Review

Arbuscular mycorrhizal fungi and heavy metal contaminated soils

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Arbuscular mycorrhizal fungi (AMF) 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 (HMs). They are reported to be present on the roots of plants growing on HM-contaminated soils and play an important role in metal tolerance and accumulation. Isolation of the indigenous and presumably stress-adapted AMF can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems. Plants grown in metal contaminated sites harbour unique metal tolerant and resistant microbial communities in their rhizosphere. These rhizo-microflora secrete plant growth-promoting substances, siderophores, phytochelators to alleviate metal toxicity, enhance the bioavailability of metals (phytoremediation) and complexation of metals (phytostabilisation). Most studies of HM toxicity to soil microorganisms have concentrated on effect where loss of microbial function can be observed and the effects on biodiversity within microbial populations and communities have not significantly been evaluated. This review highlights the interaction between HM-contaminated soils and AMF.

Key words: Activity, arbuscular mycorrhizal fungi, biodiversity, functionality, heavy metal, soil contamination.

INTRODUCTION

Heavy metals (HMs) occur naturally in the environment and constitute a potential hazard for waters, soils, plants and sediments. Numerous studies have indicated that agroecosystems receive inputs of HMs from the increased use of agrochemicals, the application of metal-containing wastes such as sewage sludge, pig manure, coal and wood ashes to soils, and from atmospheric deposition (Mhatre and Pankhurst, 1997). Although some of these metals are essential plant micronutrients and are required or are beneficial for plant growth and develop-ment (Zn, Cu, Fe, Mn, Ni, Mo, Co), high contents and/or long-term presence of HMs, in soils, are generally considered a matter of concern to society as they may adversely affect the quality of soil and water, and com-promise sustainable food production (Pandolfini et al., 1997; Keller et al., 2002; Voegelin et al., 2003; Kabata-Pendias and Mukherjee, 2007). Therefore in recent

years, increasing attention has been paid to the remediation of polluted soils, among which the use of plants and microbes to remove hazardous metals ions is particularly emphasized. Phytoremediation is the use of plants to remove pollutants from environment, while microremediation refers to the use of microbes. These two approaches are preferred to chemical/physical remediation, because of their cost-effectiveness, environmental friendliness and fewer side effects.

Phytoremediation cannot be done alone by the plant, just as there is always a close interaction between the microorganisms in the rhizosphere and the plant which leads to an increased activity related to soil remediation (Compant et al., 2010). Overall a searching for and application of hyperaccumulating plants in combination with a beneficial rhizo- and/or endo-spheric microbial community holds great promise for low cost cleaning of contaminated sites.

Arbuscular mycorrhizal fungi (AMF) are one of the important endophytic fungi living in the roots of most terrestrial plants. This symbiosis confers benefits directly

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to the host plant's growth and development through the acquisition of phosphorous and other mineral nutrients from the soil by the fungus. In addition, they may also enhance the plant's resistance to biotic and abiotic stresses (Harrier and Sawczak, 2000). Potential roles of AMF associations have repeatedly been demonstrated to alleviate metal stress of plants (Hildebrandt et al., 2006). Poor or absent mycorrhizal inoculum were found in some of mine soils, which could explain the lack of mycorrhizal colonization. However, mycorrhizal rather than non-host plants could colonize polluted mining sites, suggesting that HM tolerance or other beneficial effects were conferred by mycorrhizal symbiosis. Besides AMF, there are other beneficial microorganisms that may contribute to the plants' tolerance to HM-contamination. Plant growth-promoting rhizobacteria (PGPR) improves plant growth through several mechanisms, such as increased nutrient uptake, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances, phytohormone production and nitrogen fixation. Although PGPR was first used for promoting the plant growth, much attention has recently been paid on the application of PGPR to remediate contaminated soils in association with plants (Huang et al., 2004b, 2005; Narasimhan et al., 2003). Nowadays, it has been shown that improvement of the interactions between beneficial rhizosphere microorganisms and plants can significantly lower the stress placed on plants by the presence of HMs, increase the availability of metal for plant uptake and subsequently are considered to be an important tool for phytoremediation technology (Glick, 2003, 2010). For example, AMF could enhance uptake of nutrient elements as well as water by host plants through their extraradical mycelial networks and protecting the host plants against HM toxicity (Leyval et al., 1997).

This AMF-induced plant nutrient uptake is of more importance in alkaline and/or calcareous soils of arid-semiarid regions in which the bioavailability of P and most of the cationic micronutrients is limited. Calcareous soils have also lower water holding capacity due to the presence of carbonates (Khodaverdiloo et al., 2011). Furthermore, Khodaverdiloo and Homaee (2008) and Davari et al. (2010) reported a significant reduction in plant transpiration with an increase in soil HM concentration. It has been suggested that heavy metals, such as Cd, can affect root hydraulic conductivity by multiple mechanisms operating on the apoplastic and/or the symplastic pathway (Shah et al., 2010). Recently, the ability of microorganisms to improve the growth of plants including canola and tomato seedlings treated with toxic concentrations of As, Cd, Ni, Pb, Se and Zn has been demonstrated (Burd et al., 1998; 2000; Belimov et al., 2005; Stearn et al., 2005).

The aims of this paper were to review

(i) The effect of HMs-contamination on microbial

population and their activity with an emphasis on AMF,
(ii) Tolerance and adaptation of AMF to HMs,
(iii) The potential benefits of AMF in phytoremediation of HM-contaminated soils.

EFFECT OF HEAVY METAL CONTAMINATION ON SOIL MICROBIAL POPULATION AND THEIR ACTIVITY

Microorganisms in the soil are responsible for nitrogen fixation, assimilation, and degradation of organic residues to release nutrients (Baath, 1989; Brookes, 1995). When HMs are retained in the soil by repeated and uncontrolled additions, they interfere with these key biochemical processes which alter ecological balance. Toxic effects of HMs on microorganisms manifests in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling (Baath, 1989), impaired enzyme synthesis and activity in soil, sediments and water. Due to their relation to soil functionality, the soil microbial population and activity have been proposed as useful indicators of soil improvement and soil degradation. In addition, soil enzyme activities are considered as sensitive and early indicators of both natural and anthropogenic disturbances (Giller et al., 1998). Besides the soil microbial function, molecular fingerprinting methods may provide a qualitative and quantitative measure of microbial diversity and community com- position in undisturbed and contaminated soils (White et al., 1998) as they reflect the status of the microbial gene pool in the investigated systems (Head et al., 1998; Van Elsas et al., 1998). Therefore, the combination of more traditional soil biochemical methods with more recent molecular techniques can provide valuable information about the microbiological status of the soil.

The soil microbial community is thought to be a sensitive bioindicator of metal pollution effects on bio-availability and biogeochemical processes (Hinojosa et al., 2005). It has been shown that HMs at certain concentrations can have long-term toxic effects within ecosystems and have a clear negative influence on biologically mediated soil processes (Lee et al., 2002). It is generally accepted that accumulation of HMs in soil reduce the amount of soil microbial biomass (Brookes and McGrath, 1984; Chander et al., 1995) and various enzyme activities, leading to a decrease in the functional diversity in the soil ecosystem and changes in the microbial community structure (Frostegard et al., 1993). However, metal exposure may also lead to the development of metal tolerant microbial populations (Ellis et al., 2003). These metal-tolerant microbial populations could potentially be used as plant helping agents in phytoremdiation of HM-contaminated environments.

Zarei et al. (2010) studied the abundance and diversity of AMF associated with dominant plant species along a transect from highly lead (Pb) and zinc (Zn) polluted to non-polluted soil at the Anguran open pit mine in Iran.

Using an established primer set for AMF in the internal transcribed spacer (ITS) region of rDNA, nine different AMF sequence types were distinguished after phylogenetic analyses, showing remarkable differences in their distribution patterns along the transect. With decreasing Pb and Zn concentration, the number of AMF sequence types increased, however one sequence type was only found in the highly contaminated area.

Morphological spore identification to the genus level also suggested that there was a reduced AMF species diversity in highly HMs-contaminated plots. To test this hypothesis, the AMF diversity in the roots of the indigenous plant species *Veronica rechingeri* was assessed by Zarei et al. (2008) in four selected plots along the same transect using molecular DNA based markers for AMF sequence type identification. The results of this study clearly showed a decline in the number of AMF sequence types colonizing the roots of *V. rechingeri*, ranging from six types in uncontaminated plots to only two types in highly HM-contaminated plots. The AMF distribution data also revealed AMF sequence types that are adapted to high HM concentrations.

AMF TOLERANCE AND ADAPTATION TO HEAVY METALS

The literature presents a range of "classic" ecological principles explaining the processes that increase the tolerance or resistance of a community (Boivin et al., 2002). Resistance refers to the ability of microorganisms to withstand the effects of a pollutant usually effective against them, while tolerance refers to the ability of microorganisms to adapt to the persistent presence of the pollutant. As stated by Leyval and Joner (2001), tolerance and resistance to the toxic effect of HMs depends upon the mechanism involved. Briefly, as mentioned in epidemiological studies (Foster and Hall, 1990; Tosun and Gönül, 2005), metal tolerance could be defined as a phenomenon by which microorganisms increase resistance towards stress resulting from exposure to HM toxicity.

Metal tolerance of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi have been assessed using several observation methods including: AM spore numbers, root colonization and the abundance of ECM fruiting bodies (Weissenhorn et al., 1993, 1994; Del Val et al., 1999). Unfortunately, such methods did not give information concerning conditions, limitations and threshold values ensuring the survival and growth of AMF, or about the genetic basis for multi-metal resistance and tolerance. Moreover, AMF coexist with other microbial communities and plant roots that can tolerate and accumulate metals, and this could confound the real interactions between AMF and metals in the medium.

More recently, to evaluate the tolerance of microorganisms in soils polluted with metals, specialists have adopted the concept of pollution-induced community tolerance (PICT) (Nikli ska et al., 2006). This perspective stipulates that with time, in an ecosystem, contamination exposure increases tolerance in microbial communities. Davis et al. (2002) used the PICT method to assess the effects of long-term exposure to Zn on the metabolic diversity and tolerance to Zn of soil microbial community. They showed that long-term exposure to Zn imposes stress on soil microbes, resulting in an increased tolerance. They concluded that the long-term accumulation of Zn in soils provides the microbial community with time to adapt to this metal. Indeed, microbial communities are often found to recover after an initial inhibition by high metal inputs (Holtan-Hartwik et al., 2002). This adaptation has been attributed to two factors (Almås et al., 2004). The first one is a gradual decrease in metal availability due to immobilization reactions occurring in the rhizosphere. The other factor is a gradual change in microbial community structure, based on changes in phospholipid fatty acid profiles (Frostegård et al., 1993) which results in more tolerant organisms.

Although metals may induce changes in the microbial community, resulting in microorganisms more resistant to metals (Almås et al., 2004), most essential and non essential metals exhibit toxicity above a certain concentration. This toxicity stress, appreciated by a threshold value (Leyval and Joner, 2001), will vary depending on many factors including the type of microorganism, the physico-chemical properties and concentration of the metal, and the edaphic and environmental conditions (Gadd, 1993).

Even though metals can exhibit a range of toxicities toward soil microorganisms (McGrath, 1994; McGrath et al., 1995; Giller et al.; 1998; Dai et al., 2004; Nikli ska et al., 2006), AMF isolates, particularly the ecotypes living in metal-enriched soils, metalliferous sites and mine spoils heavily polluted with metals, can, depending on intrinsic and extrinsic factors, tolerate and accumulate HMs (Gildon and Tinker, 1981; Weissenhorn et al., 1993, 1994; Joner and Leyval, 1997; Leyval et al., 1997; Smith and Read, 1997). Field investigations have indicated that AMF can colonize plant in metal contaminated sites (Díaz and Honrubia, 1993; Pawlowska et al., 1996) and in agricultural soils contaminated with metals of different origins, including atmospheric deposition from smelter and sludge amendments. Mycorrhizal fungi have also been shown to be associated with metallophyte plants on highly polluted soils. Nevertheless, it should be kept in mind that in some extreme metal conditions, AMF inoculation can be entirely inhibited (Weissenhorn et al., 1994). Del Val et al. (1999) reported that spore numbers decreased with the increasing amounts of HMs, whereas specie richness and diversity increased in soils receiving an intermediate rate of sludge contamination but decreased in soils receiving the highest rate of HM-contaminated sludge.

Several reports and reviews suggested that AMF from

metal-contaminated sites have developed tolerance against metal toxicity and are well adapted (Weissenhorn et al., 1993, 1994; Leyval and Joner, 2001; Toler et al., 2005; Sudova et al., 2007). The evolution of metal tolerance is showed to be rapid in MF. As stated by Sudova et al. (2007), tolerant strains of some MF may develop within one or two years (Weissenhorn et al., 1994; Tullio et al., 2003). Gonzalez-Chavez et al. (2002a, b) reported that AMF have evolved arsenate resistance and conferred enhanced resistance on Holocus lanatus. Heavy metal concentration may decrease the numbers and vitality of AMF as a result of HM toxicity (Dixon, 1988) or may have no effect on mycorrhizal colonization (Leyval et al., 1997). Biró et al. (2005) studied the stress buffer effect of the AMF and their colonization behaviour in metal spiked soil on a long-term level in controlled conditions. The soils used were collected after a 12 year metal-adaptation process, where 13 trace element salts, such as Al, As, Ba, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sr and Zn were applied in four gradients (0, 30, 90 and 270 mg/kg dry soil). Barley (*Hordeum vulgare L.*) was used as a test plant. They found a strong dose dependency at the arbuscular richness in general. The sporulation of the AMF was found as the most sensitive parameter to long-term metal(loid) stress. They reported that Al, As, Ba, Cd, Cr, Cu, Pb, Se, Sr and Zn reduced significantly the spore-numbers of the AMF, while the Ni loadings (at 36 g/soil) increased mycorrhizal sporulation.

At present, potential interaction mechanisms between AMF and metals, and the cellular and molecular mechanisms of HM tolerance in AMF, are poorly understood (Leyval and Joner, 2001; Martin et al., 2007). Metal transporters and plant-encoded transporters are involved in the tolerance and uptake of HMs (Göhre and Paszkowski, 2006; Hildebrandt et al., 2007) from extra-cellular media, or in their mobilization from intracellular stores (Gaither and Eide, 2001). Göhre and Paszkowski (2006) hypothesized that metals could be released at the pre-arbuscular interface and then taken up by plant-encoded transporters.

ROLE OF AMF IN PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOILS

In nature, some plants hyperaccumulate HMs. Although hyperaccumulator plants are widely used in phytoex-traction, they are generally of low biomass, inconvenient for phytoremediation of HM-contaminated soils. In other words, phytoremediation usually is time consuming, mostly as a result of low bioavailability of HMs in the soil environment and/or low biomass of hyperaccumulators (Khodaverdiloo and Homaee, 2008) . Mobility and bioavailability of HMs and therefore their possible phytotoxicity is strongly affected by affinity of the soil for sorption of a given metal, which in turn depends on soil properties (Khodaverdiloo and Samadi, 2011). Low bioavailability of cationic HMs are of most

importance in the young, low-developed calcareous soils of arid/semi-arid regions that have high affinity to irrever-sibly sorb HMs, most likely, through metal-carbonate precipitation (Khodaverdiloo and Samadi, 2011). Numerous studies have indicated that AMF can decrease the metal uptake of the host plants, thus protecting them against HMs toxicity (Leyval et al., 1997; Zaidi and Musarrat, 2004).

The number of spores and subsequently root coloniza-tion of host plants are often reduced by soil disturbance (Waaland and Allen, 1987). However, AMF species adapted to local soil conditions could be able to stimulate plant growth better than non-indigenous species. Indi-genous AMF ecotypes result from long-term adaptation to soils with extreme properties (Sylvia and Williams, 1992; Bae et al., 2003; Rahmanian et al., 2011). Therefore, inoculation of plants with indigenous and presumably stress-adopted AMF can be a potential biotechnological tool for successful restoration of degraded ecosystems (Dodd and Thompson, 1994; Mathur et al., 2007; Rahmanian et al., 2011).

Lasat (2002) observed that the effect of AMF asso-ciations on metal root uptake appears to be metal and plant specific. Greater root length densities and presumably more hyphae enable plants to explore a larger soil volume thus increasing access to cations (metals) not available to non-mycorrhizal plants (Mohammad et al., 1995).

Rahmanian et al. (2011) showed that the introduction of HM-resistant microbes caused a significant decline in plant biomass. They attributed his reduction to the increased access of plants to the relatively immobile Cd existed in the soil and also to more metal contaminants absorption caused by the microbes.

Gharemaleki et al. (2010a, b) reported that co-inoculation of plant growth- promoting rhizobacteria (PGPR) and AMF resulted in increasing Cd and Zn uptake and their accumulation by corn (*Zea mays*) with comparison to sterile condition. However, in the given level of soil contamination, the plant accumulated higher Cd and Zn in PGPR inoculated soil rather than AMF inoculated soil. They concluded that in plant-microorganism system for co-remediation of Cd and Zn, PGPR was effective than AMF.

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