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Role of arbuscular mycorrhizal (AM) fungi in phytoremediation of soils contaminated: A review

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ABSTRACT

Pollution of the soil environment with toxic materials from fossil burning, mining and smelting of metalliferous ores, disposal of sewage, fertilizers and pesticides, etc. has increased dramatically since the onset of industrial revolution. Application of plants with ability of absorbing heavy metals is a low-cost alternative for eliminating soils from heavy metals. Phytoremediation uses plants to remove pollutants from the environment. Arbuscular mycorrhizal Fungi provide an attractive system to advance plant based environmental clean-up. 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. This review highlights the potential of AM fungi for enhancing phytoremediation of heavy metal contaminated soils.

Key words: Arbuscular mycorrhizae; Heavy metal; Phytoremediation; Pollutants

INTRODUCTION

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriagu, 1979). The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, metallurgical industries, municipal wastes, fertilizers, pesticides, and sewage (Alloway, 1990). In addition to sites contaminated by human activity, natural mineral deposits containing particularly large quantities of heavy metals are present in many regions of the globe (Memon et al., 2001). Application of plants with ability of absorbing heavy metals is a low-cost alternative for eliminating soils from heavy metals. Application of plants for remediation of soils and waters contaminated with organic and mineral pollutants is called phytoremediation which is known as a new method for in-situ remediation of contaminated soils. Phytoremediation technology is using plants for replacement, transfer or stabilization of heavy metals in contaminated soils with low to average contamination in the area of root development. This technique originally was used for groundwater containing contaminated material and then it was applied on contaminated soils and air (Khan et al., 2000).

Recently it was demonstrated that phytoremediation can be enhanced by the use of appropriately selected microorganisms, such as mycorrhizal fungi (Hildebrandt et al., 1999). The fungi provide nutrients and water otherwise not accessible for plants (Cui and Nobel, 2006; Nadian et al., 1997;

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George et al., 1992) and facilitate the establishment and survival of vegetation under stress conditions (Smith et al., 1998; Jasper et al., 1989). The compounds produced by the extraradical mycelium can also take part in heavy metal chelation. According to the calculations by Söderström (1979), the surface of interaction between fungi and soil is up to 0.14 m2 in 1 g of soil. They can remove metals from the wastes both by metabolism dependent (bioaccumulation) or independent (biosorption) processes (Gadd, 1993).

According to the plant species and to the growing practices and conditions, mycorrhizae provide different benefits to the plants and to the environment (Kumar and Kumar, 2011):

► Increase yields and crop quality

► Reduce disease occurrence

► Enhance flowering and fruiting

► Increase plant establishment and survival at seedling or transplanting

► Produce more vigorous and healthy plants

► Improve drought tolerance, allowing watering reduction

► Optimize fertilizers use, especially phosphorus

► Increase tolerance to soil salinity

► Contribute to maintain soil quality and nutrient cycling

► Contribute to control soil erosion

Phytoremediation

In recent years, phytoaccumulation/phytoextraction, i.e., the use of plants to clean up soils contaminated with non-volatile hydrocarbons and immobile inorganics is showing promises as a new method for *in situ* cleanup of large volumes of low to moderately contaminated soils. Plants can be used to remove, transfer, stabilize and/or degrade heavy metal soil contaminants (Kling, 1997; Kumar et al., 1995). In the case of non-degradable pollutants such as heavy metals and metalloids, the precise terms covering the involved aspects of phytoremediation are rhizofiltration (metals in water), phytoextraction (metals in soil), phytovolatilization (metals that may be volatilized: e.g. Se and Hg) and phytostabilization (control of spread by erosion or leaching). When organic, biodegradable pollutants are the target, phytoremediation may comprise rhizodegradation (microbial degradation in the rhizosphere), phytodegradation (degradation of compounds absorbed by the plant), and hydraulic control (limiting the spread of a plume in soil by plant evapotranspiration) (EPA, 2000; Flathman et al., 1998).

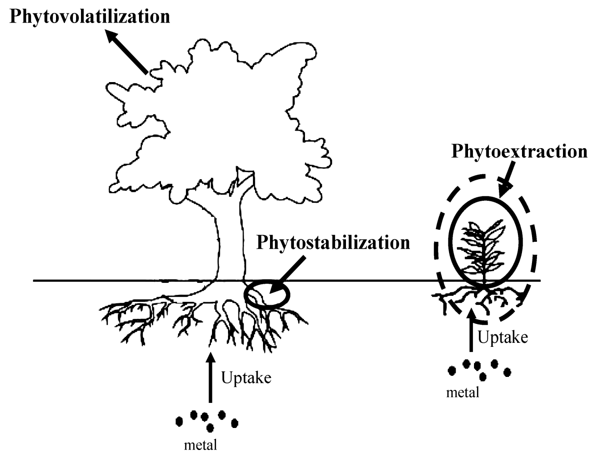

Figure 1. Types of soil phytoremediation (Sing et al., 2003).

Advantages and disadvantages of phytoremediation

Table 1 gives a summary of the advantages and disadvantages of phytoremediation.

Table 1. General advantages and disadvantages of phytoremediation (Raskin and Ensley, 2000)

Advantages	**Disadvantages**
Cost	***Time***
Low capital and operating cost	Slower compared to other techniques and seasonally dependent
Metal recycling provides further economic advantages	Most of the hyperaccumulators are slow growers
Performance	***Performance***
Permanent treatment solution	Not capable of 100% reduction
In situ application avoids excavation	May not be functional for all mixed wastes
Applicable to variety of contaminants	High contaminant concentration may be toxic to plants
Eliminate secondary air or water borne wastes	Soil phytoremediation is applicable only to surface soils
Other	***Other***
Public acceptance due to aesthetic reasons	Regulators are unfamiliar with this new technology
Compatible with risk-based remediation	Lack of recognized economic performance data
Can be used for site investigation or after closure	Groundwater and wastewater application requires large surface area

Mycorrhiza

Mycorrhizas are ubiquitous root-fungus symbioses that comprise three major groups: ectomycorrhizas (ECM: formed mainly by forest trees), ericoid mycorrhizas (formed by heather plants like the *Ericaceae*) and arbuscular mycorrhizas (AM: formed mainly by herbaceous plants) (Smith and Read, 1997). The major function of mycorrhizas is nutrient transport. Extra-radical hyphae anchored in the root thus exploit soil outside the root where it absorbs mineral nutrients (mainly N, P and micronutrients), translocate them back to the root, and transfer them to the host plant in exchange for phytosynthetically fixed C in the form of sugars. The fact that these hyphae are fed with C and energy from the host plant gives them an advantage over other microorganisms with respect to growth and active metabolism in nutrient-poor substrates. In a biodegradation context, it is important to note that the three groups of mycorrhiza have very different saprophytic capacities. The ericoid mycorrhizal fungi are potent degraders, ECM fungi are moderately capable, while AM fungi are obligate symbionts with little or no capacity for degradation of organic materials (Michelsen et al., 1998; Michelsen et al., 1996). All groups of mycorrhiza do, however, interact with and modify the microbial communities that the hyphae encounter in soil, and in this manner they may all affect microbial degradation processes indirectly. Mycorrhiza transport water and mineral nutrients from the soil to the plant while the fungus is benefiting from the C compounds provided by the host (Figure 2).

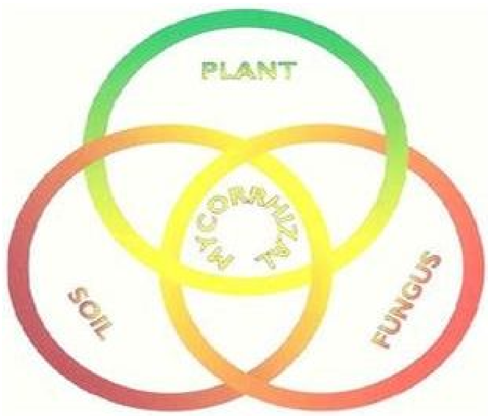

Figure 2. Mycorrhizal association, showing the interactions between fungus, plant and soil (Brundrett et al., 1996).

Significance of arbuscular mycorrhizae fungi (AMF)

Arbuscular mycorrhizae associations are important in natural and managed ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders (Xavier et al., 2002). AMF are known to improve plant growth and health by improving mineral nutrition (Table 2), or increasing resistance or tolerance to biotic and abiotic stresses (Clark et al., 2000). AMF modify the quality and abundance of rhizosphere microflora and alter overall rhizosphere microbial activity. Following host root colonization, the AMF induces changes in the host root exudation pattern, which alters the microbial equilibrium in the mycorrhizosphere. Their potential role in phytoremediation of heavy metal contaminated soils and water is also becoming evident (Jamal et al., 2002; Chaudhry et al., 1998).

Table 2. Positive effects of AMF in nutrient's absorption

Nutrient	References
Phosphorus	Chandreshekara et al., (1995); Harley and Smith (1983)
Nitrogen	Liu et al., (2002)
Potassium	Liu et al., (2002)
Magnesium	Gildon and Tinker (1983)
Copper	Gildon and Tinker (1983)
Zinc	Jamal et al., (2002)
Calcium	Liu et al., (2002)
Iron	Caris et al., (1998)
Cadmium	Gonzalez et al., (2002); Guo et al., (1996)
Nickel	Jamal et al., (2002); Guo et al., (1996)
Uranium	Rufyikiri et al., (2002)

Role of arbuscular mycorrhizae in phytoremediation

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. 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 (Leyval et al., 1997). The influence of AMF on metal plant uptake depends on many factors such: "fungal genotype, uptake of metal by plant *via* AM symbiosis, root length density, competition between AMF communities, the rhizosphere (pH, CEC, etc.), the metal itself, concentrations of available metals, soil contamination conditions (contaminated or artificially contaminated vs non-contaminated soil, interactions between P and metals (addition of P fertilizers), experimental conditions (light intensity, plant growth stage, available N and P), litter inputs, plant species and plant size" (Giasson et al., 2008). According to Gadd (1993), both live and dead components of the fungal cell wall can be involved in HM binding with help of free amino, hydroxyl, carboxyl and other groups. AMF form extraradical mycelium and intraradical hyphae that penetrate the intercellular spaces and enter cortical root cells. In the case of reduction of HM uptake, an important role in retention, binding and immobilization seems to be associated to *fungal vacuoles*. They are involved in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metal ions. The fungal cell wall, respectively chitin and glomalin from the fungal wall (Christie et al. 2004), are also important due to the presence of free amino, hydroxyl, carboxyl and other functional groups (Gadd, 1993).

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 (Del Val et al., 1999; Weissenhorn and Leyval, 1995). Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition (Figure 3). The extramatrical fungal hyphae can extend several cm into the soil and uptake large amounts of nutrients, including heavy metals, to the host root. The effectiveness of AM root colonization in terms of nutrient acquisition differs markedly between AM fungi and host plant genotype (Ahiabor and Hirata, 1995; Marschner, 1995). Mycorrhizae have also been reported in plants growing on heavy metal contaminated sites (Chaudhry et al., 1998; Shetty et al., 1995) indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the site. Noyd et al. (1996) reported that AM fungal infectivity of native prairie grasses increased over three seasons on a coarse taconite iron ore tailing plots which helped to establish a sustainable native grass community that will meet reclamation goals. The reported symbiotic associations in the plants colonizing heavy metal contaminated soils further suggests a selective advantage for these plants as pioneering species on such sites and that they may be largely responsible for the successful colonization of such habitats. Also, Gali et al. (1994) suggested that mycorrhizae can play a crucial role in protecting plant roots from heavy metals. The efficiency of protection, however, differs between distinct isolates of mycorrhizal fungi and different heavy metals.

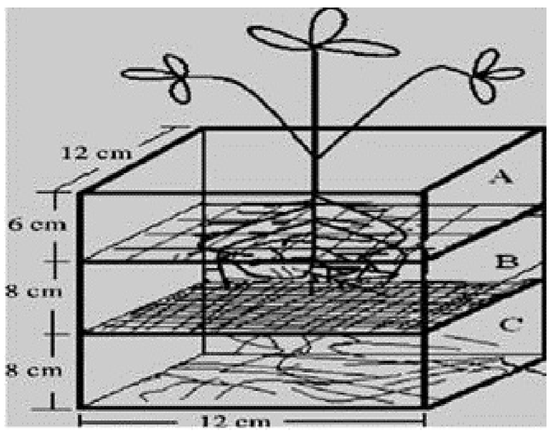

Figure 3. Area covered by mycorrhizal fungi hyphae. This figure shows how mycorrhizal fungi increase the surface area of plant roots and thus help in remediation. The ordinary plant root did not go farther than compartment B; however the fungi hyphae extended into compartment C (Adapted from Gao et al., 2010).

Particular effects of AM fungi on different HM

The alleviation of **Zn** toxicity towards plants by using AMF was reported in Christie et al. (2004) and Chen et al. (2004), and this phenomenon was shown to be dependent on direct and indirect mechanisms. As an example for a direct mechanism, Zn was bound in mycorrhizal structures and immobilized in mycorrhizosphere, while for an indirect effect, an influence of mycorrhiza on the plant's mineral nutrition, especially for P, lead to increased plant growth and enhanced metal tolerance. The mobility of Zn is greatly affected by the changes in soil pH. The Zn immobilization through the fungal activity might be an effect of these changes, contributing to the inhibition of Zn uptake into the mycorrhizal plant by storage in the arbuscles, but also in hyphae (Christie et al., 2004). In highly contaminated soil, Zn was found in higher concentration in roots while a decrease in the shoots was seen as effect of AMF. When Zn amounts in soil increased, a critical threshold exists, below which Zn uptake is enhanced, while above this level Zn translocation to the above-ground parts of host plants is inhibited. In some plant species, higher translocation rates may occur, but at the cost of poor plant biomass development and probable early death of the individuals (Chen et al., 2005). Turnau (1998) studied the localization of heavy metals within the fungal mycelium and mycorrhizal roots of *Euphorbia cyparissias* from Zn contaminated wastes and found higher concentrations of Zn as crystaloids deposited within the fungal mycelium and cortical cells of mycorrhizal roots.

Studies related by Rufyikiri at al. (2004) demonstrated that the mobility of **U** in soil depends on the organic compound content, the bioavailability being highly dependent on soil pH. The same author found that the most mobile U forms are U(VI) salts, predominantly as $UO2^{2+}$ and carbonate complexes, while other forms are less bioavailable and remain bound to soil particles. The role of AM fungi in translocating U as uranyl cations to roots through fungal tissues is related to fungal mycelium HM binding capacity (Chen et al., 2005). Chen *et al.* (2005 cited by Babula at al., 2008) performed and confirmed such studies using *Medicago trunculata* as a model plant, inoculated with *Glomus intraradices.* They found higher concentrations of U in roots than in shoots of mycorrhizal plant, suggesting that the AM fungus has a potential to reduce the translocation of U from roots to shoots.

Yu et al. (2010) reported that in the case of **Hg,** the uptake is lower by mycorrhyzal than by non-mycorrhyzal roots of maize, and AMF inoculation significantly decreased the total and extractable Hg concentrations in soil as well as the ratio of extractable to total Hg. Calculating mass balances for Hg in soil indicated a loss of Hg which can be attributed to Hg volatilization as a result of AMF influence. No significant difference of Hg concentrations was found between mycorrhizal and non-mycorrhizal shoots of maize which suggest that contribution of root uptake to shoot accumulation of Hg is very limited. The release of Hg into soil gases or into the atmosphere is a result of methylation (CH_3Hg^+), which leads to phytovolatilization, seen also with As and other metalloids.

Some research has been carried out on **Cs**, with, e.g., Leyval et al. (2002) reporting that ^{134}Cs radioactivity increased twofold in leaf tissue of *Paspalum notatum* in symbiosis with AMF while, in the case of mycorrhizal *Mellilotus officinalis* 1.7 to 2 times increased ^{137}Cs was found. *Sorgum Sudanese* revealed only insignificantly increase. A significant decrease of ^{137}Cs in mycorrhyzal *Festuca ovina* and *Agrostis tenuis* was found; this finding underlining that soil fungi represent a potential for Cs immobilization. On the other hand, Rosén et al. (2005) working with mycorrhizal ryegrass and leek found an enhanced ^{137}Cs uptake by leek, but no effect on the uptake by ryegrass. Similar studies were performed on mycorrhizal *Festuca ovina* in which shoots showed higher ^{137}Cs concentration than roots, as well as on *Trifolium repens,* and AM plants took up less Cs with no increase in translocation of ^{137}Cs to the shoots being found. In conclusion, AMF seems to play a role, with regard of both immobilization and phytoextraction being represented depending on plant species. Specifically grasses seem to respond with decreased uptake into shoot biomass.

CONCLUSION

Phytoremediation is a newly emerging as a biobased and low cost, alternative technology in the cleanup of contaminated soils. Arbuscular mycorrhizal fungi provide an attractive system to advance plant-based environmental clean-up. During symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up HM from an enlarged soil volume. The efficiency of this method of

Phytoremediation depends on the species and origin of the fungi used, the type of plant colonized, and the type and concentration of the pollutants. However, more research is needed in order to harness the benefits of this method of soil phytoremediation.

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