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 roots[1]. About 95% of the world's plant species belong to characteristically mycorrhizal families[2] and potentially benefit from AM fungus-mediated mineral nutrition[3] due to the fundamental role played by these glomalean fungi in biogeochemical element cycling[3]. AM symbiosis occurs in almost all habitats and climates[4], including disturbed soils[5,6] and those derived from mine activities[7–9].

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 ponds[18].

Soil degradation produces changes in the diversity and abundance of AM fungal populations[19]. This is critical because of the role of mycorrhizal fungi in plant establishment and survival[20]. Such elimination of AM fungi populations can lead to problems with plant establishment and survival[21]. Even if AM fungi are ubiquitous in terrestrial ecosystems, mechanical or chemical disturbance of the soil can substantially reduce AM fungal population vigour and functioning[22]. The number of spores and root colonization of plants occurring at sites are often reduced by soil disturbance[23]. 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 properties[22]. Therefore, isolation of indigenous stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants in disturbed ecosystems[24]. 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 $5\,\mathrm{g\,cm^{-3}}$, able to form sulphides[25]. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni and Co[26], 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 immobilized[27]. Traditionally, remediation of heavy metal contaminated soils involves either on-site management or excavation, and subsequent disposal to a landfill site[28]. 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 environment[29]. Soil washing for removing contaminated soil is an alternative to excavation and disposal to landfill[30]. 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 present[33]. 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 plants[32]. Some reports indicate higher concentrations of heavy metals in plants due to AM[34], whereas others have found a reduced plant concentration; for example, Zn and Cu in mycorrhizal plants[35]. 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 tolerance[36] 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 $\mathrm{mg\,kg^{-1}}$ of Zn have been reported in fungal tissues of *Glomus mosseae* and over 600 $\mathrm{mg\,kg^{-1}}$ 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 metals[5], due to better nutrition[42,43], water availability[44] 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 soil[19], 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 Tinker[48] isolated a mycorrhizal strain which tolerated 100 $\mathrm{mg\,kg^{-1}}$ 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 $\mathrm{mg\,kg^{-1}}$ (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 grow[54]. 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 soils[55]. A *Glomus* sp. isolated from the roots of the violet plant improved maize growth in a polluted soil[56] and reduced root and shoot heavy metal concentrations in comparison to a common *Glomus* isolate or non-colonized controls[15]. 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 sites[56].

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 $\mathrm{mg\,kg^{-1}}$) and Pb (895 $\mathrm{mg\,kg^{-1}}$) 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. mossese* group were isolated from two heavy metal-polluted soils in France[50]. The two cultures isolated from Cd-polluted soils were found to be tolerant to Cd concentrations of approximately 50–70 $\mu g l^{-1}$ and 200–500 $\mu g l^{-1}$ respectively. Highly functional AM symbiosis was reported from southern Poland, in plants colonizing calamine spoil mounds rich in Cd, Pb and Zn[58]. 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 years[57]. *Glomus claroideum*, another ecotype, potentially adapted to increased metal concentration in soil was isolated from plots receiving 300 $m^3 ha^{-1} yr^{-1}$ of contaminated sludge[59]. 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 bedrock) have been shown to germinate better in heavy-metal polluted soil compared to spores from non-polluted soils[60]. 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 capacity[68]. 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 soil[69], chelation by such compounds as siderophores and metallothionens released by fungi or other rhizosphere microbes, and sequestration by plant-derived compounds like phytochelatins or phytates[34]. 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 pH[70], microbial communities[71] and root-exudation patterns[72].

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 capacity[65] 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 Leyval[34] 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 ^{109}Cd, 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 experiments[54], 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 controls[15]. 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 Zn[75].

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 tolerant[36]. 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 soil[76].

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 hyphae[77], but the fungus may constitute a biological barrier against transfer of heavy metals to shoots[34]. 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 inoculation[40]. 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 plants[32]. Mycorrhiza functioning depends on exploitation of non-rhizosphere soil by extraradical hyphae[82]. 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 toxicity[34]. 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 mycorrhi-

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

Heavy metal	Tolerant fungi	Host used	Reference
Cd	*Glomus mosseae*	*Trifolium repens*	10
	Glomus sp. and *Gigaspora* sp.	*Hordeum vulgare*	66
	G. mosseae	*Trifolium subterraneum*	34
	G. mosseae	*Allium porrum*	50
Ni	*Gigaspora* sp. and *Glomus tenue*	*Berkheya coddii*	73
Zn	*Glomus* sp.	*Viola calaminaria*	15, 54, 56
	Glomus sp.	*Fragaria vesca*	52
	Mixed AM inocula	*T. repens*	38
	Glomus constrictum, Glomus ambisporum, Scutellospora pellucida	*Andropogin gerardii*	75
	Scutellospora dipurpurescens	*Agrostis capillaris*	53
	Glomus fasiculatum	*Festuca rubra* and *Calamagrostis*	62
Pb	*Glomus intraradices*	*A. capillaris, Zea mays*	40
Cd, Cu	*Glomus caledonium*	*Z. mays*	80
Zn, Pb	*G. mosseae, Glomus macrocarpum*	*Lygeum spartum*	81
Cd, Zn	*G. mosseae*	*T. subterraneum*	65
	Mixed AM inocula	*Glycine max*	35
Cd, Zn, Pb	AM fungi	*Biscutella laevigata, Plantago lanceolata*	58
Cd, Zn, Cu	*G. mosseae*	*T. subterraneum*	79
	G. mosseae	*Phaseolus vulgaris*	77
Zn, Cd, Cu, Ni, Pb	*G. caledonium*	*A. porrum, Sorghum bicolor*	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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1. Barea, J. M. and Jeffries, P., Arbuscular mycorrhizas in sustainable soil plant systems. In *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology* (eds Hock, B. and Varma, A.), Springer-Verlag, Heidelberg, 1995, pp. 521–559.
2. Smith, S. E. and. Read, D. J., *Mycorrhizal Symbiosis*, Academic Press, San Diego, USA, 1997.
3. Jeffries, P. and Barea, J. M., Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant soil systems. In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems* (eds Gianinazzi, S. and Schuepp, H.), Birkhauser, Basel, 1994, pp. 101–115.
4. Barea, J. M., Azco'n-Aguilar, C. and Azcon, R., Interactions between mycorrhizal fungi and rhizosphere microorganism within the context of sustainable soil–plant systems. In *Multitrophic Interactions in Terrestrial Systems* (eds Gange, A. C. and Brown, V. K.), Cambridge, United Kingdom, 1997, pp. 65–77.
5. Enkhtuya, B., Rydlová, J. and Vosátka, M., Effectiveness of indigenous and non-indigenous isolates of arbuscular mycorrhizal fungi in soils from degraded ecosystems and man-made habitats. *Appl. Soil Ecol.*, 2002, **14**, 201–211.
6. Estaún, V., Savé, R. and Biel, C., AM inoculation as a biological tool to improve plant revegetation of a disturbed soil with *Rosmarinus officinalis* under semi-arid conditions. *Appl. Soil Ecol.*, 1997, **6**, 223–229.
7. Bi, Y. L., Li, X. L., Christie, P., Hu, Z. Q. and Wong, M.H., Growth and nutrient uptake of arbuscular mycorrhizal maize in different depths of soil overlying coal fly ash. *Chemosphere*, 2003, **50**, 863–869.
8. Weiersbye, I. M., Straker, C. J. and Przybylowicz, W. J., Micro-PIXE mapping of elemental distribution in arbuscular mycorrhizal roots of the grass, *Cynodon dactylon*, from gold and uranium mine tailings. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 1999, **158**, 335–343.
9. Bundrett, M. C., Ashwath, N. and Jasper, D. A., Mycorrhizas in the Kadau region of tropical Australia. II. Propagules of mycorrhizal fungi in disturbed habitats. *Plant Soil*, 1996, **184**, 173–184.
10. Vivas, A., Marulanda, A., Gómez, M., Barea, J. M. and Azcón, R., Physiological characteristics (SDH and ALP activities) of arbuscular mycorrhizal colonization as affected by *Bacillus thuringiensis* inoculation under two phosphorus levels. *Soil Biol. Biochem.*, 2003, **35**, 987–996.
11. Yao, Q., Li, X., Weidang, A. and Christie, P., Bi-directional transfer of phosphorus between red clover and perennial ryegrass via arbuscular mycorrhizal hyphal links. *Eur. J. Soil Biol.*, 2003, **39**, 47–54.
12. Rillig, M. C. and Steinberg, P. D., Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification. *Soil Biol. Biochem.*, 2002, **34**, 1371–1374.
13. Steinberg, P. D. and Rillig, M. C., Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. *Soil Biol. Biochem.*, 2003, **35**, 191–194.
14. Dehn, B. and Schuepp, H., Influence of VA mycorrhizae on the uptake and distribution of heavy metals in plants. *Agric. Ecosyst. Environ.*, 1989, **29**, 79–83.
15. Kaldorf, M., Kuhn, A. J., Schroder, W. H., Hildebrandt, U. and Bothe, H., Selective element deposits in maize colonized by a

heavy metal tolerance conferring arbuscular mycorrhizal fungus. *J. Plant Physiol.*, 1999, **154**, 718–728.

16. Marx, D. H., Mycorrhizae and the establishment of trees on strip-mined land. *Ohio J. Sci.*, 1975, **75**, 88–297.
17. Marx, D. H. and Altman, J. D., *Pisolithus tinctorius* ectomycorrhiza improve survival and growth of pine seedlings on acid coal spoil in Kentucky and Virginia. In *Mycorrhizal Manual*, Springer, Berlin, 1979, pp. 387–399.
18. Wilson, G. W. T., Hetrick, B. A. D. and Schwab, A. P., Reclamation effects on mycorrhizae and productive capacity of flue gas desulfurization sludge. *J. Environ. Qual.*, 1991, **20**, 777–783.
19. Koomen, I., McGrath, S. P. and Giller, K. E., Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. *Soil Biol. Biochem.*, 1990, **22**, 871–873.
20. Haselwandter, K. and Bowen, G. D., Mycorrhizal relations in trees for agroforestry and land rehabilitation. *For. Ecol. Manage.*, 1996, **81**, 1–17.
21. Pfleger, F. L., Stewart, E. L. and Noyd, R. K., Role of VAM fungi in mine land revegetation. In *Mycorrhizae and Plant Health* (eds Pfleger, F. L. and Linderman, R. G.), The American Phytopathological Society, MN, USA, 1994, pp. 47–82.
22. Sylvia, D. M. and Williams, S. E., Vesicular–arbuscular mycorrhizae and environmental stresses. In *Mycorrhizae in Sustainable Agriculture* (eds Bethlenfalvay, G. J. and Linderman, R. G.), ASA No. 54, Madison, USA, 1992, pp. 101–124.
23. Waaland, M. E. and Allen, E. B., Relationships between VA mycorrhizal fungi and plant cover following surface mining in Wyoming. *J. Range Manage.*, 1987, **40**, 271–276.
24. Dodd, J. C. and Thompson, B. D., The screening and selection of inoculant arbuscular mycorrhizal and ectomycorrhizal fungi. *Plant Soil.*, 1994, **159**, 149–158.
25. Adriano, D. C., *Trace Elements in Terrestrial Environments*, Springer, Berlin, 1986.
26. Marschner, H. and Romheld, V., Strategies of plants for acquisition of iron. *Plant Soil.*, 1995, **165**, 262–274.
27. Kroopnick, P. M., Vapor abatement cost analysis methodology for calculating life cycle costs for hydrocarbon vapor extracted during soil venting. In *Remediation of Hazardous Waste* (eds Wise, D. L. and Trantolo, D. J.), Marcel Dekker, New York, 1994, pp. 779–790.
28. Parker, R., Environmental Restoration Technologies. *EMIAA Yearbook*, 1994, pp. 169–171.
29. Williams, G. M., Integrated studies into ground water pollution by hazardous wastes. In *Land Disposal of Hazardous Waste, Engineering and Environmental Issues* (eds Gronow, J. R., Schofield, A. N. and Jain, R. K.), Horwood Ltd, Chichester, UK, 1988.
30. Elliott, H. A., Linn, J. H. and Shields, G. A., *Haz. Waste Haz. Mater.*, 1989, **6**, 223–228.
31. Zak, J. C. and Parkinson, D., Initial vesicular-arbuscular mycorrhizal development of the slender wheatgrass on two amended mine soils. *Can. J. Bot.*, 1982, **60**, 2241–2248.
32. Leyval, C., Turnau, K. and Haselwandter, K., Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza*, 1997, **7**, 139–153.
33. Pawlowska, T. B., Blaszkowski, J. and Rühling, A., The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza*, 1996, **6**, 499–505.
34. Joner, E. J. and Leyval, C., Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytol.*, 1997, **135**, 353–360.
35. Heggo, A., Angle, J. S. and Chaney, R. L., Effects of vesicular arbuscular mycorrhizal fungi on heavy-metal uptake by soybeans. *Soil Biol. Biochem.*, 1990, **22**, 865–869.
36. del Val, C., Barea, J. M. and Azcòn-Aguilar, C., Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Appl. Soil Ecol.*, 1999, **11**, 261–269.
37. Jamal, A., Ayub, N., Usman, M. and Khan, A. G., Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soyabean and lentil. *Int. J. Phytoremed.*, 2002, **4**, 205–221.
38. Zhu, Y. G., Christie, P. and Laidlaw, A. S., Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere*, 2001, **42**, 193–199.
39. Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S. and Hayes, W. J., Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 2000, **41**, 197–207.
40. Malcova, R., Vosátka, M. and Gryndler, M., Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L. *Appl. Soil Ecol.*, 2003, **23**, 55–67.
41. Chen, B., Christie, P. and Li, L., A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. *Chemosphere*, 2001, **42**, 185–192.
42. Feng, G., Song, Y. C., Li, X. L. and Christie, P., Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Appl. Soil Ecol.*, 2003, **22**, 139–148.
43. Taylor, J. and Harrier, L. A., A comparison of development and mineral nutrition of micropropagated *Fragaria* × *ananassa* cv. Elvira (strawberry) when colonized by nine species of arbuscular mycorrhizal fungi. *Appl. Soil Ecol.*, 2001, **18**, 205–215.
44. Auge, R. M., Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 2001, **11**, 3–42.
45. Kabir, Z. and Koide, R. T., The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. *Agric. Ecosyst. Environ.*, 2000, **78**, 167–174.
46. Medina, A., Probanza, A., Gutierrez Mañero, F. J. and Azcón, R., Interactions of arbuscular-mycorrhizal fungi and *Bacillus* strains and their effects on plant growth, microbial rhizosphere activity (thymidine and leucine incorporation) and fungal biomass (ergosterol and chitin). *Appl. Soil Ecol.*, 2003, **22**, 15–28.
47. Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R. and Barea, J. M., Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Appl. Soil Ecol.*, 2003, **22**, 29–37.
48. Gildon, A. and Tinker, P. B., A heavy metal-tolerant strain of a mycorrhizal fungus. *New Phytol.*, 1981, **95**, 263–268.
49. Gildon, A. and Tinker, P. B., Interactions of vesicular-arbuscular mycorrhiza infections and heavy metals in plants. II. The effects of infection on uptake of copper. *Trans. Br. Mycol. Soc.*, 1983, **77**, 648–649.
50. Weissenhorn, I., Leyval, C. and Berthelin, J., Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant Soil*, 1993, **157**, 247–256.
51. Sambandan, K., Kannan, K. and Raman, N., Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu. *J. Environ. Biol.*, 1992, **13**, 159–167.
52. Turnau, K., Ryszka, P., Gianinazzi-Pearson, V. and van Tuinen, D., Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza*, 2001, **10**, 169–174.
53. Griffioen, W. A. J., Iestwaart, J. H. and Ernst, W. H. O., Mycorrhizal infection of *Agrostis capillaris* population on a copper-contaminated soil. *Plant Soil*, 1994, **158**, 83–89.
54. Tonin, C., Vandenkoornhuyse, P., Joner, E. J., Straczek, J. and Leyval, C., Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza*, 2001, **10**, 161–168.
55. Baker, A. J. M., Accumulators and excluders – strategies in the response of plants to heavy metals. *J. Plant Nutr.*, 1981, **3**, 643–654.

56. Hildebrandt, U., Kaldorf, M. and Bothe, H., The zinc violet and its colonization by arbuscular mycorrhizal fungi. *J. Plant Physiol.*, 1999, **154**, 709–717.
57. Weissenhorn, I., Leyval, C. and Berthelin, J., Bioavailability of heavy metals and abundance of arbuscular mycorrhiza in a soil polluted by atmospheric deposition from a smelter. *Biol. Fertil. Soil*, 1995, **19**, 22–28.
58. Orlowska, E., Zubek, S., Jurkiewicz, A., Szarek-ukaszewska, G. and Turnau, K., Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. *Mycorrhiza*, 2002, **12**, 153–160.
59. del Val, C., Barea, J. M. and Azcòn-Aguilar, C., Diversity of arbuscular mycorrhizal fungus populations in heavy metal contaminated soils. *Appl. Environ. Microbiol.*, 1999, **99**, 718–723.
60. Leyval, C., Singh, B. R. and Joner, E. J., Occurrence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties. *Water Air Soil Pollut.*, 1995, **84**, 203–216.
61. Weissenhorn, I., Leyval, C., Belgy, G. and Berthelin, J., Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza*, 1995, **5**, 245–251.
62. Dueck, T. A., Visser, P. and Ernst, W. H. O., Vesicular-arbuscular mycorrhizae decrease zinc toxicity to grasses growing in zinc-polluted soil. *Soil Biol. Biochem.*, 1986, **18**, 331–333.
63. Tao, H. Q., Effect of arbuscular mycorrhiza on resistance of red clover to heavy metal Zn and Cd pollution, MS thesis, China Agricultural University, Beijing, 1997.
64. Li, X. L. and Christie, P., Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. *Chemosphere*, 2000, **42**, 201–207.
65. Joner, E. J., Leyval, C. and Briones, R., Metal binding capacity of arbuscular mycorrhizal mycelium. *Biol. Fertil. Soils*, 2000, **226**, 227–234.
66. Tullio, M., Pierandrei, F., Salerno, A. and Rea, E., Tolerance to cadmium of vesicular arbuscular mycorrhizae spores isolated from a cadmium-polluted and unpolluted soil. *Biol. Fertil. Soils*, 2003, **37**, 211–214.
67. Turnau, K., Kottke, I. and Oberwinkler, F., Element localization in mycorrhizal roots of *Pteridium aquilinum* L. Kuhn collected from experimental plots treated with cadmium dust. *New Phytol.*, 1993, **123**, 313–324.
68. Zhou, J. L., Zn biosorption by *Rhizopus arrhizus* and other fungi. *Appl. Microbiol. Biotechnol.*, 1999, **51**, 686–693.
69. Joner, E. J., Ravnskov, S. and Jakobsen, I., Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radiolabelled inorganic and organic phosphate. *Biotechnol. Lett.*, 2000, **22**, 1705–1708.
70. Li, X. L., George, E. and Marschner, H., Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New Phytol.*, 1991, **119**, 397–404.
71. Olsson, P. A., Francis, R., Read, D. J. and Söderström, B., Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids. *Plant Soil*, 1998, **201**, 9–16.
72. Laheurte, F., Leyval, C. and Berthelin, J., Root exudates of maize, pine and beech seedlings influenced by mycorrhizal and bacterial inoculation. *Symbiosis*, 1990, **9**, 111–116.
73. Turnau, K. and Mesjasz-Przybylowicz, J., Arbuscular mycorrhiza of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. *Mycorrhiza*, 2003, **13**, 185–190.
74. Schüepp, H., Dehn, B. and Sticher, H., Interaktionen zwischen VA-Mykorrhizen und Schwermetallbelastungen. *Angew. Bot.*, 1987, **61**, 85–96.
75. Shetty, K. G., Hetrick, B. A. and Schwab, A. P., Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environ. Pollut.*, 1995, **88**, 307–314.
76. Weissenhorn, I., Glashoff, A., Leyval, C. and Berthelin, J., Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. *Plant Soil*, 1994, **167**, 189–196.
77. Guo, Y., George, E. and Marschner, H., Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean and maize plants. *Plant Soil*, 1996, **184**, 195–205.
78. Weissenhorn, I., Mench, M. and Leyval, C., Bioavailability of heavy metals and arbuscular mycorrhiza in a sewage sludge amended sandy soil. *Soil Biol. Biochem.*, 1995, **27**, 287–296.
79. Joner, E. J. and Leyval, C., Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. *Biol. Fertil. Soils*, 2001, **33**, 351–357.
80. Liao, J. P., Lin, X. G., Cao, Z. H., Shi, Y. Q. and Wong, M. H., Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. *Chemosphere*, 2003, **50**, 847–853.
81. Diaz, G., Azcón-Aguilar, C. and Honrubia, M., Influence of arbuscular mycorrhiza on heavy metal (Zn and Pb) uptake and growth of *Lygedum spartum* and *Anthyllis cytisoides*. *Plant Soil*, 1996, **180**, 241–249.
82. Pearson, J. N. and Jakobsen, I., The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with ^{32}P and ^{33}P. *New Phytol.*, 1993, **124**, 489–494.

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