metal contaminated soils involves either on-site management or excavation, and subsequent disposal to a landfill site²⁸. However, this method of disposal merely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment²⁹. Soil washing for removing contaminated soil is an alternative to excavation and disposal to landfill³⁰. This method is, however, costly and produces a residue rich in heavy metals, which will require further treatment

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or burial. Moreover, these physio-chemical technologies used for soil remediation render the land useless as a medium for plant growth, as they remove all biological activities. Therefore, sustainable on-site techniques for remediation of heavy metal contaminated sites need to be developed. Phytoremediation, the use of plants to remediate or clean-up contaminated soils can be used as a promising method to remove and/or stabilize soils contaminated with heavy metals. Moreover, it is essential to create a stabilizing plant cover composed of herbaceous species which are active in phytoremediation processes and produce high levels of root and shoot biomass^{31,32} to prevent intensive wind erosion. Plants which appear spontaneously in such places are frequently devoid of mycorrhizal symbiosis and are mostly characterized by poorly developed root and shoot biomass when heavy metals are present³³. The lack of mycorrhiza can hamper the revegetation of the metal-contaminated mine spoil or other degraded sites. The introduction of an AM fungal inoculum into these areas could be a strategy for enhancing the establishment of mycorrhizal herbaceous species. AM fungal isolates differ in their effect on heavy metal uptake by plants³². Some reports indicate higher concentrations of heavy metals in plants due to AM³⁴, whereas others have found a reduced plant concentration; for example, Zn and Cu in mycorrhizal plants³⁵. Thus, selection of appropriate isolates could be of importance for a given phytoremediation strategy. AM fungal species can be isolated from areas which are either naturally enriched by heavy metals or are old mine/industry waste sites in origin. In this context, AM fungi constitute an important functional component of the soil-plant system that is critical for sustainable productivity in degraded soils.

AM fungi are of importance as they play a vital role in metal tolerance³⁶ and accumulation^{37,38}. External mycelium of AM fungi provides a wider exploration of soil volumes by spreading beyond the root exploration zone^{39,40}, thus providing access to greater volume of heavy metals present in the rhizosphere. A greater volume of metals is also stored in the mycorrhizal structures in the root and in spores. For example, concentrations of over 1200 mg kg⁻¹ of Zn have been reported in fungal tissues of *Glomus mosseae* and over 600 mg kg⁻¹ in *G. versiforme*⁴¹. Another important feature of this symbiosis is that AM fungi can increase plant establishment and growth despite high levels of soil heavy metals⁵, due to better nutrition^{42,43}, water availability⁴⁴ and soil aggregation properties^{12,45} associated with this symbiosis. AM fungus is significant in the ecological improvement of rhizosphere^{46,47}.

Heavy metal-tolerant AM fungi

Heavy metals have been reported to reduce or eliminate AM infection at high concentrations of heavy metals in the soil¹⁹, thus interfering with possible beneficial effects

of the mycorrhizal association. On the other hand, there are reports of AM fungi from metal-contaminated soils, which suggests a potential adaptation of the indigenous AM populations. Several heavy metal-tolerant AM fungi have been isolated from polluted soils, which can be useful for reclamation of such degraded soils as they are found to be associated with a large number of plant species in heavy metal-polluted soil. Gildon and Tinker⁴⁸ isolated a mycorrhizal strain which tolerated 100 mg kg⁻¹ of Zn in the soil. Considerable amount of AM fungal colonization was also reported in an extremely polluted metal mining area with HCl-extractable Cd soil concentration of more than 300 mg kg⁻¹ (ref. 49). Similarly, Weissenhorn *et al.*⁵⁰ isolated mycorrhizal fungi from two heavy metal-polluted soils, which were found to be more resistant to Cd than a reference strain. Sambandan *et al.*⁵¹ reported 15 AM fungal species from heavy metal-contaminated soils from India. Of the 15 AM species isolated, *Glomus geosporum* was encountered in all the sites studied. The percentage colonization ranged from 22 to 71% and spore count was as high as 622 per 100 g soil.

Turnau *et al.*⁵² analysed the community of AM fungi in roots of *Fragaria vesca* growing in Zn-contaminated soil. Seventy per cent of the root samples containing positively stained fungal hyphae were found to be colonized by *G. mosseae*. Another unique AM fungal species, *Scutellospora dipurpurascens* has been reported by Griffioen *et al.*⁵³ from the rhizosphere of *Agrostis capillaris* growing in contaminated surroundings of a zinc refinery in the Netherlands. This indicates that these fungi have evolved Zn and Cd tolerance and that they might play an important role in conferring Zn and Cd tolerance in plants.

Mycorrhizal fungi have also been shown to be associated with metallophyte plants on highly polluted soils, where only adapted plants such as *Viola calaminaria* (violet) can grow⁵⁴. This yellow-violet plant is described as an absolute metallophyte plant, which usually colonizes Zn- and Pb-rich soils, and accumulates remarkably low levels of metal or none at all, despite the elevated levels of metal in these soils⁵⁵. A *Glomus* sp. isolated from the roots of the violet plant improved maize growth in a polluted soil⁵⁶ and reduced root and shoot heavy metal concentrations in comparison to a common *Glomus* isolate or non-colonized controls¹⁵. Tonin *et al.*⁵⁴ reported AM fungal spores from the violet rhizosphere and from violet roots which were characterized by polymerase chain reaction (PCR) amplification of the SSU rDNA. At least four different *Glomus* species were found in the violet rhizosphere analysed using terminal-restriction fragment length polymorphism. Other reports have also shown higher mycorrhizal colonization in strongly contaminated sites⁵⁶.

Weissenhorn *et al.*⁵⁷ suggested a high tolerance of indigenous AM fungal population to elevated metal concentrations in soil and inside the roots. AM fungal colonization up to 40% was reported in spite of high Cd (1220 mg kg⁻¹) and Pb (895 mg kg⁻¹) concentrations.

They further reported abundance of AM fungi (100 spores per 50 g soil) in two agricultural soils close to a Pb–Zn smelter. Spores belonging to the *G. mosseae* group were isolated from two heavy metal-polluted soils in France⁵⁰. The two cultures isolated from Cd-polluted soils were found to be tolerant to Cd concentrations of approximately 50–70 $\mu\text{g l}^{-1}$ and 200–500 $\mu\text{g l}^{-1}$ respectively. Highly functional AM symbiosis was reported from southern Poland, in plants colonizing calamine spoil mounds rich in Cd, Pb and Zn⁵⁸. Mycorrhizal colonization was much higher in *Plantago lanceolata* (up to 90%) compared to *Biscutella laevigata* (up to 40%). Besides vesicles and coils, arbuscules were also observed.

del Val *et al.*³⁶ determined AM fungal diversity in a long-term experiment amended with sewage-sludge containing heavy metals such as Zn, Cd, Cu, Ni and Pb. The total number of AM fungal spores decreased with long-term sludge application and with increasing amounts of heavy metals, but the AM fungal spores never disappeared completely in soils amended with the highest rates of sludge, suggesting a certain adaptation of these indigenous AM fungi to such environment stress. Six AM fungal ecotypes were found in the experimental soils, showing consistent differences with regard to their tolerance to the presence of heavy metals. AM fungal ecotypes ranged from very sensitive to the presence of metals to relatively tolerant to high rates of heavy metals in soil. Other studies have also demonstrated the abundance of AM fungi in long-term sewage-sludge field trials, where contaminated sludge was applied for 18 years⁵⁷. *Glomus claroideum*, another ecotype, potentially adapted to increased metal concentration in soil was isolated from plots receiving 300 $\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$ of contaminated sludge⁵⁹. This isolate produced a significantly higher root colonization level (20% in *Allium porrum* and 15% in *Sorghum bicolor*) in the heavy metal-contaminated soil than produced by other isolates (isolated from non-contaminated soil). Furthermore, *G. claroideum* was the most effective fungus in improving the growth of *A. porrum* and *S. bicolor* in heavy metal-contaminated soil. Spores from naturally polluted soils (collected from contaminated sites close to a Zn smelter and a site overlying with alum shale bed-rock) have been shown to germinate better in heavy-metal polluted soil compared to spores from non-polluted soils⁶⁰. Thus, indigenous AM fungi showed tolerance to heavy metals as they germinated in soils containing 6060 mg kg^{-1} Pb, 24,410 mg kg^{-1} Zn and 1630 mg kg^{-1} Cu.

AM fungi confer tolerance to plants grown in heavy-metal contaminated soil

Alleviation of Zn and Cd toxicity by AM has been reported^{35,57,61–63}. It has been suggested that heavy metal-tolerant AM fungi could protect plants against harmful

effects of excessive heavy metals^{22,32}. Several biological and physical mechanisms have been proposed to explain metal tolerance of AM fungi and AM fungal contribution to metal tolerance of host plants. Immobilization of metals in the fungal biomass is one such mechanism involved^{38,64}. Reduced transfer, as indicated by enhanced root/shoot Cd ratios in AM plants, has been suggested as a barrier in metal transport^{65,66}. This may occur due to intracellular precipitation of metallic cations with PO_4^- . Turnau *et al.*⁶⁷ have demonstrated greater accumulation of Cd, Ti and Ba in fungal structures than in the host plant cells. Uptake into hyphae may be influenced by absorption on hyphal walls as chitin has an important metal-binding capacity⁶⁸. Thus, AM fungal metal tolerance includes adsorption onto plant or fungal cell walls present on and in plant tissues, or onto or into extraradical mycelium in soil⁶⁹, chelation by such compounds as siderophores and metallothionens released by fungi or other rhizosphere microbes, and sequestration by plant-derived compounds like phytochelatins or phytates³⁴. Other possible metal-tolerance mechanisms include dilution by increased root or shoot growth, exclusion by precipitation onto polyphosphate granules, and compartmentalization into plastids or other membrane-rich organelles^{15,67}. Indirect mechanisms include the effect of AM fungi on rhizosphere characteristics such as changes in pH⁷⁰, microbial communities⁷¹ and root-exudation patterns⁷².

AM fungi associated with metal-tolerant plants, e.g. metallophytes, may contribute to the accumulation of heavy metals in plant roots in a non-toxic form. Occurrence of AM fungi has been reported in rhizosphere of metallophytes such as *Viola calaminaria*⁵⁴ and *Berkheya coddii*⁷³ in Zn/Pb- and Ni-rich soils respectively. The accumulation of heavy metals in the fungal structures as suggested by their high heavy metal-binding capacity⁶⁵ could represent a biological barrier. Reduced Cd translocation from roots to shoots in the presence of AM fungi in roots of *Trifolium subterraneum* has been shown^{34,74}. In experiments involving compartmented pots, Joner and Leyval³⁴ reported that a large proportion of increased Cd content of mycorrhizal plants was sequestered in the roots. The mycorrhizal status of the plants did not influence shoot concentration of ¹⁰⁹Cd, but concentration in roots was increased in mycorrhizal plants. Cd-root:shoot ratio was found to be 3.15 in mycorrhizal plants compared to 1.66 in non-mycorrhizal plants. They concluded that extraradical hyphae of AM fungus can transport Cd from soil to plant, but transfer from fungus to plant is restricted due to fungal immobilization. In other experiments⁵⁴, subterranean clover was grown in pots containing a soil supplemented with Cd and Zn salts and inoculated with AM fungal spores extracted from the rhizosphere of *V. calaminaria* and spores of Cd-tolerant *G. mosseae*. The AM fungal inoculum from the metal-tolerant plant (*V. calaminaria*) was efficient in sequestering metals in the roots. Clover roots inoculated with AM fungi from

the rhizosphere of this plant contained eightfold higher Cd and threefold higher Zn concentrations compared to non-inoculated plants, without any significant difference in plant biomass and concentration of metals in shoots. Hildebrandt *et al.*⁵⁶ reported that *Glomus* Br1 isolated from the roots of *V. calaminaria* improved maize growth in a polluted soil and reduced root and shoot heavy-metal concentration in comparison to a common *Glomus intraradices* isolate or non-colonized controls¹⁵. Cd-tolerant *G. mosseae* from metallophyte plant enhanced growth of maize, alfalfa, barley, etc. in heavy metal-rich soils. Thus, mycorrhizal fungi adapted to elevated soil metal concentration can significantly improve the growth and plant P nutrition under metal stress. By maintaining a higher shoot P/Zn concentration ratio mycorrhizal plants are able to alleviate the negative effects of Zn⁷⁵.

Different AM fungi have been shown to differ in their susceptibility and tolerance to heavy metals. del Val *et al.*³⁶ reported six AM fungal ecotypes with consistent differences with regard to their tolerance to the presence of heavy metals. AM fungal ecotypes ranged from very sensitive to the presence of metals to relatively tolerant to high rates of heavy metals in soil. *Glomus* sp. (isolated from non-polluted soil) was shown to be the most sensitive fungus, while one strain of *G. claroideum* (isolated from the most contaminated soil) was the most tolerant³⁶. The effectiveness of the different AM fungal isolates in improving plant growth also depends on the level of heavy metals in soil. Furthermore, AM fungi from different soils may differ in their metal susceptibility and both metal-specific and nonspecific tolerance mechanisms may be selected in metal-polluted soil⁷⁶.

Contribution of AM fungi to uptake of heavy metals

AM fungi supply plants with essential nutrients from the soil through uptake by extraradical hyphae. Toxic elements like Cd may also be transported by hyphae⁷⁷, but the fungus may constitute a biological barrier against transfer of heavy metals to shoots³⁴. Thus, there are different effects of AM fungi on heavy-metal uptake. In some cases, AM fungi reduce excess plant uptake of trace elements like Zn, Cd and Mn^{35,64}, whereas in other cases they enhance or have no effect on the uptake^{37,78-80}. Kaldorf *et al.*¹⁵ showed that maize grown in two different heavy-metal soils contained lower metal concentration (including Pb) in roots and shoots when colonized with heavy metal-tolerant *Glomus* isolate, compared to plants grown with common *Glomus* isolate. Diaz *et al.*⁸¹ observed lower Pb concentration in the shoots of plants inoculated with an AM isolate from contaminated soil (*G. mosseae*) than in the shoots of plants inoculated with an isolate from non-contaminated soil (*G. macrocarpum*).

The effect of AM fungal inoculation on metal accumulation has been shown to vary among plant species. The

inoculation with two *G. intraradices* isolates significantly reduced Pb concentration in maize plants, while Pb concentration in *Agrostis* plants was not changed or even increased by inoculation⁴⁰. Dependence of heavy metal-AM fungal interaction on plant species was also shown in two studies on AM isolates from the rhizosphere of a metallophyte zinc violet, *V. calaminaria*^{15,54}. Kaldorf *et al.*¹⁵ found much lower concentrations of heavy metals in maize plants inoculated with *Glomus* Br1 isolate than in non-inoculated control plants. Tonin *et al.*⁵⁴ reported that colonization of clover roots with mixed population of AM fungi from the zinc-violet rhizosphere significantly increased Cd and Zn concentration in clover roots, without significantly affecting heavy-metal concentration in the shoots. A range of factors like fungal properties, inherent heavy metal-uptake capacity of plants and soil absorption/desorption characteristics can influence heavy-metal uptake by mycorrhizal plants³². Mycorrhiza functioning depends on exploitation of non-rhizosphere soil by extraradical hyphae⁸². Functional mycorrhizas can reduce excessive passive uptake of potentially harmful elements through the roots while maintaining an adequate supply of the other elements like N and P through active hyphal uptake. The hypha are also less sensitive than roots with respect to heavy-metal toxicity³⁴. Thus, hyphal growth and nutrient uptake may be maintained when roots are impaired.

Conclusion

The prospect of AM fungi existing in heavy metal-contaminated soils has important implications for phytoremediation which are summarized in Table 1. Since heavy-metal uptake and tolerance depend on both plant and soil factors, including soil microbes, interactions between plant root and their symbionts such as AM fungi can play an important role in successful survival and growth of plants in contaminated soils. Mycorrhizal associations increase the absorptive surface area of the plant due to extramatrical fungal hyphae exploring rhizospheres beyond the root-hair zone, which in turn enhances water and mineral uptake. AM fungi can further serve as a filtration barrier against transfer of heavy metals to plant shoots. The protection and enhanced capability of uptake of minerals result in greater biomass production, a prerequisite for successful remediation. Indigenous AM isolates existing naturally in heavy metal-polluted soils are more tolerant than isolates from non-polluted soils, and are reported to efficiently colonize plant roots in heavy metal-stressed environments. Thus, it is important to screen indigenous and heavy metal-tolerant isolates in order to guarantee the effectiveness of AM symbiosis in restoration of contaminated soils. It is further suggested that the potential of phytoremediation of contaminated soil can be enhanced by inoculating hyper-accumulator plants with mycorrhizal

Table 1. Summary of AM fungi-heavy metal interactions and application in phytoremediation

Heavy metal	Tolerant fungi	Host used	Reference
Cd	<i>Glomus mosseae</i>	<i>Trifolium repens</i>	10
	<i>Glomus</i> sp. and <i>Gigaspora</i> sp.	<i>Hordeum vulgare</i>	66
	<i>G. mosseae</i>	<i>Trifolium subterraneum</i>	34
Ni	<i>G. mosseae</i>	<i>Allium porrum</i>	50
	<i>Gigaspora</i> sp. and <i>Glomus tenue</i>	<i>Berkheya coddii</i>	73
Zn	<i>Glomus</i> sp.	<i>Viola calaminaria</i>	15, 54, 56
	<i>Glomus</i> sp.	<i>Fragaria vesca</i>	52
	Mixed AM inocula	<i>T. repens</i>	38
	<i>Glomus constrictum</i> , <i>Glomus ambisporum</i> , <i>Scutellospora pellucida</i>	<i>Andropogin gerardii</i>	75
	<i>Scutellospora dipurpurescens</i>	<i>Agrostis capillaris</i>	53
Pb	<i>Glomus fasciculatum</i>	<i>Festuca rubra</i> and <i>Calamagrostis</i>	62
	<i>Glomus intraradices</i>	<i>A. capillaris</i> , <i>Zea mays</i>	40
Cd, Cu	<i>Glomus caledonium</i>	<i>Z. mays</i>	80
Zn, Pb	<i>G. mosseae</i> , <i>Glomus macrocarpum</i>	<i>Lygeum spartum</i>	81
Cd, Zn	<i>G. mosseae</i>	<i>T. subterraneum</i>	65
Cd, Zn, Pb	Mixed AM inocula	<i>Glycine max</i>	35
	AM fungi	<i>Biscutella laevigata</i> , <i>Plantago lanceolata</i>	58
Cd, Zn, Cu	<i>G. mosseae</i>	<i>T. subterraneum</i>	79
	<i>G. mosseae</i>	<i>Phaseolus vulgaris</i>	77
Zn, Cd, Cu, Ni, Pb	<i>G. caledonium</i>	<i>A. porrum</i> , <i>Sorghum bicolor</i>	36

zal fungi most appropriate for the contaminated site. It is therefore of great importance that we combine selected plants with specific AM fungal isolates adapted to high concentrations of heavy metal in future research for phytoremediation programmes. However, there is need to develop new methods and optimize the conditions to grow in large quantities and characterize, develop and screen large number of AM fungi for tolerance to metals. The lack of correlation between colonization rates and a beneficial or detrimental host response perhaps suggests the need to look more closely at the diversity and competition among AM fungi-colonizing roots. Identifying and culturing the most effective isolates could then be undertaken to select or develop genetically improved strains customized for a particular set of conditions or host plants.

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