Chapter 4

ARBUSCULAR MYCORRHIZAE AND ALLEVIATION OF SOIL STRESSES ON PLANT GROWTH

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Abstract: Within the last decade, inventories of the soil's productive capacity indicate severe degradation and loss of arable lands as a result of soil erosion, cultivation, salinization, over-grazing, land clearing, desertification, soil pollution, and atmospheric pollution. Large areas of land have been, and continue to be, contaminated by trace metals, and petroleum hydrocarbons. Many technologies using physical and chemical treatment methods have been developed to remediate contaminated soils. Recently, phytoremediation has been thought to provide an environmentally friendly alternative for the treatment of polluted soils. In phytoremediation of metal-contaminated soils, bioavailability and metal uptake are important factors. Among soil-plant factors controlling metal uptake, the rhizosphere flora is known to play a special role in the phyto-availability of trace elements. In this regard, arbuscular mycorrhizal fungi (AMF), which are among the most common components of soil rhizosphere flora, is of great interest to soil and environmental scientists, from a phyto-remediation and an environmental standpoint. AMF play important roles in the restoration of contaminated ecosystems and are increasingly used in many countries to improve plant nutrition and fertility of degraded land. As AMF are becoming commercially available, their use will also provide further avenues for reducing pollution from agriculture. This chapter reviews the role, the importance, and the application of AMF in ecologically remediating contaminated soils (mycorrhizoremediation). Emphasis is given to the effects of AMF on growth and yield, and on the uptake of trace metals by plants (rhizo-availability) from agricultural and metal-contaminated soils. The chapter also addresses the AMF's potential for improving or sustaining soil fertility.

Keywords: Arbuscular mycorrhizal fungi; nutrient availability; mycorrhizoremediation; rhizoextraction; metal pollution; heavy metals.

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1 INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are important soil micro-organisms (Liu and Lianfeng, 2008) that play a key role in facilitating nutrient uptake by crops in a variety of agroecosystems, particularly in low-input farming systems, and in revegetation and rhizomeredіation processes (Barea and Jeffries, 1995; Barea *et al.*, 2002; Atkinson *et al.*, 2002; Lombi *et al.*, 2001; Gadd, 2005; Jansa *et al.*, 2008). Many studies in glasshouse and fields have assessed the positive effects of AMF on plant uptake, and plant growth and yield. Enhancing the mycorrhizal system of a low-fertility or degraded soil helps the root system acquire more nutrients (Roesti *et al.*, 2005). It is widely acknowledged that AMF play an important role in improving the uptake of low mobile ions, in phosphate (PO_4^{3-}) and in ammonium (NH_4^+) phases (Smith and Read, 1997; Marschner, 2007; Martin *et al.*, 2007). AMF not only increase the rate of nutrient transfer from the roots to the host plant, but they also increase resistance to biotic and abiotic stresses (Smith and Read, 1997; Khan, 2006; Singh, 2006; Martin *et al.*, 2007). In polluted soils, AMF adapted to the high toxic metal concentrations can restore the biomass values. This chapter aim to provide a synopsis on the role of AMF in rhizoremediation of low-fertility land and polluted soils.

2 WHAT ARE ARBUSCULAR MYCORRHIZAL FUNGI (AMF)?

2.1 Arbuscular mycorrhizal associations

Arbuscular mycorrhizal fungi (AMF) or endomycorrhizae, including fungi belonging to the recently established phylum Glomeromycota (Schüßler *et al.*, 2001), are a normal part of the root system (Gregory, 2006) in most natural and agroecosystems, including polluted soils (Göhre and Paszkowki, 2006). It is postulated that arbuscular mycorrhizae are the ancestral and predominant form of mycorrhizae (Wang and Qiu, 2006). They occur in the soil rhizosphere as spores, hyphae and propagules (Martin *et al.*, 2007). Arbuscular mycorrhizal fungi are considered as obligate symbiotic biotrophs, in that they cannot grow without a host plant supplying them with carbohydrates (glucose and sucrose) (Muchovej, 2001; Harrison, 2005; Martin *et al.*, 2007; Hamel and Plenchette, 2007). In this symbiotic association, the fungus colonizes the plant's root hairs by entering the cortex cells and acts as an extension of the root system (Douds and Millner, 1999; Muchovej, 2001). This type of association is characterized by the formation of arbuscles (finely branched hyphal structures) in the region of the root cortex that may function as nutrient organs (or nutrient exchange sites between the symbionts) and also for fungal multiplication (Muchovej, 2001; Gregory, 2006). According to Douds and Millner (1999), the AMF genera *Gigaspora* and *Scutellospora* produce only arbuscules and extensive intraradical and extraradical hyphal networks (Smith and Read, 1997), whereas *Glomus*, *Entrophospora*, *Acaulospora*, and *Sclerocystis* also produce vesicles (formerly known as vesicular-arbuscular mycorrhizal [VAM] fungi (Martin *et al.*, 2007)). Kistner and Parniske (2002) suggested that the genes involved in arbuscular mycorrhizae and rhizobial symbioses are common in both infection processes. The formation of mycorrhizae induces great changes in the physiology of the roots, in the internal morphology of the plant, and in the mycorrhizosphere, i.e., the soil surrounding the roots (Leyval and Joner, 2001; Gregory, 2006; Martin *et al.*, 2007). The symbiotic association of AMF and plant roots has been considered to be the oldest symbiosis of plants and is suspected to ecologically be the most important symbiotic relationship between microorganisms and higher plants (Paszkowski, 2006).

Arbuscular mycorrhizal associations are reported to occur in about 80% of terrestrial plants including trees, shrubs, forbs and grasses (Gregory, 2006). Many plants are able to establish symbiotic relationships with AMF. The plants are called mycorrhizal crops. However, crop plants from *Brassicaceae*, *Chenopodiaceae*, and *Polygonaceae* do not form mycorrhizal associations. The reader is referred to Varma and Hock (1999), Brundrett

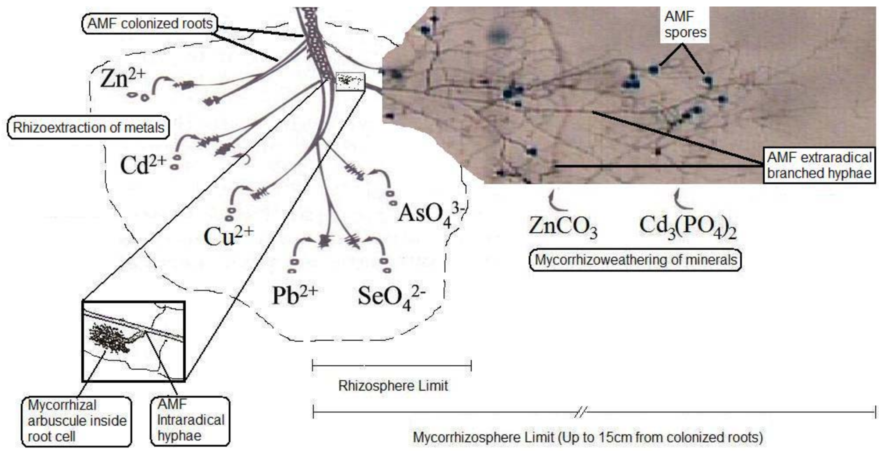

Fig. 1. Rhizosphere and mycorrhizosphere interactions with heavy metals in soils. Mycorrhizal extraradical hyphae release organic acids that weather rocks and minerals in soils. Heavy metals are sequestered and extracted by AMF colonized roots. Nutrients and metals can be exchanged between the fungus and the host plant via mycorrhizal arbuscules inside the root cell.

and Abbott (2002), and Martin *et al.* (2007), for a detailed description and occurrence of AMF.

In the mycorrhizosphere, microscopic fungi naturally occur in soil to form a symbiosis with plant roots and produce a highly elaborated mycelium network (hyphae) (see Fig. 1). These fungal associations could grow into the soil some 5–15 cm from the infected root, reaching farther and into smaller pores than could the plant's own root hairs (Brady and Weil, 2008). AMF have the capability of penetrating extremely small pores in soil and of accessing contaminants contained within (Hutchinson *et al.*, 2003).

2.2 Role of AMF in improving plant metal nutrition

The role of AMF on nutrient uptake (N, P and microelements), on the growth of AM crops, as well as on possible mechanisms of nutrient uptake, have been widely studied, as recently reviewed by Jeffries *et al.* (2003), Al-Karaki (2006), Cardoso and Kuyper (2006), Göhre and Paszkowki (2006), Gregory (2006), Martin *et al.* (2007), and Cavagnaro (2008). It is now generally recognized that AMF enhance the uptake of nitrogen (N) and of relatively immobile soil nutrients such as phosphorus (P), sulfur (S), copper (Cu), zinc (Zn), and boron (B).

AMF increase the plant contact area with soil. They were shown to enhance root absorption area up to 47-fold (Smith and Read, 1997). By colonizing the roots, the fungus enhances plant growth by making soil elements more accessible (George *et al.*, 1992; Nadian *et al.*, 1997; Gregory, 2006; Siddiqui, 2006) and by improving water absorption (Sweat and Davis, 1984; Cui and Nobel, 1992). Accordingly, mycorrhizal colonization improves vegetation establishment and survival particularly in adverse conditions such as in low fertility and arid soils (Jasper *et al.*, 1989; Allen *et al.*, 1996; Smith *et al.*, 1998). Knowing that contaminated sites are generally poor in nutrients and contain a highly altered soil structure, mycorrhizal fungi are suspected to play an important role in vegetation establishment for phytoremediation purposes.

Nutrients are taken up via the fungal hyphae by specific uptake systems and can be mobilized and transported to the plant via continuous fungal extra- and intracellular structures (Göhre and Paszkowki, 2006). It is suggested that constitutive expression or induction of nutrient transporters during symbiosis could improve translocation to the plant (Harrison *et al.*, 2002). However, some studies have reported decreased nutrient uptake or growth of mycorrhizae in certain circumstances (Kucey and Janzen, 1987; Arines *et al.*, 1990). For example, arbuscular mycorrhizal colonization of plants may depend on edaphic properties and environmental factors such as rainfall and sunlight hours. Lingfei *et al.* (2005) found that arbuscular mycorrhizal colonization were negatively correlated with total N, total P, available P and soil organic matter but positively correlated with soil pH. Karanika *et al.* (2008) found, in a field experiment, that AMF colonization was negatively affected by P and positively affected by N addition. However, the response varied among different plant species. In fact, they observed that P addition, in the field experiment, increased the colonization level of the high P demanding annual forb (non-leguminous dicot) such as *Galium lucidum*, decreased hyphal abundance of the forb *Plantago lanceolata* and the grass *Agrostis capillaris*, and appeared to have a negligible effect on the forb *Prunella vulgaris* and on leguminous species.

Other studies have shown a negative impact of AMF on the uptake of some nutrients, probably due to dilution effects (Burleigh *et al.*, 2003) and complex interactions between nutrients (e.g., P and Zn) within AMF at the cellular/sub-cellular levels (Cardoso and Kuyper, 2006; Christie *et al.*, 2004; Cavagnaro, 2008). Antagonistic reactions between nutrients exist under deficiency stress (e.g., P/Zn interaction, Cd/Zn interaction, etc.) (Kabata-Pendias, 2001).

In sum, under low soil nutrient concentrations, improvements in mineral nutrition of mycorrhizal crops can be attributed to the following factors (Burleigh *et al.*, 2003; Christie *et al.*, 2004; Cardoso and Kuyper, 2006; Cavagnaro *et al.*, 2007; Cavagnaro, 2008; Jackson *et al.*, 2008): (1) uptake of available nutrients via the mycorrhizal pathway; (2) differing P uptake kinetics in hyphae from those of roots, possibly through a higher affinity (lower K_m); (3) morphological and physiological changes in roots induced by AMF colonization; (4) differing ways in which roots and hyphae explore microsites, especially small patches of organic matter; (5) changes in edaphic conditions (e.g., pH and others soil variables) favourable to AMF colonization and nutrient solubility and mobility; (6) microbial communities (e.g., activity of mycorrhizal-helper bacteria); (7) nutrient cycling.

3 MYCORRHIZAL RELATIONSHIPS WITH TRACE ELEMENTS

3.1 Heavy metals or trace metal elements

Heavy metals (HM) occur naturally in the environment. Many definitions and interpretations of the term “heavy metal” exist (Duffus, 2002; Karam, 2007). Although imprecise and thoroughly objectionable (Phipps, 1981), the term “heavy metal” has been used increasingly in various publiccations and in legislation related to chemical hazards and the safe use of chemicals (Duffus, 2002) to identify metals with atomic weights greater than 40 (Rand *et al.*, 1995) and densities or specific gravities greater than about

5.0 g/cm^3 (Lozet and Mathieu, 1991; Morris, 1992). This term is often used as a group name for metals and metalloids (semimetals) that have been associated with contamination and potential toxicity (Duffus, 2002). Some authors proposed that this term "heavy metal" be abandoned in favour of "trace element". The later commonly refers to mineral elements that are present in soil in low concentrations, relative to the more abundant element in both the soil solution and the plant (Pandolfini *et al.*, 1997). Here the terms "metal", "heavy metal" and "trace metal" will be used interchangeably to indicate trace metal elements such as arsenic (As), cadmium (Cd), chromium (Cr), Cu, manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), and Zn.

Numerous studies have indicated that agroecosystems receive inputs of heavy metals from the increased use of commercial fertilizers and biocides, from 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; Kabata-Pendias, 2001; Kabata-Pendias and Mukherjee, 2007). Although some of these metals are essential plant micronutrients since they are required for plant growth and development (Zn, Cu, Fe, Mn, Ni, Mo, Co), high contents of heavy metals, as well as the long-term presence of potentially toxic metals (Cd, Pb) and metalloids (As) in surface horizon of agricultural soils, are generally considered a matter of concern to society as they may adversely affect the quality of soils and surface water, and compromise sustainable food production (Pandolfini *et al.*, 1997; Kabata-Pendias, 2001; Keller *et al.*, 2002; Voegelin *et al.*, 2003; Kabata-Pendias and Mukherjee, 2007). The soil microbial community is thought to be a sensitive bioindicator of metal pollution effects on bioavailability and biogeochemical processes (Hinojosa *et al.*, 2005).

Metal forms in soils are basically characterized by their differential solubilities in various chemical extractants. The majority of fractionation schemes (Tessier *et al.*, 1979; Ma and Rao, 1997) group soil metal fractions into: "soluble", "exchangeable", "carbonate bound", "sesqui-oxides bound", "organic matter bound/sulfides" and "residual".

All metals present in a soluble form in the soil solution can be taken up by microorganisms and terrestrial plants (Cataldo and Wildung, 1978; Pandolfini *et al.*, 1997; Kabata-Pendias, 2001; Naidu *et al.*, 2003; Boruvka and Drabek, 2004). Many soil and environmental factors influence metal solubility and phytoavailability (Jackson and Alloway, 1992; Pandolfini *et al.*, 1997; Leyval and Joner, 2001; Karam *et al.*, 2003; Kabata-Pendias and Mukherjee, 2007). These factors can be summarized as follows: (1) nature of soil types; (2) nature of the metal species and their interaction with soil colloids and other soil components (sorption-desorption processes; complexation; diffusion; occlusion; precipitation); (3) concentration and chemical form of the metal entering the soil; (4) mineralogical composition (e.g., clay minerals and other aluminosilicates, oxides and hydroxides, carbonates, phosphates, sulphides, sulphates, and chlorides); (5) sorptive properties of soils or binding capacity; (6) physical, chemical, and biological soil properties (e.g., soil texture, soil moisture content and temperature, soil pH, redox potential, cation-exchange capacity, exchangeable cations, salt content, amount and type of clay, organic mater and oxides and hydroxides of Fe and Mn, free carbonates, and microbial activity); (7) biological activity of the rhizosphere; (8) duration of contact with the surface binding these metals; (9) chemical composition of the soil solution; (10) plant type and plant exudate.

Many studies have demonstrated that in neutral or alkaline substrates (soils, mine tailings, etc.) metals are more intensively adsorbed and chelated in unavailable forms relative to acidic substrates. Moreover, in soils rich in calcium carbonate and phosphate, in well-aerated soils with S compounds, and in soils and mine tailings amended with organic materials, metals are less mobile and available, or are associated with substrate constituents in unavailable forms (Kabata-Pendias, 2001; Kabata-Pendias and Mukherjee, 2007; Karam and De Coninck, 2007).

3.2 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 heavy metals 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 heavy metal 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.*, 1999b). 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) (Niklińska *et al.*, 2006). This perspective stipulates that with time, in an ecosystem, contamination exposure increases tolerance in microbial communities. Davis *et al.* (2004) 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-Hartwig *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; Gadd, 2005; Niklińska *et al.*, 2006), AMF isolates, particularly the ecotypes living in metal-enriched soils, metalliferrous sites and mine spoils heavily polluted with metals, can, depending on intrinsic and extrinsic factors, tolerate and accumulate HM (Gildon and Tinker, 1981, 1983a, b; Weissenhorn *et al.*, 1993, 1994; Joner and Leyval, 1997; Leyval *et al.*, 1997; Smith and Read, 1997; Gadd, 2005). Field investigations have indicated that mycorrhizal fungi can colonize plant in metal contaminated sites (Díaz and Honrubia, 1994; Pawlowska *et al.*, 1996) and in agricultural soils contaminated with metals of different origins, including atmospheric deposition from smelter and sludge amendments (Weissenhorn *et al.*, 1995b, c). 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.* (1999b) reported that spore numbers decreased with the increasing amounts of heavy metals, whereas specie richness and diversity increased in soils receiving an intermediate rate of sludge contamination but decreased in soils receiving the highest rate of heavy metal-contaminated sludge.

Several reports and reviews suggested that mycorrhizal fungi (MF) from metal-contaminated sites have developed tolerance against metal toxicity and are well adapted (Weissenhorn *et al.*, 1993, 1994; Del Val *et al.*, 1999a; 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 arbuscular mycorrhizal fungi have evolved arsenate resistance and conferred enhanced resistance on *Holocus lanatus*. HM concentration may decrease the numbers and vitality of AMF as a result of HM toxicity (Dixon, 1988; Dixon and Buschena, 1988) or may have no effect on mycorrhizal colonization (Wilkins, 1991; 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 heavy metals (Göhre and Paszkowski, 2006; Hildebrandt *et al.*, 2007) from extracellular 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.

The ability of an organism to tolerate and to resist metal toxicity may involve more than one of the following mechanisms (Gadd, 1993, 2005; Leyval and Joner, 2001; Lux and Cumming, 2001; Ouziad *et al.*, 2005; Sudová and Vosátka, 2007):

- Fungal gene expression
- Extracellular metal sequestration and precipitation
- Production of metallothioneins (metal binding proteins)

- Avoidance of metals (reduced uptake or increased efflux, formation of complexes outside cells, release of organic acids, etc.)
- Intracellular chelation (synthesis of ligands such as polyphoshates and metallothioneins)
- Compartmentation within leaf vacuoles
- Loss of leafs during dry or cold seasons
- Phosphorus plant status or interaction between P and metals (increased P uptake by host plant)
- Biological sorption via glomalin
- Volatilization.

The expression of several protein encoding genes potentially involved in heavy metal tolerance varied in their response to different heavy metals. Such proteins included a Zn transporter, a metallothionein, a 90 kD heat shock protein and a glutathione *S*-transferase (all assignments of protein function are putative). Studies on the expression of the selected genes were also performed with roots of *Medicago truncatula* grown in either a natural, Zn-rich heavy metal "Breinigerberg" soil, or in a non-polluted soil supplemented with 100 μM $ZnSO_4$. The transcript levels of the genes analyzed were enhanced up to eightfold in roots grown in the heavy metal-containing soils. The data obtained demonstrate the heavy metal-dependent expression of different AMF genes in the intra- and extraradical mycelium. The distinct induction of gene coding for proteins possibly involved in the alleviation of damage caused by reactive oxygen species (a 90 kD heat shock protein and a glutathione *S*-transferase) might indicate that heavy metal-derived oxidative stress is the primary concern of the fungal partner in the symbiosis.

In a soil environment, levels and persistence of metal tolerance of the AMF (Leyval and Joner, 2001; Jamal *et al.*, 2002; Turnau and Mesjasz-Przybylowicz, 2003; Toler *et al.*, 2005; Fomina *et al.*, 2005; Biró *et al.*, 2005; Sudová *et al.*, 2007) depends on a number of factors:

- AM community ecotype or diversity of AM fungi
- Specific properties of host plant and conditions of plant growth
- Nature of the metal
- Level of soil metal contamination, particularly available or extractable HM
- Cultivation regime
- Colonization conditions (axenic culture vs symbiotic conditions)
- Activities related to land disturbance
- Seasonal variations.

3.3 Heavy metal uptake by AMF

Many studies have shown that metals are sorbed in the soil system by microbial biomass, such as fungi, yeast, bacteria, algae and cyanobacteria (Lepp, 1992; Mullen *et al.*, 1992; Morley and Gadd, 1995; Kapoor and Viraraghavan, 1998; Zhou, 1999). In general, mobilization of metals by soil microorganisms can be achieved by protonation, chelation, and chemical transformation (Gadd, 2005). The exudates, such as citric acid and other organic compounds, released from both plant roots and soil microorganisms, are very effective in solubilizing and releasing metals from soil components (Murphy and Levy, 1983; Gadd, 1990).

Arbuscular mycorrhizae have often been reported to sequester and to accumulate metals in their biomass as well as in the roots of host plants (Burke *et al.*, 2000; Joner *et al.*, 2000; Leyval and Joner, 2001; Gadd, 2005; Martin *et al.*, 2007). It is reported that intracellular and extraradical mycelium of AM and ectomycorrhizal (ECM) fungi would have potential for metal sorption (Marschner *et al.*, 1998; Joner *et al.*, 2000). Most of the metals were demonstrated to be bound to the cell wall components like chitin, cellulose, cellulose derivatives and melanins of ecto-and endomycorrhizal fungi (Galli *et al.*, 1994). High sorption capacity of fungal mycelium for some metals such as Pb was also confirmed for ECM fungi (Marschner *et al.*, 1998).

Recently, much evidence indicates that AMF exhibit great activity in the mobilization of metals that are bound by soil components (Leyval and Joner, 2001; Gadd, 2005; Göhre and Paszkowski, 2006). AMF can also act as a «barrier» in the uptake or transport of metals. However, little work has been performed to assess the effect of AMF colonization on metal fractionation (metal pools) and labile fractions of metal in soils and mine tailings. The chemical form of metals in the hyphae of AMF has received little investigation. There is no information on the chemical form of many toxic metals in AMF. Besides, all physical parameters inherent to binding sites remain to be elucidated. Much still remains to be learned about factors determining metal uptake by AMF.

Gonzalez-Chavez *et al.* (2002a, b) designed a set of experiments to investigate the characteristics of sorption and accumulation of Cu by the extraradical mycelium (ERM) of different *Glomus* spp. (*Glomus caledonium* BEG133, *Glomus claroideum* BEG134, *Glomus mosseae* BEG132) isolated from a highly Cu-polluted mine soil and grown on sorghum (*Sorghum vulgare* L.) under controlled conditions. Copper localization and compartmentalization was done using Transmission and Scanning Electron Microscopy equipped with energy dispersive X-ray analysis. They observed that ERM of AMF is able to sorb and accumulate Cu. Their experiments demonstrated and concluded the following:

- ERM of AMF from polluted soils accumulated Cu in the mucilaginous outer hyphal wall zone, cell wall and inside the hyphal cytoplasm.
- The accumulated Cu was mainly associated with Fe in the mucilaginous outer hyphal wall zone and in the cell wall.
- Copper was associated with traces of arsenate inside the cytoplasm of the ERM of *Glomus mosseae* BEG134.
- Arsenate may be accumulated inside the cytoplasm in the same way as polyphosphates.
- Different Cu and arsenate uptake and accumulation strategies (tolerance mechanisms) exist between the three AMF isolated from the same polluted soil.

In another set of experiments with excised mycelium of four *Glomus* spp. with different histories of exposure to heavy metals (Cd and Zn), Joner *et al.* (2000) confirmed the capacity of extraradical hyphae of *Glomus* spp. to fix metal ions. The results showed the following sorption features:

- Sorption was fast and sorbed Cd was achieved within 30 minutes.
- Sorption was concentration dependent and, at the highest solution concentrations, the amounts sorbed seem too high to obey a monolayered Langmuir adsorption model.
- *G. mosseae* P2 (metal-tolerant strain from soil with a 60-year history of industrial metal pollution, and grown on subterranean clover, *Trifolium subterraneum*, c.v. Mount Barker) sorbed significantly more Cd than *G. lamellosum* (from non-contaminated soil, grown on ryegrass, *Lolium perenne*, cv. Barclay) and *G. mosseae* Gm (non-metal tolerant strain, BEG 12, grown on ryegrass).

It would seem likely that AMF behave similarly as ECM and other soil filamentous fungi. AMF have metal binding sites and are able to produce intracellular and extracellular with high affinity for metals. Binding sites vary with AMF species.

Although the mycorrhizal mechanisms for enhancing uptake are not entirely known, some of them could be the following (Gadd, 1990, 1993; Joner *et al.*, 2000; Gonzalez-Chavez *et al.*, 2004):

- Transfer of metals to the hyphae by cation exchange and chelation (non-metabolic binding of metals to cell walls).
- Interacting with hyphal synthetized products or metabolites that act as biosorption agents such as chitin and glomalin, an insoluble glycoprotein. The thin hyaline layer of the spore wall of *Glomus geosporum* AMF is composed mainly of chitin (Sabrana *et al.*, 1995).
- Chelation of metals inside the fungus.
- Intracellular precipitation with phosphate (PO_4).

Uptake of metals is controlled by or depends on different factors (Gadd, 1990, 1993; Laheurte *et al.*, 1990; Joner *et al.*, 2000; Leyval and Joner, 2001), including the following:

- AM species
- Metabolite composition
- Fungal biomass CEC
- Edaphic and environmental conditions
- Metal pools
- Metal electrochemical properties
- Competition between metals for mycorrhizal surface adsorption sites
- Nature of the host plant
- Root exudation patterns.

3.4 Effects of AMF on growth and uptake of trace metals by plants

Recent general reviews concerning the transport of metals to plants by mycorrhizal fungi have been published elsewhere (see Leyval and Joner, 2001; Singh, 2006). The following paragraphs provide a synthesis of the factors that contribute to the divergent influences of AMF on heavy metal status in host plant. As mentioned earlier, an important factor determining the phytoavailability of a trace metal is its binding capacity to soil constituents. Plants readily take up trace metals from soils (or other growth media) through the roots, mainly in a soluble form. The specific properties of the mycorrhizosphere are known to accelerate the immobilization of metals and to accelerate the weathering at the root-soil surface relative to the bulk soil (Mench and Martin, 1991; Courchesne *et al.*, 2001). Mycorrhizal fungi can affect the transformation of trace metals in the soil in several ways (Leyval and Joner, 2001) including: (i) altering the pH of the soil (i.e., acidification), (ii) immobilization (by adsorption, chelation, or absorption of free metallic species in the soil solution) and (iii) modification of root exudation. It is important to note that acidification caused by organic acids secreted by AMF facilitates the mobilization of trace metals.

A number of studies have been carried out on trace metal uptake by mycorrhizal plants and the results vary with each experiment and each host plant. However, it can be generalized that, as demonstrated for ectomycorrhizal and ericoid mycorrhizal fungi, AMF can increase the uptake and accumulation of metals in host plants (Davies *et al.*, 2001, 2002; Hovsepyan and Greipsson, 2004; Rufyikiri *et al.*, 2002, 2003) even when the metals are present at toxic levels. Cheung *et al.* (2008) found that inoculation of jute (*Corchotus capsulari*, a higher plant) with *G. mosseae* and *G. intraradices* improved plant growth. However, in other situations, where AM fungi exude enzymes that participate in the immobilization process of metals, AMF colonization decreases the uptake and accumulation of metals in host plants (Joner *et al.*, 2000; Leyval *et al.*, 1997; Weissenhorn *et al.*, 1993). Deram *et al.* (2008) observed that AMF colonization disappeared when Cd concentrations in soil increased. Arbuscular mycorrhizae have also been found to sequester metals in the roots of plants and prevent translocation to the shoot (Burke *et al.*, 2000). In studying the effect of AMF on the accumulation and transport of Pb from an anthropogenically-polluted substrate to root and shoot biomass of maize plants, Sudová and Vosátka (2007) found that Pb concentrations increased in highly colonized root segments, whereas they decreased in the shoots of maize. They hypothesized that Pb was immobilized in the fungal mycelium due to intraradical fungal structures. AM may also protect their host plants from the toxicity of excessive metal or metalloid (Zhu *et al.*, 2001; Bai *et al.*, 2008) through: (i) P nutrition by activating P; (ii) chemical precipitation in the soil; (iii) tissue dilution due to increased plant biomass, (iv) hyphal sequestration of metal; and (v) root immobilization.

The AMF have variable effects on metal uptake (translocation and accumulation in plant tissues) and growth of host plant. Most of these variations could be summarized as follows: (i) metal uptake into the host plant is enhanced or repressed (Kothari *et al.*, 1990; Li *et al.*, 1991; Ietswaart *et al.*, 1992; Bürkert and Robson, 1994; Weissenhorn *et al.*, 1995a; Jamal *et al.*, 2002; Bai *et al.*, 2008); (ii) metal accumulation by plant shoots is reduced under elevated soil metal concentrations while increased under normal metal conditions (Toler *et al.*, 2005); (iii) metal acquisition by plant is reduced and plant growth is enhanced (Weissenhorn *et al.*, 1995b); (iv) metal concentration in shoots is lower at the highest soil metal concentrations (Leyval *et al.*, 1991); (v) metal uptake was either not affected by or not enhanced in mycorrhizal plants, depending on the nature of the metal (Weissenhorn and Leyval, 1995); and (vi) metal accumulation in root and dry matter yield of shoot and root increased (Bai *et al.*, 2008).

Many factors contribute to the divergences of AMF on metal plant uptake, plant growth and plant biomass production (Leyval and Joner, 2001; Citterio *et al.*, 2005; Wang *et al.*, 2005; Audet and Charest, 2006; Deram *et al.*, 2008; Jansa *et al.*, 2008; Piotrowski *et al.*, 2008). These include: (i) fungal genotype; (ii) uptake of metal by plant via AM symbiosis; (iii) root length density, (iv) competition of the AMF communities; (v) seasonal variation in AM; (vi) association with soil microorganisms; (vii) chemical properties of the soil outside the rhizosphere (pH, CEC, etc.); (viii) the metal itself; (ix) concentrations of available metals; (x) soil contamination conditions (contaminated or artificially contaminated vs non-contaminated soil); (xi) interactions between P and metals (addition of P fertilizers); (xii) experimental conditions (light intensity, plant growth stage, available N and P); (xiii) litter inputs; and (xiv) plant species and plant size. Besides, since AMF cannot be grown without a host plant (Leyval and Joner, 2001) and may coexist with other microbial communities (Roesti *et al.*, 2005; Toljander, 2006) that can tolerate and accumulate metals (Lepp, 1992), this would obscure the interaction between AMF and metals in the substrate.

4 AMF FOR MYCORRHIZOREMEDIATION OF CONTAMINATED SOILS AND MINE SITES

4.1 Metal hyperaccumulators

In nature, some plants hyperaccumulate heavy metals. For example, *Viola calaminaria* and *Thlaspi calaminare* grow over calamine deposits in Aachen, Germany and contain over 1% (dry weight) zinc in their tissues. Also, some *Alyssum* species like *A. bertolinii* grow on serpentine soils in Tuscany, Italy and contain over 1% (dry weight) nickel. These species are respectively called calamine and serpentine flora. *Thlaspi caerulescens* from the *Brassicaeae* family can also hyperaccumulate both Zn and Cd (Brooks, 1998). As classified by McIntyre (2003), Zn and Cd hyperaccumulators contain these metals at minimal levels respectively of 10,000 and 100 μg/g.

Heavy metal complexes in hyperaccumulators plants are mainly associated with carboxylic acids like citric, malic and malonic acids. These organic acids are implicated in the storage of heavy metals in leaf vacuoles. Amino acids like cysteine, histidine glutamic acids, and glycine also form heavy metal complexes in hyperaccumulators (Homer *et al.*, 1997). These complexes are more stable than those with carboxylic acids. They are mostly involved in heavy metal transport through xylem. Moreover, hyper-accumulator plants can increase availability of metals like Fe and also Zn, Cu and Mn by releasing chelating phytosiderophores. Hyperaccumulation mechanisms may then be related to rhizosphere processes such as to the release of chelating agents (phytosiderophores and organic acids) and/or to differences in the number or affinity of metal root transporters (Lombi *et al.*, 2001).

Although hyperaccumulator plants are widely used in phytoextraction, they are generally of low biomass, inconvenient for phytoremediation. However, arbuscular mycorrhizae fungi (AMF), especially *Glomus intraradices*, colonized *Festuca* and *Agropyron* species have shown higher heavy metal (Zn, Cd, As and Se) content than non-colonized controls (Giasson *et al.*, 2006). As for hyperaccumulators, fungi can synthesize cysteine-rich metal binding proteins called metallothioneins (Gadd and White, 1989). AMF might therefore be directly implicated in heavy metal hyperaccumulation in plants.

4.2 Mycorrhizosphere and phytoextraction of metals

Phytoremediation has already proven its potential in numerous applications around the world (Baker *et al.*, 1988; Kumar *et al.*, 1995; Giasson and Jaouich, 1998; Salido *et al.*, 2003). There are several processes associated with phytoremediation of heavy metal polluted soils. Phytostabilization is the reduction of the mobility, bioavailability and/or toxicity of the pollutant in the rhizosphere, while the process of phytoaccumulation is the sequestration, by plant roots, of the contaminants, typically heavy metals, and then translocation to their aerial parts. The most common heavy metals found in polluted soils are Pb, As, Cr, Cd, Ni and Zn. In phytoremediation, the contaminant mass is not destroyed but ends up in the plant shoots and leaves, which can then be harvested and disposed of safely.

The relatively low potential cost of phytoremediation allows for the decontamination of many sites that cannot be treated with currently available methods. In addition, it has aesthetic advantages and long term applicability: it preserves the topsoil and reduces the amount of hazardous materials generated during cleanup (Schnoor, 1997; Ensley, 2000). However, research in this field must be pursued to enhance biomass and heavy metals accumulation in plants. In this way, mycorrhizal fungi may be very helpful (see Fig. 1).

Since the early eighties, many researchers have shown that mycorrhizal colonization can have an impact on heavy metal assimilation by plants (Bradley *et al.*, 1981; Gildon and Tinker, 1983a, b). Dehn and Schüepp (1989) have found that mycorrhizal infection enhances heavy metal accumulation in lettuce roots but not in shoots. However, Angle *et al.* (1988), Lambert and Weidensaul (1991), and Jamal *et al.* (2002) have shown that mycorrhizae enhance heavy metal accumulation in legume shoots like soybeans, alfalfa and lentils. Killham and Firestone (1983), Hetrick *et al.* (1994), Mohammad *et al.* (1995), Burke *et al.* (2000), and Bi *et al.* (2003) have found similar results with grasses. In the case of cesium (Cs) and strontium (Sr), Entry *et al.* (1999) have indicated that mycorrhizal plants produce higher biomass and higher Cs and Sr content in plant tissues than non-mycorrhizal plants.

Moreover, Turnau and Mesjasz-Przybylowicz (2003) have found that *Berkheya coddii*, a hyperaccumulator from the Asteraceae family, cultivated with well-developed mycorrhization, which includes arbuscule formation, increased not only the shoot biomass of the plant but also strongly increased the Ni content of shoots. Ni shoot content of *B. coddii* colonized with *Glomus intraradices* was 1.3% of dry weight, while in nonmycorrhizal plants it was below 0.5%.

In a glasshouse experiment, Giasson *et al.* (2006) studied four commonly found AMF species well adapted to North American soils: *Glomus intraradices*, *Glomus mossae*, *Glomus etunicatum*, and *Gigaspora gigantea*. *Glomus* spp. and *Gigaspora* spp. are AMF species identified in metal rich soils (Chaudry *et al.*, 1999). A grass mixture of *Festuca rubra* and *F. eliator* (70%), *Agropyron repens* (25%), and *Trifolium repens* (5%) was used. This vegetation mix is used in land reclamation in Eastern Canada to revegetate mine tailings. *Festuca* species like *F. rubra* are considered characteristic species on metalliferous soils and can accumulate excessive amounts of metals (Smith and Bradshaw, 1979; Pichtel and Salt, 1998). Also, this grass mixture can be harvested several times per year because the articulated stubble can renew itself constantly (Marie-Victorin, 1964). In this study, AMF mycorrhizal root infection varied from 30% to 70% for all heavy metal treatments. Relative arbuscular richness varied from 38% to 84%. Arbuscules are the internal structures in the root cells that facilitate nutrient exchange between the fungus and the host plant. Well developed mycorrhization, which includes arbuscule formation, has shown to increase the metal content in shoots (Turnau and Mesjasz-Przybylowicz, 2003). Absence of arbuscular structures can indicate altered host physiology and carbon allocation, or can be a sign of stress in the mycorrhizal fungus. In their glasshouse study, Giasson *et al.* (2006) found the following results regarding heavy metal extractions by AMF colonized vegetation:

- There is interspecific variation between AMF regarding translocation of metals to plants.
- Arbuscule relative richness in Zn treatment was the highest (75%) vs other metal treatments.
- Zn, Cd, As, and Se extractions by *Glomus intraradices* colonized plants are generally higher than in non-mycorrhizal plants, depending on the metal concentration in the soil and whether this heavy metal interacts with other metals in that soil.
- Grasses colonized by *Glomus intraradices* had greater Zn, Cd, As, and Se mass extracted than for non inoculated vegetation because of higher plant biomass.
- When in interaction with other metals in the soil, Se is extracted more readily by AMF colonized plants. With time, however, Se in plants is lost in part by volatilization of the dimethyl diselenide form.
- For all four metal treatments (Cd, Zn, As and Se), there is a positive linear correlation between metal in plant tissues and metal content in soils. When soil metal content is increased tenfold metal in plant tissues is also increased by 10, for both colonized and control treatments.
- Metal extraction reaches a plateau after 80 days showing no further phytoaccumulation or sometimes slightly diminishes because of either phytovolatilization (As and Se) or necrosis in plants (Zn) caused by high heavy metal levels. This observation suggests that *G. intraradices* colonized perennial grasses may be harvested after a two-month

period allowing for two to three harvests per year in Canadian latitudes. In this way, phytoremediation can be accelerated two- to threefold.

Lasat (2002) observed that the effect of AMF associations 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).

As related by other studies (Shetty *et al.*, 1994), AMF alters the pattern of Zn translocation from root to shoot in *Festuca arrundinaceae*. Zinc hyphal uptake and translocation are known to be similar to P transport (Cooper and Tinker, 1978; Weissenhorn *et al.*, 1995a). In their *in vitro* experiment, Giasson *et al.* (2005b) observed that zinc adsorption at spore propagules was weak – approximately 9.6 μg of Zn per gram of spore in the 500 μg/g Zn treatment because mycorrhizal hyphae vacuoles and arbuscules contain phosphorus in the form of polyphosphate. Additionally, Zn is transferred to the plant host though AMF hyphae and arbuscules. Arbuscules are involved in this transfer by providing a considerable increase in fungus and plant contact surface area (Smith and Read, 1997). Frequent degeneration of fungal arbuscules in the root thus allows Zn content to be transferred directly into the host cell (Gildon and Tinker, 1983a) reducing Zn concentrations in fungi. Turnau and Mesjasz-Przybylowicz (2003) found that well-developed mycorrhization, containing arbuscule formations, increased the metal content in plant shoots. Zn can then be accumulated in leaves as a citrate complex in the vacuole (Salt *et al.*, 1999).

Phosphate is central to mycorrhizal symbiosis. In P deficient soils, plant roots exude chemical signals to attract AMF. In such environments, AMF have developed an active phosphate transporter (Meharg *et al.*, 1994). Arsenate (As(V)) is chemically similar to phosphate and can enter cells via arsenite (As(III)) translocating ATP'ase (Jun *et al.*, 2002). The presence of AMF can therefore enhance both phosphate and arsenate uptake in such conditions (Martin *et al.*, 2007)

Also, at high levels of P, mycorrhizal colonization may be reduced with consequent reductions in uptake and cause deficiencies of essential metals like Cu and Zn. Interactions such as these may be involved in the apparent alleviation of Zn toxicity in polluted sites (Dueck *et al.*, 1986). If the sites are P deficient, then mycorrhizal P uptake can result in increased growth and dilution of Zn in the tissues (Smith and Read, 1997).

In an *in vitro* study using transformed carrot roots (*Daucus carota* L.) growing in a phytagel (M media), Giasson *et al.* (2005b) found that even without pressure, AMF hyphae passed from the proximal to the distal side of the Petri dish into the M media containing low and high concentrations of Zn and Cd. The hyphal network was well developed and sporulation was high in the low heavy metal level side (100 μg/g Zn and 5 μg/g Cd). More than 16,000 spores per half Petri plates were counted for the low Cd and Zn treatments.

In the same experiment, Giasson *et al.* (2005b) observed that at high heavy metal levels in the media (500 μg/g Zn and 20 μg/g Cd), hyphal network was less developed (taking spiral shapes) and sporulation was weaker. The spore population was approximately 1,500 per half Petri plates for the 20 μg/g Cd treatment and 1,300 for the 500 μg/g Zn treatment. The results are revealing. Essential cation (Zn) and nonessential cation (Cd) translocation from substrate (phytagel) to plant occurred through mycorrhizae hyphae, even at high (toxic) heavy metal concentrations. This is in accordance with Chen *et al.* (2003), who found that Zn is taken up and transferred to a host plant via extraradical hyphae. Root over growth media accumulation factors reached 5:1 and 18:1 for Zn and Cd, respectively. With over 90 μg/g cadmium and 550 μg/g zinc found in the roots, the presence of *G. intraradices* caused carrots to become cadmium hyperaccumulators and Zn accumulators.

Cadmium, like other nonessential metals, is generally of low abundance in the biosphere and should therefore not compete with specific transport systems for essential metals (Gadd and White, 1989). However, as a result of human activities, nonessential metals are concentrated in certain areas at very high levels. Toxic and nonessential metals, such as Cd, generally bind more strongly to ligands compared with essential metals thereby displacing essential metals from their normal sites, and exerting toxic effects by binding to other sites (Hughes and Poole, 1989).

Furthermore, Cd (0.97 Å) has a similar ionic radius to calcium (Ca) (0.99 Å), and so there is the possibility of metal-for-metal substitution in the predominantly oxygen-containing ligand sites preferred by Ca. Also, because of cadmium's position in the Periodic Table (Group IIB), it bears a chemical resemblance to Zn. Competition among Cd, Ca, and Zn ions for adsorption sites on AM hyphae seem to favour Cd over Ca and Zn (Joner *et al.*, 2000). In microbes, Cd competes with both Mn and Zn transport systems. Cadmium appears to enter via the Mn transport system and is rapidly diffused from resistant cells, via antiporter genes, exchanging cadmium for hydrogen and cation-translocating ATPase (Silver *et al.*, 1989).

Cadmium will also bind at sites normally occupied by Zn containing either a soft ligand, like sulphur (for example, cysteine or metallothionein) or a hard ligand, like nitrogen (for example, histidine) and oxygen (Rayner and Sadler, 1989). A common metal-induced response in fungi is the intracellular synthesis of cysteine rich metal-binding proteins called metallothioneins (MT), which have functions in metal detoxification and also in the storage and regulation of intracellular metal ion concentrations (Gadd and

White, 1989). Fungal cells have certain mechanisms to maintain metal homeostasis and prevent metal toxicity. Glutathione (GSH), metal-binding peptides, metallothionein-like peptides, and sulphide ions play a role in such mechanisms. Cellular metal stress triggers the biosynthesis of some of these molecules, regulated via intracellular metal sensors (Singh, 2006).

There are also small peptides called phytochelatins (PC) in microbes and plants that bind metals such as Cd via cysteinyl residues. These peptides protect plant cells from metal poisoning (Baker *et al.*, 1988). Joner and Leyval (1997) suggested that sequestration of Cd in fungal structures could be responsible for the retention of Cd in the roots. It is likely however that the extent of this retention mechanism is restricted due to the relatively small biomass of the fungi. Giasson *et al.* (2005b) found Cd adsorption on spore propagules to be at concentrations below the detection limit of a chromatograph detector (HPLC). According to Colpaert (1998), once Cd saturation occurs in the fungi, increased translocation to shoots is thought to occur. Hughes and Poole (1989) found that some heavy metals appear to enter cells directly, possibly through a lesion in the cell membrane, as a result of the strong binding of the cation.

In an *in vitro* study, Giasson *et al.* (2005b) found that heavy metal accumulation by colonized carrot roots seemed to reach a plateau: 550 μg Zn/g and 90 μg Cd/g, independently of the initial growth media heavy metal concentrations. This could be explained by heavy metal saturation in vegetation after a two-month exposure period (Giasson *et al.*, 2006). Furthermore, Rayner and Sadler (1989) demonstrated that when cadmium levels are increased, adaptation results thereby in increasing the growth rate and reducing the extent of cadmium accumulation from the medium.

These conclusions are worth considering for phytoremediation of heavy metal–contaminated soils enhanced by mycorrhizal inoculation.

4.3 Mycorrhizostabilization of metals

Phytostabilization and mycorrhizostabilization reduce the mobility, bioavailability and/or toxicity of the pollutant in the rhizosphere. Mycorrhizal fungi can enhance soil structure by secreting a glycoprotein slime called glomalin. Fungi glomalin production enhances aggregate formation and may also create larger pores for better growth of hyphae (Thomas *et al.*, 1993; Jastrow *et al.*, 1998). A lack of large pores can restrict fungal growth in soils, however glomalin production was found to be higher in small pores (0.1 mm) than in large ones allowing for more indirect fungal contact with soil (Brady and Weil, 2008). Glomalin can sequester heavy metals such as Cu, Cd, Pb and Mn in polluted soils. Gonzalez-Chavez *et al.* (2004) found that glomalin from hyphae of an isolate of *Gigaspora rosea* sequestered up to 28 mg Cu/g *in vitro* media.

Mycorrhization can also improve plant resistance towards heavy metal phytotoxicity by biosorption (Dueck *et al.*, 1986; Weissenhorn *et al.*, 1995a). Turnau *et al.* (1993) suggested that sequestration of metals like Cd, titanium (Ti) and barium (Ba) by polyphosphate in fungal structure might be important in minimizing transfer to the plant. Fungal sorption of heavy metals is a passive mechanism of ion immobilization on the surface of microbial cells including processes like adsorption, ion-exchange, complexation, precipitation, and crystallization on and within what may often be a multilaminate, microfibrillar cell wall rich in negatively charged ligands such as phosphoryl, carboxyl, sulfhydryl, hydroxyl, and phenolic groups (Leyval and Joner, 2001).

Lead has low mobility in soil (less than Cd and Zn) (Orlowska *et al.*, 2002) and it seems to form organic complexes with soil organic matter considering it is unavailable for plants. Also, plants have mechanisms to precipitate Pb in highly insoluble forms in the rhizosphere, such as the $PbSO_4$ (Brooks, 1995). Furthermore, sequestration of Pb in roots was found to be correlated with an increase in the number of fungal vesicles in highly colonized species. Fungal vesicles may be involved in storing toxic compounds and, thereby, could provide an additional detoxification mechanism (Göhre and Paszkowski, 2006).

4.4 Mycorrhizae and phytovolatilization of metals

A number of the elements in subgroups II, V and VI of the Periodic Table, like Hg, As and Se, form volatile hydrides or methyl derivatives that can be liberated in the atmosphere, probably as a result of the action of bacteria or soil fungi (Brooks, 1998). Metals can also be mycotransformed by such mechanisms as reduction, methylation and dealkylation.

Metalloids and some metals (e.g., As, Se, Hg, Sn, Pb) can be transformed by fungi into their methylmetal form which causes their volatilization in soil gazes and eventually in the atmosphere. In a greenhouse study, Giasson *et al.* (2006) suggested that phytoaccumulation of As and Se can slightly diminish because of phytovolatilization.

As showed by Zayed *et al.* (2000) and Giasson *et al.* (2006), Se may be lost in part by phytovolatilization in the dimethyl diselenide ($CH_3SeSeCH_3$) form. Dimethyl arsenic ($AsO(CH_3)_2(OH)$), methyl mercury (CH_3Hg^+) and tetramethyl lead ($Pb(CH_3)_4$) are the most common methylated forms of As, Hg and Pb that can also be phytovolatilized.

4.5 Mycorrhizoweathering of soil rocks and minerals

Bioavailability and toxicity of heavy metals in soils depend on their form rather than on total amounts. The availability of the eight metal fractions can be divided into three groups: (1) easily extractable and exchangeable, including water-soluble, exchangeable, and bound to reducible Fe and Mn oxides fractions; (2) potentially extractable and exchangeable, including strongly bound to minerals or weakly bound to organic matter (OM), strongly chelated by OM, bound to or occluded by carbonates, and bound to or occluded by sulphides fractions; and (3) nonextractable and nonexchangeable, found in residue fraction (Tessier *et al.*, 1979; Ma and Rao, 1997; Dinel *et al.*, 2000).

Heavy metals bound to or occluded by carbonates are more difficult to extract by vegetation. Carbonates can be the dominant heavy metal sink in a particular soil. Heavy metals may co-precipitate with carbonates incurporated in their structure, or may be sorbed by oxides (mainly Fe and Mn) that were precipitated onto the carbonates or other soil particles (Kabata-Pendias and Mukherjee, 2007). On the other hand, accumulation of heavy metals – Zn, Cd, As and Se – in plants can be enhanced by inoculation of roots by arbuscular mycorrhizal fungi (AMF) (Giasson *et al.*, 2006). Fungi produce protons, organic acids, phosphatases, and other metabolites for solubilization and complexation of metal cations (Singh, 2006).

Moreover, mycorrhizal fungi are able to acidify the rhizosphere by releasing organic acids like citric and oxalic acids (see Fig. 1) (Leyval and Joner, 2001). Oxalic acid is a leaching agent for a variety of metals, such as Al, Fe and Li, forming soluble metal oxalate complexes (Singh, 2006). The most important mechanisms for regulating heavy metal behavior by carbonates are related to variations in soil pH. Carboxylic acids released by AMF can solubilize heavy metals bound to carbonates and enhance their phytoaccumulation (Giasson *et al.*, 2005a).

Zinc and Cd speciation concentrations measurements from contaminated soil near a zinc smelter in Canada show that the metal fraction distribution is similar for Zn and Cd. In fact, the easily extractable and exchangeable fractions represent less than 27% for both Zn and Cd, which is not interesting for a phytoremediation technology. On the other hand, the two first metal fraction groups, consisting of easy and/or potentially extractable and exchangeable fractions including carbonate fraction, regroup around 86% of the metal total concentration for both Zn and Cd.

To determine if mycorrhizal fungi play a role in the speciation of heavy metals (biochemical weathering), Giasson *et al.* (2005a) used *in vitro* compartmented systems to study the mechanisms implicated in heavy metal (essential and non-essential) absorption by AMF colonized plant roots. The goal of their experiment was to determine whether mycorrhizal hyphae are directly involved in sequestration and uptake of essential Zn and non-essential Cd by plant roots, while these heavy metals were present in toxic concentrations in the Petri media. They wanted to verify the effects of endomycorrhizal (*Glomus intraradices*) hyphae on speciation of essential (Zn) and nonessential (Cd) heavy metals in order to change this water-insoluble carbonate form to a soluble and phytoavailable form.

Their results indicate that there is a solubilization of $ZnCO_3$ by hyphae and translocation to roots. Zinc saturation was reached in the *G. intraradices* colonized roots at approximately 400 µg/g, independently of initial $ZnCO_3$ concentrations. In the cadmium treatment, Cd saturation was not reached. In the lower Cd treatment, the plant to media metal ratio was 3:1, and in the higher treatment, the ratio was 1:1 (Giasson *et al.*, 2005a). In fact, mycorrhizal fungi are able to acidify the rhizosphere by releasing organic acids like citric and oxalic acids (Leyval and Joner, 2001). These organic acids can form coordination compounds or complexes with metals.

If the organic acids (e.g., citric and oxalic acids) contain two or more electron donor groups so that ring-like structures are formed, then the resulting complexes are metal chelates (Gadd, 2000). Berthelin *et al.* (2000) showed that releases of organic acids by ectomycorrhizae are efficient in weathering and solubilization of minerals by the following complexation dissolution processes:

$$M^{+}\ (\mathrm{Mineral})^{-} + \mathrm{H}L \rightarrow \mathrm{H}^{+}\ (\mathrm{Mineral})^{-} + ML \quad (1)$$

$$\mathrm{H}L + LM \rightarrow L_2M + \mathrm{H}^{+} \quad (2)$$

where L = organic ligands and M^{+} (Mineral)$^{-}$ are carbonates, phosphates, silicates and so on.

Because P availability is strongly controlled by dissolution of mineral P that can constitute a considerable portion of the available P, soil pH is a major factor in determining the relative importance of mycorrhizae in P uptake. Mineral phosphorous has greatest availability at slightly acid to near-neutral pH. At low pH, phosphorous solubility is limited by the low solubility of Fe and Al phosphates, whereas at alkaline pH phosphorous forms insoluble Ca and Mg phosphate minerals (Crowley and Alvey, 2002).

The availability of Cd from rock and mineral phosphates (apatite) can be enhanced with the release of organic acids such as tartaric acid by ectomycorrhizal fungi. *Suillus granulatus* was more efficient than *Pisolithus tinctorius* in that matter (Leyval and Joner, 2001). Mycorrhizoweathering of soil minerals (silicates, carbonates, phosphates) can enhance the availability of metals in the rhizosphere thereby enhancing plant uptake.

4.6 AMF and plant stress alleviation on mine sites

One of the main objectives in mine site reclamation is revegetation. This mining environment is characterized by poor physical and chemical conditions, poor nutrient (N, P) and organic matter contents, very low or very high pH, drought and high surface temperatures. Mycorrhizal colonization could improve vegetation establishment and survival particularly in such adverse conditions.

Young seedlings have to be protected from extremely high surface temperatures to prevent heat girdling of stems (Danielson, 1985). By colonizing the roots, the fungus enhances plant growth by making soil elements more accessible (George *et al.*, 1992; Nadian *et al.*, 1997; Gregory, 2006) and by improving water absorption (Sweat and Davis, 1984; Cui and Nobel, 1992). Accordingly, mycorrhizal colonization improves vegetation establishment and survival particularly in adverse conditions such as low fertility and arid soils (Jasper *et al.*, 1989; Allen *et al.*, 1996; Smith *et al.*, 1998).

Mine spoils may be extremely acidic or alkaline. Acid mine drainage (AMD) is very frequent, especially in sulphide metal ore tailings, where rain water reacts with sulphide to form sulphuric acid (H_2SO_4). Leachate pH exiting from the tailings could be as low as 1. Plant roots can be colonized with mycorrhizae at pH values as low as 2.7, the critical pH for 95% maximum colonization of cassava roots varying with species from 4.4 to 4.8 (Ballen and Graham, 2002).

Hyphae of AMF may extend 8 cm from the root surface, but rhizomorphs of *Pisolithus* may extend 4 m into the soil, a result that suggests ectomycorrhizae are better adapted to long-distance transport than AMF (Danielson, 1985). Relatively few species of ectomycorrhizal symbionts have been identified as occurring on mine wastes, and of those, even fewer have been properly quantified with respect to their actual importance. To determine the degree of fungal symbiont adaptation to mine waste conditions, infection levels of each species must be quantified (Danielson, 1985). Ectomycorrhizae *Pisolithus tinctorius*, *Telephora terrestris*, and *Cenococcum geophilum* have been successfully field tested on spoils and tailings.

In their experiment, Chen *et al.* (2007) provided evidence for the potential use of local plant species in combination with AMF for ecological restoration of metalliferous mine tailings. It appears that considerable strain differences exist among AMF, and it would be profitable to screen isolates for adaptability to mine spoils. Old mine spoils with established vegetation may prove to be valuable sources of inoculum of adapted strains (Danielson, 1985).

5 CONCLUSION

Although usually considered important primarily for P uptake, AMF can improve assimilation of other non metallic nutrients such as N, K, S, B as well as of metallic nutrients (Zn, Cu, Mn, and others), particularly in unpolluted soils of low nutrient status. It has been suggested that mycorrhizae may benefit plant growth by increasing the availability of P from non-labile sources. The response to AMF colonization may vary among the different plant species. However, it should be considered to introduce mycorrhizae inoculums tolerant to metallic nutrients (e.g., Zn, Cu, Mn or others) into low-input agricultural soils in order to facilitate the recycling of organic, industrial and urban wastes on agricultural fields that would otherwise be extremely dangerous to agricultural ecosystems (Weissenhorn *et al.*, 1995c). For environmental considerations, mycorrhizal associations should be managed to attenuate the possibility of contaminating the soil and surface water (Jeffries *et al.*, 2003).

In order to exploit microbes as biofertilizers, biostimulants and bio-protectants against pathogens and heavy metals, ecological complexity of microbes in the mycorrhizosphere needs to be taken into consideration and optimization of rhizosphere/mycorrhizosphere systems need to be tailored (Khan, 2006). There is interspecific variation between AMF regarding translocation of metals to plants. As observed by Lasat (2002), effect of AMF associations 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 nonmycorrhizal plants (Mohammad *et al.*, 1995).

6 FUTURE RESEARCH

Arbuscular mycorrhizal fungi have great potential in the remediation of disturbed land and low fertility soil but the use of these mycorrhizae, and other beneficial microbial communities, by farmers in their fields is still lacking. Further experiments are needed to assess the ability of AMF to continue growing in the presence of multiple toxic metal or metalloid cations, either alone or in combination.

The understanding of interactions occurring between AMF and its biotic and abiotic environment is still in its infancy. The characterization of the composition of AMF exudates and the effects of these compounds on soil microbial community, plant nutrition, metal accumulation in plant shoots and shoot biomass production have implications for sustainable soil management and land rehabilitation.

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