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Potential of arbuscular mycorrhizal fungi for bioremediation

C. Leyval[1], E. J. Joner[1], C. del Val[2] and K. Haselwandter[3]

[1] *Laboratoire des Interactions Microorganismes-Minéraux-Matière Organique dans les Sols, CNRS, F-54501 Vandoeuvre-les-Nancy, France*
[2] *Depto. Microbiologia del Suelo y Sistemas Simbioticos, Estacion Experimental del Zaidin, CSIC, Profesor Albareda 1, E-18008 Granada, Spain*
[3] *Universität Innsbruck, Institut für Mikrobiologie, Technikerstr. 25, A-6020 Innsbruck, Austria*

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Introduction

Bioremediation is the use of organisms for the treatment of soil pollution. Root colonizing symbiotic microorganisms such as arbuscular mycorrhizal fungi (AMF) are mainly involved in phytoremediation, that uses plants for soil remediation. Phytoremediation comprises a set of technologies that use various plants as a containment, destruction or extraction technique (EPA 2000). These techniques have received considerable interest in recent years because of potential cost savings compared to conventional non biological techniques. Different strategies of phytoremediation can be applied depending on the kind of pollutants. In all cases, vegetation reduces infiltration of water and erosion. Heavy metals cannot be degraded and can only be extracted (phytoextraction) from the soil or immobilized in a non toxic form (phytostabilization). AMF can help alleviate metal toxicity to plants by reducing metal translocation from root to shoot (Leyval et al. 1997). Therefore they may contribute to plant establishment and survival in heavy metal polluted sites and could be used as a complement to immobilization strategies. Phytoextraction mainly uses plants accumulating high concentrations of heavy metals, which can be harvested, discarded and even extracted to recover metals. For this purpose plants with various capacities for metal accumulation are used, like members of the *Brassicaceae*, which are generally considered non mycorrhizal, but also other accumulators producing higher biomass, which can be mycotrophic. Organic pollutants such as polycylic aromatic hydrocarbons (PAH) can be transformed or degraded through microbial activity, which is commonly enhanced in the root zone (rhizodegradation). It is not known whether this enhanced degradation in the rhizosphere is due to plant exudates including enzymes, surfactants, and other physical/chemical effects, and/or to increased microbial activity. Another possible mechanism for degradation of organic pollutants could be directly dependent on plant metabolism. However, this is not a quantitatively important route for PAH (Binet et al. 2000a), and will not be discussed here. AMF may be beneficial for PAH rhizodegradation because they affect root exudation and root associated microbial populations and because, in some ways, they act as an extension of the roots outside the rhizosphere. They may potentially also have a direct effect on PAH degradation. Finally, AMF can be used in bioassays of soil quality or soil toxicity, due to their sensitivity towards a range of soil pollutants. In this way, they could be used to show that adequate soil quality has been re-established after remediation.

The objective of this chapter is to review recent findings concerning the potential of AMF for bioremediation of soils polluted with heavy metals, radionuclides and PAH as representatives of organic pollutants. In each case, it will underline obstacles, and outline the perspectives for a successful application of AMF in phytoremediation strategies.

Potential of AMF for phytoremediation of heavy metal polluted soils

Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants (Birch and Bachofen 1990). AMF are among the most common soil microorganisms and constitute an important functional component of the soil-plant system occurring in almost all habitats and climates (Barea et al. 1997), including disturbed soils (McGonigle et al. 1996; Bundrett et al. 1996). Degraded soils do, however, suffer from changes in diversity and abundance of AM fungal populations (Koomen et al. 1990; Jasper et al. 1991; Loth 1996).

More specifically, it has been shown that AMF can be affected by heavy metal toxicity, but in many cases mycotrophic plants growing in soils contaminated with heavy metals are colonized by AMF (see e.g. review by Leyval et al. 1997). Many reports concerning this have quantified spores and estimated root colonization *in situ*. Others have gone further and described metal tolerant AMF in heavy metal polluted soils (Gildon and Tinker 1983; Weissenhorn et al. 1995; del Val 1999a; Hildebrandt et al. 1999).

In the last few years, research interest has focused on the diversity and tolerance of AMF in heavy metal contaminated soils trying to understand the basis underlying adaptation and tolerance of AMF to heavy metals in soils, since this could facilitate the management of these soil microorganisms, for restoration/bioremediation programs. Vandenkoornhuyse (1998) showed that AMF species diversity associated with maize plants in a long term field experiment did not differ between three plots that had received different levels of heavy metals-containing sewage sludge. However, the number of spores of each species was lower in the soil with the highest concentration of heavy metals. Using the same long-term field experiment and the same plant variety, but more acidic soils, del Val et al. (1999) found a reduction of number, but also of diversity of AMF spores in the soil receiving the highest rate of sludge.

On a highly polluted soil in northern France where only adapted plants can grow, *Arrhenaterum elatius* roots were collected along a gradient of heavy metal concentration. Up to three different *Glomus* species were identified inside *Arrhenaterum* roots, which differed along the gradient of metals (Leyval et al. unpublished data). The contribution of these AMF to plant tolerance to heavy metals or heavy metal accumulation by plants has not been established. Four *Glomus* species were also found in the rhizosphere of another metal tolerant plant, *Viola calaminaria*, growing on a soil highly contaminated with heavy metals (20,961 and 41 mg kg^{-1} Zn and Cd, respectively) (Tonin et al. 2001). Only one of these fungi colonized clover roots growing in pots supplemented with Cd and Zn salts. This *Glomus* sp. increased Cd and Zn concentrations in clover roots, but not in shoots, and did not affect plant growth. On the contrary, a *Glomus* isolate from the rhizosphere of *Viola calaminaria* increased the growth of maize and lucerne in heavy metal polluted soils and reduced Zn concentration in roots and shoots (Hildebrandt et al. 1999; Kaldorf et al. 1999).

Although AMF have been recovered from numerous metal enriched habitats, their role in plant interaction with toxic metals is not well understood. At high metal concentrations reports show variations in metal accumulation and inter-plant translocation depending on the fungi, host-plant, root density, soil characteristics, metals and their availability (El-Kerbawy et al. 1989; Leyval et al. 1997; Joner and Leyval 2001b). Large variations have also been found between AM fungal species due to differences in hyphal growth outside the rhizosphere (Bürkert and Robson 1994; Joner and Leyval 1997). Metal-tolerant AMF isolates can decrease metal concentration in shoots or in roots, or decrease translocation from root to shoots (Diaz et al. 1996; Joner and Leyval 1997; Joner and Leyval 2001b; del Val et al. unpublished data). The latter could be due to the high metal sorption capacity of these fungi, which could ‘filter’ metal ions during uptake (Joner et al. 2000). The high concentrations of heavy metals in the intracellular hyphae of a heavy metal tolerant AMF colonizing maize roots (Kaldorf et al. 1999) and in phosphate rich material in hyphal vacuoles of mycorrhizal roots of *Pteridium aquilinum* (Turnau et al. 1993) strengthen the hypothesis of a sequestration of metals by AMF structures. However, the competitivity of such metal tolerant AMF in the field is often unknown and should be investigated. Further, the potential benefit of a consortium of AMF, which corresponds to the situation in the roots, to improve phytoremediation, should be considered.

Phytoextraction studies often use hyperaccumulators (plants accumulating high concentrations of heavy metals, e.g. 1% Zn in their dry matter), which are in most cases non-mycotrophic plants belonging to the *Brassicaceae*. One objective is to use plants with high concentrations of heavy metals in shoots, which may limit the potential use of AM plants. However, many of these hyperaccumulating plants, such *Thlaspi caerulescens,* are small and grow slowly, which limits phytoextraction rates. Other accumulators producing a higher biomass, such as sunflower and willow, are now receiving attention and these are mycorrhizal plants. Highly productive crops associated with metal-tolerant AMF may therefore be considered for decontamination of slightly contaminated soils (Ernst 2000).

The use of non-mycorrhizal plants for phytoextraction such as *Thlaspi caerulescens* can also change the glomalean community and reduce the propagule number in soil (Pawlowska et al. 2000). In this situation, re-inoculation with AMF after phytoextraction would be a possible option. Using metal-accumulating plants able to form mycorrhizas instead of non-host-plants should also improve the establishment of a metal-intolerant vegetation.

Phytostabilization refers to promoting plant growth to reduce or eliminate the bioavailability of heavy metals and various grasses such as *Festuca rubra* and *Agrostis tenuis* have been used commercially (Salt et al. 1995; van Tichelen et al. 1996). Physical immobilization of heavy metals in soil is often performed using various amendments such as beringite combined with planting of metal tolerant species. Within this context, AMF can play an important role by improving plant establishment, promoting plant growth and reducing metal translocation to shoots. The beneficial effect of the mycorrhizal symbiosis has been shown in the facultative mycotroph *Festuca arundinacea* when grown in mine spoils (Hetrick et al. 1994). The possible contribution of AMF to the success of restoration programmes developed for polluted soils is also outlined by Turnau and Haselwandter (in this book).

Further studies should be carried out with plants susceptible of mycorrhizal colonization and already known for their capacity to translocate heavy metals. There are some reports which show that plant species, including maize and *Sonchus solearaceus*, can extract considerable amounts of lead from soil (Huang et al. 1996). Other species, like barley, which are also potentially mycorrhizal, are able to extract Zn as efficiently as can the non-mycorrhizal *Brassica juncea* (Ebbs and Kochian 1998).

Potential of AMF for bioremediation of radionuclides

It is well known that mycorrhizal infection affects the mineral nutrition of plants (George et al. 1994; Smith and Read 1997). Nevertheless, remarkably little research has been carried out on the effect of AMF on plant uptake of (radio)cesium. The few results obtained so far appear controversial (Haselwandter et al. 1994).

McGraw et al. (1979) reported that root infection of *Paspalum notatum* by two of ten species of AMF resulted in a twofold increase of the radioactivity in leaf tissue 48 h after injection of ^{134}Cs into soils. A study carried out by Rogers and Williams (1986) also indicated that some AMF may enhance the radiocesium uptake. They found that the ^{137}Cs concentration in mycorrhizal *Melilotus officinalis* was 2.0 and 1.7 times that in the non-AM clover at the first (65 d) and second (93 d) harvest, respectively. Additionally, in the case of

Sorghum sudanense, the radiocesium concentration was greater in the AM than in the non-AM grass, albeit not significantly.

In case the biosphere is subjected to contamination with radiocesium, one possible strategy involves remediation of contaminated areas by using plants that accumulate large amounts of Cs. Obviously, at least some AMF species have the capacity to enhance the radiocesium uptake by plants and, hence, the mycorrhizal symbiosis can be very useful. Based on the use of a plant species considered to be most efficient in ^{137}Cs phytoextraction and not mycorrhizal, Ebbs et al. (2000) have estimated to achieve a 75% decrease of ^{137}Cs contamination in approximately 15 years. It appears essential to compare such a potential with that of a mycorrhizal plant species.

The alternative strategy aims at preventing radioactive Cs isotopes from entering the food chain, and relies on crops that do not accumulate substantial amounts of radiocesium. With regard to this strategy it is of interest to note that AMF can also lead to a significant decrease in ^{137}Cs content e.g. of *Festuca ovina*. After three and five weeks of growth, shoot tissue radioactivity in the mycorrhizal grass was about half that of the non-mycorrhizal controls (Haselwandter and Berreck 1994). Similar results were obtained in pot experiments with *Agrostis tenuis*, in which AM colonisation by *Glomus mosseae* led to a significant decrease in the Cs uptake from the first (4 weeks) to the third (8 weeks) harvest (Berreck and Haselwandter 2001). The reduction in Cs content of mycorrhizal *versus* non-mycorrhizal plants was between 18 and 23%. In the study quoted above, the effect of K fertilization on Cs uptake by AMF was monitored over a growth period of 10 weeks. Potassium was applied at a rate recommended as one of the countermeasures after contamination of grassland with radiocesium (Konoplev et al. 1993; Segal 1993). Potassium addition resulted in a significant decrease of Cs uptake of both AMF and non-AMF grass. The fertilized non-AMF plants contained 56 to 78% of the Cs concentration of the non-fertilized plants, whereas fertilized AM plants contained 61 to 78% of the non-fertilized AM plants (Berreck and Haselwandter 2001). Thus the findings summarized above corroborate the hypothesis that soil fungi represent a potential pool of Cs immobilization (Dighton et al. 1991).

It is clear that AMF play a key role with regard to both of the strategies, be it soil remediation on basis of plants accumulating large amounts of radiocesium or the prevention of Cs from entry into the food chain. Hence, AMF deserve particular interest with regard to the development of countermeasures for controlling the radionuclide uptake by plants. As Cs^+ transport across the plasma membrane of root cells determines the potential of a plant either to accumulate or exclude radiocesium it was envisaged that genetic modifications would allow for the development of means to control the uptake rates (White and Broadley 2000). However, as almost all plants are normally associated with mycorrhizal fungi which, obviously, are at least as crucial for cesium uptake as the plant uptake system *per se*, it seems feasible to suggest exploitation of mycorrhizal potential first. Ernst (2000) expressed a similar view with regard to metal (hyper)accumulation by plants.

Role of AMF in PAH-polluted soils

Polycyclic aromatic hydrocarbons (PAH) are hydrophobic organic molecules consisting of two or more fused benzene rings. A selection of 16 PAH are commonly quantified for characterization and monitoring of these pollutants, while in fact 200–300 PAH compounds and their derivatives are commonly extracted and may putatively be identified in polluted soil samples. The concern for PAH pollution derives from their ubiquitous distribution, their recalcitrance towards degradation and their proven or suspected mutagenic properties (WHO 1983). The origin may partially be natural (organic residues after fire) or anthropogenic (mainly processing and incomplete combustion of fossile fuels). Thus, oil spills and industrial sites, e.g. for coke distillation etc., commonly give rise to extreme pollution events for which physical, chemical and biological remediation strategies are employed for clean-up. These include, among others, bio-venting, land-farming, bio-augmentation and phytoremediation. The latter is only applicable when pollution levels and physical conditions of the polluted matrix permit the establishment of plants, but offers a cost-effective and efficient treatment that simultaneously restores an ecosystem, limits erosion and improves the esthetical impression of a polluted site (Cunningham et al. 1996; Schwab and Banks 1994; Wilson and Jones 1993).

Arbuscular mycorrhiza fungi may play a role in two aspects of bioremediation of PAH: the establishment of a plant cover on polluted soil and modification of PAH degradation rates or pathways.

Improved plant establishment on fallowed, degraded or polluted soils, waste materials or soil-waste mixtures in the presence of AMF is well known (Reddell and Milnes 1992; Shetty et al. 1994,Thompson 1994; Leyval and Binet 1998). The major mechanism behind the success of AM under such conditions is an improvement of plant nutrient acquisition, with additional benefits due to improved water relations, pollutant tolerance and sequestration. The impact of the latter factors are often difficult to distinguish due to the confounding effect of plant nutrition on plant size, but, in the case of enhanced survival in the absence of competitors/predators, the role of AM may be clear. Identification of the symbiotic features that have permitted continued subsistence is however difficult, and such investigations are still in their infancy. One mechanism that may be involved is the oxidation of the contaminant by activated oxygen species and concomitant enhancement of oxidoreductases to protect the plant from oxidative stress. Indeed, enhanced levels of hydrogen peroxide in AM roots (Salzer et al. 1999) as well as enhanced levels of peroxidase activity in mycorrhizal roots and the rhizosphere (Criquet et al. 2000) has been demonstrated.

One peculiarity of PAH polluted soil that may be overcome by AM plants is the hydrophobicity and resulting limitations in uptake of water and waterdissolved inorganic nutrients (Leyval and Binet 1998). Again confounding effects of improved mineral nutrition prevents the distinction of mycorrhizal effects on uptake of water and mineral nutrients, but even if the effect is limited to mineral uptake it is of no less importance.

Effects of AMF on PAH degradation in the rhizosphere may be direct or indirect. As PAH are not absorbed by plants (Binet et al. 2000b; Schwab and Banks 1994) and are metabolized intracellularly, all degrading activity would take place in soil or inside soil organisms other than AM. Furthermore, AMF have poor saprophytic capacities, so the only probable direct effect of AMF on PAH degradation would be through enhanced production of extracellular peroxidases. Indirect effects would be due to changes in the microbial community e.g. due to stronger competition for mineral nutrients, direct antagonistic or synergistic effects of AMF, or changes in root exudation patterns, phenomena that are well documented.

Binet et al. (2000b) found no effect of AM on the dissipation on light compounds like anthracene (3 rings) in spiked soil on the short term (<40 days). Continued phytoremediation treatments of spiked soil resulted in an almost complete (98%) dissipation of anthracene after only 56 days in the rhizosphere of clover/ryegrass (Joner et al. unpublished data). Heavier compounds (4–6 rings) are more persistent and often rely on degradation by co-metabolism rather than direct metabolism (Cutright and Lee 1994; Perry 1979). The rhizospheric effect on dissipation of these compounds is thus more pronounced than for the lighter PAH (Binet et al. 2000a) as the root exudates may drive co-metablism. In addition, we have observed an enhanced dissipation of 4–5 ring PAH in mycorrhizal treatments compared to non-mycorrhizal treatments after prolonged periods (>100 days) of phytoremediation (Joner et al. submitted). Mycorrhizal effects on PAH dissipation was in this case accompanied by a modification in biomass partitioning (but not total plant biomass) between two co-occurring plant species (clover and ryegrass) (Joner and Leyval 2001a), as well as changes in soil microbial community structure. The effect of colonization by mycorrhizal fungi on the competitive ability of clover on PAH-polluted soil (Joner and Leyval 2001a) is perhaps similar to that observed in non-polluted soil (Bolan et al. 1987), i.e. a phenomenon linked to the improved nutrient acquisition of the mycotrophic legume. The modification of the soil microbial community may on the other hand either be due to inherent qualitative (Germida et al. 1998) or quantitative (Griffiths et al. 1999) differences in root exudation between the two plant species coupled with a proportional change in plant biomass (indirectly mediated by the mycorrhizal effect on competitive ability/plant size of the two plants), or more direct effects of AMF, i.e. alterations in root exudation as a consequence of AMF colonization (Graham et al. 1981; Laheurte et al. 1990) and direct antagonistic/synergistic effects between the AMF hyphae and the soil microflora (Linderman 1988; Linderman 1991). The exploitation of root-free soil by AMF hyphae has a potential for modifying microbial composition (Olsson et al. 1996; Ravnskov et al. 1999) and activity. Ephemeral hyphae serving as a source of C outside the rhizosphere (see e.g. Schreiner and Bethlenfalvay 1995) may ultimately result in a microbial community with an improved capacity for PAH degradation (Joner et al. submitted).

A limited number of AMF was used in the above cited experiments on phytoremediation of PAH. The selection of fungi as well as of associated host-plants for improving the dissipation of PAH should be performed. Since the half-life of most of the PAH is high, longer-term and field experiments should also be carried out.

Potential use of AMF in bioassays for soil pollution

Arbuscular mycorrhizas are not only an aid to ecosystem remediation. They should also be considered as a key indicator for soil pollution or soil quality because:

(i) mycorrhizal fungi are ubiquitous microorganisms,
(ii) many plants are highly dependant on mycorrhizas for their growth,
(iii) they provide a direct link between soil and roots, and
(iv) are involved in the transfer of elements including pollutants from soil to plants.

Furthermore, AMF can be affected by pollutants in soil and can be more sensitive to pollutants than plants (Weissenhorn and Leyval 1995).

The toxicity of compounds such as xenobiotics, PAH and heavy metals on AMF has been studied using techniques based on estimation of spore germination (Weissenhorn et Leyval 1996), mycorrhizal colonisation of roots in pot cultures using nested PCR (Jacquot et al. 2000), mycorrhizal infectivity (Leyval et al. 1995) and mycorrhizal colonization of Ri T-DNA transformed roots (Wan et al. 1998). AMF spore germination in soil can be used as an early indication of the toxicity towards the ecosystem, while mycorrhizal colonisation of roots indicates toxicity at a later stage of the association between symbiotic fungi and roots. AMF can be affected by pollutants but also by soil properties such as pH and phosphorus content (Leyval et al. 1995). The lack of specificity towards heavy metals or other pollutants is often the case for other indicator organisms such as earthworms, algae, fish and plants, which are used for the assessment of ecological risk due to toxic substances. AMF could be included as additional indicator organisms in the existing battery of bioassays. There is a need for a standardized technique using arbuscular mycorrhizas as a bioassay, which should be made easier since commercial AMF inoculum is now available (Feldmann and Grotkass in this book, von Alten et al. in this book).

Conclusions

Some of the limitations for phytoremediation are the contact between roots and the pollutants, the root growth rate, and the toxicity of some of the pollutants. In that respect, AM should be beneficial because they increase the volume of soil explored by roots, improve plant growth, and contribute to alleviate toxicity of pollutants such as heavy metals.

For bioremediation of heavy metal polluted soils, metal tolerant AMF have been identified. Promising results have been obtained under laboratory conditions and in short-term experiments, showing that AMF may contribute to the phytoextraction or phytostabilisation of heavy metals and radionuclides, depending on the plant-fungus partners. Since it is often difficult to extrapolate from laboratory experiments to field situations, longer-term and field experiments should be performed to confirm the potential benefit of AMF for phytoremediation. Improving the knowledge on competitivity and survival of introduced AMF, AMF community structure and dynamics *in situ,* genetic diversity and functioning after soil disturbance by heavy metals and/or the introduction of selected endophytes, may be necessary to overcome for making the application of AMF in phytoremediation programmes successful. Methods to identify and recognise particular AMF isolates *in situ* should also be further developed. Soil parameters such as pH and P content may be as crucial for mycorrhizal establishment and efficiency as heavy metal concentrations (Weissenhorn and Leyval 1996) and should be checked before the set-up of any field experiment.

The impact of AM on PAH polluted soil is still quite uncertain, and the few results that have been obtained need verification with a wider range of soil, plants and fungi. The potential, however, is substantial as both plant establishment, survival and degradation would independently confer large advantages, and in some cases even justify the substantial cost that a field inoculation may represent. AM implication in plant tolerance to PAH does not seem improbable, as these compounds are found naturally in soil e.g. after fire. Fire is a phenomenon that occurs relatively regularly in some ecosystems, and probably frequently enough to exert a selection pressure on fungal genera that largely propagate clonally, like the Glomales.

There is still a need to improve our understanding of the mechanisms involved in the transfer and immobilisation of heavy metals by AMF, and in their contribution to organic pollutant availability and degradation. This is necessary if we are to improve the chances of successful application under practical conditions, including application in soils where both kinds of pollutants are present.

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