12 Arbuscular Mycorrhizal Fungi and Heavy Metals: Tolerance Mechanisms and Potential Use in Bioremediation

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CONTENTS

12.1 INTRODUCTION

The arbuscular mycorrhizal (AM) symbiosis is commonly found in more than 80% of plant species. This association results in multiple benefits to the plants: improvement of plant growth; protection against root pathogens; adaptation to survive in extreme soil conditions; etc. Only recently have a role in soil conservation and a contribution to determine plant community structure in natural ecosystems been recognized for this symbiosis [1,2]. In contrast, its role in altered soil conditions, such as contaminated soils, is not completely understood yet.

Occurrence of arbuscular mycorrhizal fungi (AMF) is common in contaminated soils; however, it has been reported that the presence of high concentrations of potentially toxic elements has a negative effect on the diversity of these fungi [3,4]. Moreover, the present literature contains contradictory results concerning effects of PTEs on mycorrhizal plants.

Some PTEs are mainly accumulated in root systems and some authors have suggested that AMF might be involved in this accumulation; however, the mechanisms of retention and allocation of PTEs have been largely ignored. In ericoid and ectomycorrhizal fungi, the binding of PTEs to the external mycelium has been proposed as a tolerance mechanism [5–8] that reduces metal translocation to the shoots, but the validity of this hypothesis had not been proved for AMF. Novel information stressing AMF's possible importance in contaminated soils is available. A better understanding of the processes involved in dealing with high concentrations of PTEs on mycorrhizal plants may have strong implications for the use of these fungi in the bioremediation of contaminated soils.

12.2 AM SYMBIOSIS

The term "mycorrhiza" implies basically the association of fungi with roots. Indeed, mycorrhiza, not roots are the chief organs of nutrient uptake by the majority of land plants [9]. The different kinds of mycorrhizae have been described on the basis of their fungal associates into those aseptate endophytes in the class Glomeromycetes [10] and those formed by septate fungi in Ascomycetes and Basidiomycetes. This chapter will consider the association formed by members of the Glomeromycetes, which have been referred to as arbuscular mycorrhiza (AM), and were formally known as vesicular–arbuscular mycorrhiza. The plant hosts of this type of mycorrhiza may belong to Bryophyta, Pteridophyta, Gymnospermae, and the majority of families in the Angiospermae [9].

Arbuscular mycorrhizae are the most common type of mutualistic symbioses with plants. The association is formed between the roots of an enormously wide variety of host plants, which have true roots, and aseptate, obligate symbiotic fungi. The name of AM is derived from characteristic structures, the arbuscules, formed by the fungi within the cortical cells of the host plants (Figure 12.1a). Arbuscules are formed by all members of the phylum of the Glomeromycetes. In contrast, vesicles (Figure 12.1b), which occur intra- or intercellularly, are formed by all members of the families Glomaceae and Acaulosporaceae, but not by the members of the Gigasporaceae. Spores are considered as structures for reproduction or propagation (Figure 12.1c). An AM has three important components: the root; the fungal structures within the cells of the root; and the external mycelium (Figure 12.1d,e) functioning as a "bridge" between the root and the soil.

12.3 IMPORTANCE OF ARBUSCULAR MYCORRHIZA IN SOILS

The importance of the arbuscular mycorrhizal fungi is due to the multiple benefits to the plant hosts. One of the most recognized roles is related to plant nutrition [11]. These fungi play a central role in nutrient uptake in nutrient-deficient soils [12], especially for nutrients with low mobility, such as phosphate, Zn^{2+}, and Cu^{2+}. In nonmycorrhizal plants, nutrient absorption is confined to the outer cell layers of the root cortex and the rhizodermal cells with root hairs. In contrast, in mycorrhizal plants, higher absorption is realized by a much greater absorptive surface, created by the external mycelium of these fungi, which can extend beyond the nutrient depletion zone formed around the plant roots [13,14].

Other benefits of AMF are protection against root pathogens and greater tolerance to stress conditions such as salinity, extreme soil pH, and drought. Recently, it has been acknowledged that AMF can affect plant community structure [1,2]. Arbuscular mycorrhizae also participate in soil stability and conservation [15] through their physical and biological activities. The physical entan-

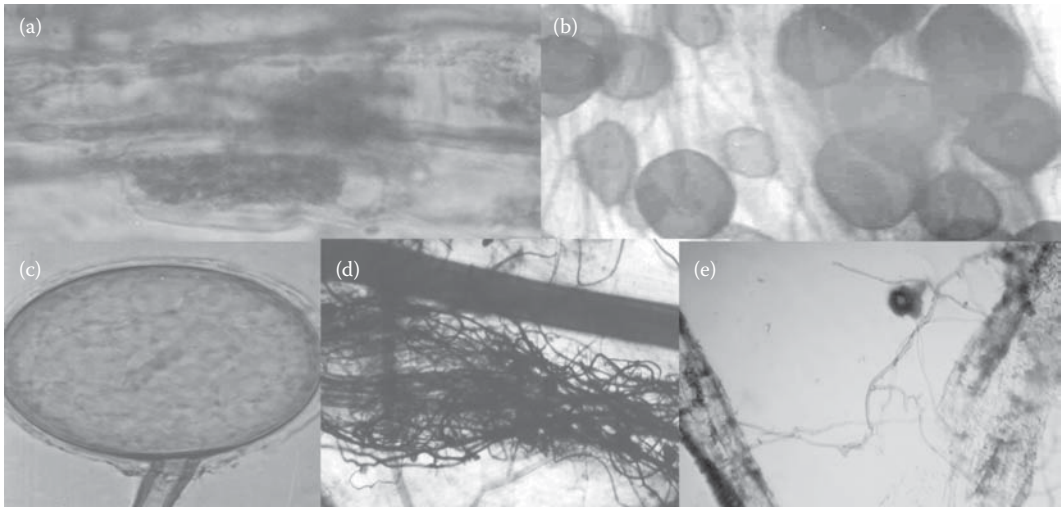

FIGURE 12.1 Fungal structures (a) arbuscules; (b) vesicles; (c) spores; and (d),(e) external mycelium.

glement of hyphae stabilizes soil aggregates. Active hyphae exudate glomalin, a glycoprotein produced abundantly by several if not all AMF, which acts as a biological cement and increases soil aggregation and resistance to soil erosion [15–17]. Additionally, Rillig et al. [18] have shown that this fungal glycoprotein significantly contributes to carbon sequestration in the soil. Nichols et al. [19] reported that glomalin, along with soil humic fraction and particulate organic matter, is a major contributor to soil organic C (up to 25%). They also concluded that this protein may be an important carbon storage pool. Glomalin production may be increased through sustainable agricultural practices.

Because the mycorrhizal condition is the normal state for the majority of the land plants, a lot of research has focused on nutritional and physiological studies in cultivated and natural ecosystems. In contrast, the importance of AM for PTE-contaminated soils has been poorly investigated. Much work has been done on MA fungi and their effect in PTE-polluted soils during the last 20 years; however, the importance of the role of mycorrhizal fungi in these soils — the fungal mechanisms dealing with PTEs — and their contribution to PTE tolerance in plants are still poorly known.

12.3.1 AMF in Contaminated Soils

Arbuscular mycorrhizae are an integral association consisting of two functioning partners: the fungus and the plant. Leyval et al. [20] stated that when interactions between fungi and PTEs are studied, two aspects should be considered: (1) the effect of PTEs on AMF; and (2) the effect of AMF on uptake and translocation of PTEs to the plant (Figure 12.2). In this relationship, fungi and plant PTE tolerance; soil and environmental factors influencing PTE availability; and ion PTEs' toxicity influence these effects.

12.3.1.1 Effect of PTEs on the Population of AMF

In recent years, more attention has been paid to understanding the effect of contaminants on soil biology. In general, PTEs seem to affect microbial species' richness, abundance, and diversity [21–23]. However, microorganisms are not uniformly affected.

Over 140 species of AMF had been described by the beginning of the 1990s [24]. In contaminated soils [25], different, identified, and, in some cases, unidentified species of AMF have been

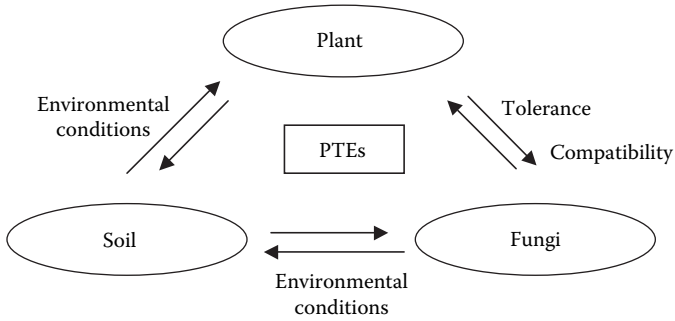

FIGURE 12.2 Interaction between potentially toxic elements (PTEs), arbuscular mycorrhizal fungi and plant. (Modified from Leyval, C. et al., *Mycorrhiza,* 7, 139, 1997.)

reported in studies investigating AMF population in contaminated sites [25]. Although identification of some AM species without validation with molecular techniques may be difficult and therefore questionable, at least one member of each of the seven genera has been found in these soils. *Glomus* is apparently the most common genus present in contaminated soils, but this should not disregard a wider distribution of species of other genera. However, predominance of one genus may be due, at least partially, to the techniques used to detect or identify these fungi (trap cultures, specific probes, etc.).

Leyval and Vandenkoornhuyse [26] showed that AMF diversity was changed by PTEs, even at concentrations below the European Community threshold values for agricultural soils. Fungal diversity was higher in a moderately contaminated soil than in a highly contaminated soil. Similar information was reported by del Val et al. [4]. These authors studied the diversity of AMF affected by the addition of different rates of sewage-amended sludge containing Pb, Cd, Cr, Cu, Ni, Hg, and Zn. The highest application rate of sludge (300 m^3 $ha^{-1}year^{-1}$) caused a significant decrease in the size and diversity of populations of AMF in soil, but diversity was increased in soils receiving intermediate rates of sludge application (100 m3 $ha^{-1}year^{-1}$).

Sambandan et al. [27] found a high level of diversity of AMF in soils (15 different species) contaminated by Zn, Cu, Pb, Ni, and Cd. Pawlowska et al. [28] reported different types of AMF in the undisturbed site of a calamine spoil (containing high concentrations of Cd, Pb, and Zn) in Poland. However, disturbance caused by surface soil removal proved to be an important factor determining frequency, distribution, and richness of AMF in the sites. They reported six different AMF on the undisturbed site and only two species on the disturbed site. *Glomus aggregatum, G. constrictum, G. pansihalos, Glomus* sp., *G. fasciculatum*, and *Entrophospora* sp. were found on the undisturbed soils; only the latter two fungi were found on the disturbed site. Apparently, *G. fasciculatum* and *Entrophosphora* sp. were less affected by disturbance.

Other authors have reported low population diversity of AMF in contaminated soils; some have observed only one to three species of AMF in the contaminated soils [29–31]. However, it is not clear whether this low diversity was really a consequence of PTE contamination or was due to other factors. PTEs tend to select species that sporulate easily in trap cultures. However, lack of sporulation does not necessarily mean absence of AMF from a soil. Other factors may be involved in situations showing low diversity of AMF in contaminated soils — for example, poor sporulation at the time of sampling; use of a restricted host range during propagation of AMF; and short propagation times to allow sporulation of those slow sporulating fungi [32]. Khan [33] reported that a soil of a Cr-contaminated site contained only spores of *Gigaspora* sp. in the rhizospheres of *Dalbergia sissoo*, *Acacia arabica*, and *Populus euroamericana*; however, their roots were also colonized by other mycorrhizal genera because vesicles were observed.

Analyzing Cd-rich slag and tailing piles (0.28 to 0.8 mg Cd-DTPA-extractable g^{-1}), González–Chávez [34] found species of *Scutellospora*, *Acaulospora*, *Entrophospora*, and *Glomus*

colonizing abundantly soil and roots of different host plants (*Dalea* sp. *Crotalaria rotundifolia*, *Trifolium goniocarpum*, *Anagallis arvensis*, *Crusea longiflora, Sida rhombifolia*, and *Lopezia racemosa*). These materials contained amazing amounts of external mycelium (Up to 26.3 mg g^{-1} dry soil).

Molecular evaluation of AM fungal populations in colonized roots and soil is a promising tool for identifying naturally occurring fungi in field sites, such as these of contaminated soils. Molecular techniques such as those used by Turnau et al. [35] nested PCR in combination with taxon-specific primers for AMF fungal species (morphotypes) seem to be highly sensitive to detecting fungi in the field. These authors observed that five different AMF species were colonizing roots of *Fragaria vesca* growing on industrial waste sites located in Chrzanow (Poland). *Glomus* sp. HM-CL4 was detected with a mean frequency of 52%; *G. claroideum* and *Glomus* sp. MH-CL5 were 32 to 34% and *G. intraradices* and *Glomus mosseae* were present less frequently (5 to 7%). However, other AM species or genus may have been present and not identified because they were not amplified by the specific primers.

Cairney and Meharg [36] suggested that, to understand the functional significance of AMF in disturbed and contaminated systems, "keystone" species (species with an important role in the ecosystem) needed to be identified in the soil system in order to study the interaction processes with the contaminants. Thus, more assessments regarding fungal diversity on time and space in PTE contaminated soils are necessary. In addition, studies of the characterization of AMF species may be helpful for a better understanding of the participation of AMF in contaminated soils (see next section).

Arbuscular mycorrhiza colonization has also been observed in several metallophyte plants, which are plants commonly found on natural contaminated soils — for example: *Armeria maritima* spp. and *Viola calaminaria* [37,38] and in coexistent facultative metallophytes such as *Campanula rotundifolia* [39]. However, studies regarding the ecological role of AMF in these plants are just starting. From all these data on AM fungi in PTE polluted soils, it is rather difficult to relate the presence of AM fungi to the toxicity of PTE because physical and chemical soil characteristics and bioavailability of the PTEs were not always estimated; also, the effect of PTEs on AM fungi cannot be separated from their effect on the host plant [20].

12.3.1.2 Effect of AMF on Plant Uptake and Translocation of PTEs

It has been observed that AMF participate in the uptake of certain essential elements by plants growing on soils with nutrient deficiencies. Phosphorus uptake has been studied extensively. Apparently, the symbiosis is also important for the uptake and translocation of other essential and relatively immobile nutrients, such as Cu and Zn [9]. However, under high concentrations of PTEs in soil, there is disagreement in the literature about the participation of AMF to PTE uptake. Some authors have reported that AMF increase the uptake of PTEs [40–44]; others have reported a decreased uptake of PTEs and a reduction of toxic effects in plants with AMF [45–48].

The inconsistency of the AMF effect on PTE uptake may be a consequence of different factors involving fungi and plant species (nutritional conditions, PTE tolerance in both organisms) and the type of PTEs (degree of toxicity, levels, and speciation) in soil. In accordance with this, precautions should be taken in the selection of plants for studies of PTE uptake because differences in nutrient uptake between plants species and even varieties have been observed. Chaney [49] reported that plants growing on the same soil showed differences in uptake. For instance, spinach contained 10 times more Zn than did tall fescue, orchard grass 15-fold more Ni than did maize, and chard 5 times more Cu than did fescue. This wide difference in the uptake of PTEs among crop plants has been reported to be due to inherent root uptake (root distribution and depth) or to soil–plant interactions.

Streitwolf–Engel et al. [50] suggested investigating the effects of different AMF with more coexisting plant species. Additionally, Raju et al. [51] and Ravnskov and Jakobsen [52] mentioned

that experiments demonstrating differential, and sometimes controversial, effects of AMF species have been carried out on single plant species and using AMF originating from different soils, which may never have naturally co-occurred with the plant under study.

Other factors affecting uptake of PTEs may be the source; amount of available metals; spatial distribution; and source and chemical speciation of the PTEs in the soils [49,53]. The source of PTEs may have a strong influence on AMF, and it may have an effect on the symbiosis. Overall, PTE cations are the most toxic inorganic metal forms, and their solubility generally increases with decreasing pH. As a result, mobility and bioavailability are enhanced and also toxicity to biological systems [54].

When PTEs are added as soluble salts, they generally show greater plant uptake and toxicity than when applied as sewage sludge or metal oxides [49]. Long incubation times are necessary to ensure that these are in equilibrium with the soil constituents and the less available bound forms complexed to organic matter and clays (which naturally takes place under field conditions). The types of PTEs have also been considered to be important for uptake and translocation by mycorrhizal plants because each PTE possesses specific chemical and physical characteristics and crop plants differ widely in uptake [49], translocation, and tolerance to PTEs [55].

12.4 MECHANISMS IN AMF TO TOLERATE PTEs

Unlike ericoid and ectomycorrhizal associations, in which the mechanisms of tolerance to PTE are more intensively studied, the participation of AMF in plant PTE tolerance is less clear. Consequently, the mechanisms involved have not been completely elucidated. An important reason is that a great proportion of AMF do not grow on conventional media; because these fungi are obligate symbionts, they cannot be cultured without a host root. This makes the studies more complicated. As a result, much of the information on the mechanisms involved has been inferred from plant response to PTEs and from observations on fungal structures in colonized roots. Thus, in some cases it is difficult to separate fungal vs. root participation.

Various mechanisms have been proposed for explaining the responses of plants to high PTE concentrations. Primary effects can be distinguished at the molecular, biochemical, and cellular level. Subsequently, effects at the physiological and organismal level can be observed [56].

Levitt [57] proposed three basic strategies for organisms dealing with high PTE concentrations: avoidance, detoxification, and biochemical tolerance. The avoidance mechanism involves PTE exclusion mechanisms, which operate at two levels: restriction of uptake and restriction of transport. The process of detoxification is essentially similar, but avoidance of toxicity results from subcellular PTE concentration or by binding. Biochemical tolerance reflects the presence of specialized metabolic pathways and enzymatic adaptations.

Research performed with microorganisms and plants has shown that the general PTE tolerance mechanisms are

- PTE-binding to cell wall
- Restricted influx through the plasma membrane
- Active PTE efflux
- Compartmentalization in the vacuole
- Chelation at the cell wall and cytoplasm

It is suggested that in plants [55,58] and in fungi [21], tolerance to PTEs may be a range of orchestrated responses and no universal pattern of response to PTEs exists because different species vary in their response to any particular metal. There is further variation in expression of tolerance to different PTEs within the same species or ecotypes.

12.4.1 Binding to Cell Wall

In related information with filamentous fungi, Gadd [21] reported that the cell wall is the first cellular site for interaction with PTEs. It is a protective barrier controlling the PTE uptake. Different components of wall structure and composition may create a variety of potential sites for sequestration at the cell wall level, including carboxyl, amine, hydroxyl, phosphate, and sulphydryl groups [59]. Polysaccharides like chitosan and chitin have been reported to participate in the binding of PTEs. However, other cell wall compounds may also participate — for example, melanins, glucans, and mannans [60–62]. Chitin and β(1–3) glucans are the main wall components in Glomus species [9].

Binding of PTEs at the cell wall level has been reported on internal hyphae of AMF-colonized roots [63] and on external mycelium (EM) of these fungi [64] grown in contaminated soils. Using scanning and transmission electron microscopy equipped with an energy dispersion system and rhodizoniate histochemical staining, Turnau [63] was able to demonstrate localization of PTEs in colonized roots of *Euphorbia cyparissias* L. collected from Zn wastes. This author reported that 80% of the total intraradical mycelium showed a high content of PTEs. On the surface of this mycelium, amorphous or crystalloid deposits could be distinguished. High levels of S, P, and As were found in the former deposits, and Ca, Fe, Zn, and less As were found in the crystalloids. Si, Pb, Cu, and Zn were found in both deposits. PTEs were below detection limits within the walls of intraradical fungal hyphae. Additionally, small deposits containing PTEs were found on the inner layer of the spore wall of some of the morphotypes found at the site.

Gonzalez et al. [64] showed that the extraradical mycelium (EM) of AMF appears to provide an efficient surface for Cu sorption. These authors found that EM was able to adsorb Cu in a range from 3 to 14 mg g^{-1} of dry mycelium. A high Cu-sorption capacity was observed in the EM of two AMF originating from the same contaminated soil (*G. mosseae* BEG-132 and *G. claroideum* BEG-134) and also in *G. mosseae* BEG-25 coming from an agricultural soil.

Spores of AMF are structures that also are involved in the binding of PTEs. Sánchez Viveros et al. [65] reported that spores of *Glomus mosseae* BEG-132 sequestered Cu in a range from 470 to 680 μg g^{-1} of Cu (spore dry weight).

12.4.2 Extracellular Chelation

Gadd [21] mentioned that, at the cellular level, many organic fungal metabolites, which are efficient PTE chelators, may interact with PTE by complexation or precipitation. Some examples of these metabolites may be oxalic, citric acids [22,66], siderophores, and riboflavin [67].

Glomalin appears to be an efficient agent sequestering PTEs, not only by hyphae from the EM, but also by the colonized roots and through deposition of glomalin in the soil. In three different experiments, Gonzalez–Chavez et al. [68] tested whether glomalin sequesters different PTEs. Glomalin was extracted from polluted soils or purified from a *Gigaspora rosea* isolate or it was extracted from two AMF grown under three different levels of Cu. The results showed that:

- Glomalin sequestered Pb, Cu, and Cd in high concentrations in two polluted soils
- Glomalin produced by *Gigaspora rosea* was able to remove Cu, Zn, Co, and Ni from solution, but not Ca, K, and Mg
- The highest amount of Cu sequestered was 28 mg Cu g^{-1} glomalin

Binding and sequestration of PTEs by different fungal structures of AMF may result in the stabilization of these elements, reducing their availability and decreasing the toxicity risk to other soil microorganisms and plants growing in these sites. Table 12.1 shows the activity of glomalin and fungal structures sequestering different PTEs. When glomalin is involved in the sequestration, the importance of stabilization phenomena increases because of its copious production and recal-

TABLE 12.1
Sequestration of Potentially Toxic Elements (PTEs) by External Mycelium, Glomalin, and Spores of A MF

Fungal structure or bioproduct	PTE involved	Amount sequestered (mg/g)	Observations	Ref.
External mycelium	Cu	3–14	Excised mycelium exposed to 30 μ*M* Cu solution for 120 min	64
Glomalin				
	Cu	1.5–4.3	Glomalin extracted from polluted soils	68
	Cd	0.2–0.08		
	Pb	0.6–1.12		
	Cu	7–28	Purified glomalin from an isolated of *Gigaspora rosea* exposed to 50 m*M* Cu solution. Precipitation occurred as soon as glomalin and Cu solution were mixed	
	Cu	1.13–1. 6	Glomalin extracted from hyphae in active growth under three levels of Cu (0.8, 10, and 20 μ*M* Cu)	
		0.3	Glomalin extracted from hyphae colonizing roots of *Sorghum vulgare*	
		0.4	Glomalin extracted from sand supporting external mycelium growth	
Spores	Cu	0.4–0.6	Spores of *Glomus mosseae* BEG-132	65

citrance in the soil [18]. Additionally, it is known that glomalin sequesters Cu not only by reversible reactions, such as ion exchange, but also by a strong and irreversible binding [68]. It is an important property of AMF and helps to understand their role in polluted soils.

12.4.3 Chelation at Cytoplasm and Vacuole Level

Chelation in the cytoplasm is an intracellular buffer system to control PTE toxicity through reduction of the concentration of cytotoxic-free PTE ions. In this system, metallothioneins (MT), metal-binding polypeptides, and/or MT-like proteins may be involved. In contrast to ectomycorrhizal fungi, the participation of these compounds in metal detoxification has not been confirmed in AMF [69,70]. However, evidence suggesting the presence of Cd-binding thiols in AMF as well as high concentrations of N and S in mycorrhizal roots exposed to Cd have been reported [71,72]. Galli et al. [72] studied the effect of Cu on the uptake, amount, and composition of Cu-binding peptides (Cu-BPs) of mycorrhizal maize plants inoculated with *Glomus intraradices*. They observed increased concentrations of the thiols cysteine, γ-glutamylcysteine, and glutathione up to an external Cu supply of 9 μg g^{-1}. However, the amount of thiols in Cu-BPs was not increased by mycorrhizal colonization in Cu-treated plants and no differences in Cu-uptake were detected between nonmycorrhizal and mycorrhizal plants.

Gonzalez [25] observed that the EM grown in a Cu-contaminated substrate presented higher tyrosinase activity (353 nmol mg^{-1} dry mycelium min^{-1}), in contrast to the tyrosinase activity in the EM propagated in noncontaminated substrate (153 n*M* mg^{-1} dry mycelium min^{-1}). The Cu substrate concentration (19 mg Cu g^{-1} substrate) induced a 2.3 times increase in tyrosinase activity in the EM of AMF tested. *Glomus caledonium* BEG-133 exhibited the highest tyrosinase activity, and *G. mosseae* BEG-132 and *G. claroideum* BEG-134 presented statistically similar activity. Comparisons using more species in different Cu levels are necessary in order to prove tyrosinase participation in Cu chelation and detoxification in AMF. This mechanism of chelation has been observed in other filamentous fungi, yeast, and ectomycorrhizal fungi [73–76].

An increase in PTE tolerance in plants has been suggested to be related to genes up-regulated in AMF-colonized roots. Rivera–Becerril et al. [77] reported that the expression of metallothionein genes increased in Cd-treated plants, but one gene (pcs) appeared to be activated specifically in Cd-treated mycorrhizal roots. In relation to these fungal PTE tolerance mechanisms, the use of visualization methods, such as transmission and scanning electron microscopy (TEM, SEM); energy-dispersive x-ray microanalysis (EDAX); and electron energy loss spectroscopy (EELS), to determine the extracellular and subcellular localization of elements has been very useful.

Gonzalez et al. [64] reported that crystal-like aggregates mainly comprising Fe were found on the mucilaginous outer hyphal wall layers of three AMF, when grown in Cu/As-contaminated soil. It was shown that, in the EM of *G. mosseae* BEG132 and *G. claroideum* BEG134, these aggregates contained significant amounts of Fe and Cu. This was not the case for *G. caledonium* BEG133; its aggregates only contained Fe.

In another comparative study, Turnau et al. [78] studied mycorrhizal roots of *Pteridium aquilinum* collected from Cd-treated experimental plots. They showed that hyphae of AMF uniformly colonizing cortical cells contained a much higher amount of PTEs than the cytoplasm of the host cells. They suggested that the ability of this plant to detoxify PTEs was due to its association with AMF. Most of the Cd was located in phosphate-rich material fungal vacuoles, which contained S, N, Al, Fe, Ti, and B. Thus, intracellular sequestration of PTE by fungal polyphosphate intracellularly into the fungus may contribute to decreasing its transfer to the plant.

Polyphosphates are produced widely by microorganisms forming an important intracellular storage of phosphate. However, these compounds may also be involved in the regulation of concentrations of PTE ion in cells [21]. Polyphosphate granules are maintaining ionic compartmentalization of PTEs and it has been observed that their biosynthesis accompanies vacuolar accumulation [79].

Gonzalez et al. [64] reported that the EM of *Glomus mosseae* BEG-132 growing in a contaminated substrate (As/Cu), contained intracellular Cu-rich bodies. In addition, traces of arsenic were also observed on the EDAX spectra of these bodies. The presence of arsenic was explained by the elevated concentrations in the soil in which the AMF were grown. Arsenate and phosphate are chemically very analogous, so this result suggests that arsenic may sequester Cu in the form of Cu-arsenate complexes in the cytoplasm of the EM of *G. mosseae* BEG132. The arsenic and Cu accumulation in *G. mosseae* BEG132 is interesting to study because detoxification of PTE within cells of several yeast species has been shown to be linked with polyphosphate granules located in the cytoplasm [79] and vacuoles [80].

12.4.4 Other Possible Mechanisms in AMF

Gonzalez et al. [81] reported that AMF isolated from a mine-spoil soil conferred enhanced tolerance to their host. AMF benefit the host on mine soils through a considerable reduction in arsenic uptake, particularly in the fraction of arsenic translocated to the shoot. The mechanisms remain unclear, but the authors suggested two alternative hypotheses:

- The fungi may have suppressed phosphate/arsenate uptake across the plasma membrane, which is the mechanism of arsenate tolerance observed in most higher plants investigated thus far [82].
- Enhanced efflux of arsenate, which is the mechanism used by an arsenate-tolerant ericoid mycorrhizal fungus (*Hymenoscyphus ericae*) and other microorganisms [83–85] may have taken place.

Elucidation of other mechanisms in AMF is required to gain knowledge about fungal participation on plant metal tolerance and their role in contaminated soils. Production of organic acids for metal chelation at the cell wall level; metallothioneins and polyphosphates for compartmentalization at the

vacuolar level; and specific proteins may be potential mechanisms for PTE detoxification in AMF. Such mechanisms have already been observed in ectomycorrhizal fungi [69,70,86–88].

When considering other fungal mechanisms, indirect effects of AMF on plant nutrition; changes in root exudation; effects on rhizosphere microbial communities; soil structure; protection from stress environmental factors; etc. should be also mentioned.

12.5 CONTRIBUTION OF AMF IN PLANT TOLERANCE TO PTEs

The contribution of AMF to plant tolerance of PTE is poorly documented compared with that for ectomycorrhizal [7,89–92] and ericoid mycorrhizal plants [5,6]. Research has mainly focused on the effect of PTEs on the growth of arbuscular mycorrhizal plants compared with nonmycorrhizal plants and their effects on colonization.

Meharg and Cairney [36] suggested three possible roles of mycorrhizal fungi in plant tolerance to PTEs:

- Enhancing plant PTE tolerance. In this case, fungal tolerance is not necessarily an important characteristic to help plants establish and survive in contaminated soil conditions. This is true for ericoid mycorrhizal fungi [5,6]. Bradley et al. [6] demonstrated fungal protection in *Calluna vulgaris* by fungi isolated from contaminated and noncontaminated soils. Thus, fungal tolerance was not an important characteristic to confer tolerance to Zn and Cu in the ericoid association. It means tolerant fungi may be found in noncontaminated soils; therefore, the fact that a fungus is isolated from noncontaminated soils does not mean that it is not tolerant.
- Just satisfying their normal role in the association. In this case, plant benefits from the symbiosis may involve uptake of P and other essential plant nutrients and protection against abiotic and biotic stresses, without any enhanced tolerance to PTEs. In some cases, it is probable that plants may not require their fungal symbiont to achieve tolerance, but plant ability to grow on contaminated soils may be strongly enhanced by being mycorrhizal.
- Fungi enhance plant tolerance to PTEs. In the second and third points, mycorrhizal fungi, like their hosts, need to be PTE tolerant. Several authors also suggested that tolerant behavior of the fungi might be an important factor conferring plant tolerance. However, the efficiency of the protection differs among fungi, and it depends more on the compatibility between fungal isolates and the host plant rather than on fungal tolerance to PTEs [7,69,87,90,93].

Arbuscular mycorrhizal fungi have been thought to participate in plant tolerance because they can modify the host PTE uptake and offer plant benefit. Gonzalez et al. [81] observed that inoculation with AMF increased shoot growth and tiller production approximately twofold compared to noninoculated plants of an arsenate-tolerant genotype of *Holcus lanatus* (a grass species commonly accumulating As and different PTEs) when growing in polluted soils. Additionally, lower As shoot accumulation (threefold) was observed in mycorrhizal than nonmycorrhizal plants. Phosphate:arsenate ratio was also threefold higher in inoculated than in noninoculated plants. These authors discussed that the ability of this grass to grow on strongly contaminated soils was due to the mycorrhizal association. Additionally, they suggested that other benefits may result form the mycorrhizal plant status, which may improve fitness and health of *H. lanatus* grown in polluted soils.

In an experiment using mycorrhizal and nonmycorrhizal excised roots, it was shown that AMF, regardless of their arsenate tolerance, reduced arsenate influx in arsenate-tolerant and -nontolerant *Holcus lanatus* plants [81]. Nontolerant plants had higher levels of AM colonization than tolerant plants for tolerant and nontolerant AMF strains (*Glomus mosseae* BEG-132 and BEG-25, respectively). This suggests that AMF may play a greater role for nontolerant plants than for tolerant plants. In another experiment, the authors observed that AMF seem to down-regulate arsenate/phos-

phate transport. Higher P concentrations in colonized roots of tolerant plants in comparison to nonmycorrhizal roots were observed when plants were grown in contaminated soil for 16 weeks. The explanation given was that the higher P levels in the roots may be regulating the kinetics of phosphate:arsenate uptake [81].

Bethlenfalvay and Franson [94] also observed significantly lower concentrations of Mn in mycorrhizal plants than in nonmycorrhizal plants and the absence of symptoms of Mn toxicity. However, they concluded that the reduced toxicity of Mn was not a direct response of colonization of *Glomus mosseae*, but probably an indirect influence on plant functions. Nogueira and Harris [95] reported similar conclusions: higher P concentrations in mycorrhizal plants probably indirectly decreased the toxic effect of Mn.

12.5.1 Importance of the External Mycelium of AMF in Plant Tolerance to PTEs

Participation of the EM of AMF, mediating almost all benefits that these fungi offer to their plant hosts, is a relevant fungal structure that should be taken into consideration. External mycelial networks link roots to the soil matrix. It is an important component in the symbiosis and participates extensively in nutrient cycling [96]. Although the EM has been neglected for many years in research of AMF, some authors have highlighted its importance [97–99].

Studies regarding the EM have shown that this fungal structure represents a fundamental component of the AM associations in noncontaminated soils. Olsson et al. [100] reported that EM may represent around 90% of the total mycelium in established arbuscular mycorrhiza. Interestingly, runner hyphae grow distantly from the colonized roots and can produce higher biomass than soil with high root densities [100].

Recently, the different methods of propagation of AMF using plants and compartmented pots, using mesh bags to keep roots in a compartment and hyphae in another compartment, have allowed studies regarding the EM and how it deals with PTEs. With compartmented pots, it is possible to obtain enough material for experimental purposes — as much as 100 mg of dry mycelium per 1-kg pot [101,102].

Excised external hyphae have the capacity to sequester Cd and Cu when exposed to solutions containing these elements [64,103]. Additionally, Gonzalez–Chavez et al. [68] showed the special ability of glomalin from hyphae of the EM to sequester Cu (1.6 mg Cu g^{-1} glomalin) when compared to glomalin extracted from roots (0.3 mg Cu g^{-1} glomalin) and sand (0.4 mg Cu g^{-1} glomalin). The hyphal capacity for sequestration increased significantly as the level of Cu increased in the growth substrate at 10 or 20 μM (1.60 and 1.63 mg Cu g^{-1} glomalin, respectively) vs. a 1.13 mg Cu g^{-1} glomalin without Cu addition.

12.6 CONSTITUTIVE AND ADAPTIVE METAL TOLERANCE IN AMF

Many plant species adapted to metalliferous soils are colonized by AMF in these environments [104]. For instance, Gildon and Tinker [29] and other researchers have reported abundant root colonization of some species, which are considered to belong as glacial relicts (10,000 to 15,000 years ago). *Viola calaminaria* is a good representative of this group [39]. Ernst [105] mentioned that evolution of the symbiosis demands coevolution of both organisms: AMF and plants. This suggests that AMF associated with plants necessarily take part in PTE tolerance and eventually plants and fungi colonize metalliferous soils. This also allows the coexistence of tolerant plants and other, less tolerant plants.

It has been suggested that AMF may evolve Zn, Cd, and As tolerance [3,29,81,106,107]. Tolerance to PTEs is typically a qualitative characteristic, which correlates with the prevailing levels of PTE availability in soil [108]. Apparently, many organisms have developed a variety of adaptive mechanisms for surviving in contaminated environments.

It was suggested that AMF isolated from contaminated soils would have a much higher PTE-binding capacity than AMF isolated from noncontaminated soils [3,106,107]; however, observations from Joner et al. [103] for Cd and Gonzalez et al. [64] for Cu show that these may have similar behavior independently of the nature of the site of isolation. Joner et al. [103] found that a Cd-tolerant *Glomus mosseae* strain possessed the highest Cd sorption capacity; two other AMF isolated from polluted soils (PS) had low sorption capabilities comparable to other strains from nonpolluted soils (NPS). Gonzalez et al. [64] observed that *G. mosseae* BEG-132 and *G. claroideum* BEG-134 (from PS) and *G. mosseae* BEG-25 (from NPS) had high Cu sorption capacity in comparison with *G. caledonium* BEG-132 (from PS) and *Gi. rosea* BEG 111 (from NPS). The fungi BEG-132, BEG-133, and BEG-134 were isolated from the same contaminated soil and they showed different responses to Cu. This suggests functional diversity of AMF.

Fungi differ in their mechanisms and capabilities to deal with PTEs, which may be related to the different strategies to control PTEs toxicity, i.e., using TEM, Gonzalez et al. [64] observed that *G. caledonium* BEG-133 appeared to avoid intracellular accumulation of As/Cu, but *G. mosseae* BEG-132 intracellularly accumulated high amounts of Cu and traces of As. Both these fungi were isolates from PS. Using spore germination test in polluted substrates, Gonzalez [25] reported that *Gigaspora rosea* BEG-111 spores were able to germinate at high concentrations of As and Cu, but retractile cytoplasm was observed in the germination tube. Sánchez–Viveros et al. [65] showed that spores of *G. claroideum* Zac-19 (isolated from NPS) germinating in polluted substrates presented negative chemotropism. This effect increased as the concentration of As/Cu increased in the substrate of germination.

Several studies with filamentous fungi and bacteria show that tolerance may be obtained by sequential exposition to PTEs [109]. However, irrefutable evidence showing that AMF present adaptive or constitutive tolerance is still lacking. Weissenhorn et al. [3] studied a fungal strain from noncontaminated soil and found that it was able to develop tolerance to Cd after 1 year of exposure to 40 mg of cadmium nitrate kg^{-1} of soil. However, when propagated under uncontaminated conditions, its tolerance was lost over a similar period. Malcová et al. [110] showed reduced Mn tolerance of AM fungal isolates due to exclusion of stress levels of Mn from the growth medium used to maintain their fungal cultures (2 years). They suggested that tolerance mechanisms are only advantageous when the selection pressure is present.

Sánchez–Viveros et al. [65] showed changes in tolerance (tested by spore germination) to As/Cu in spores of fungi propagated continuously or not in free-pollution substrate (Figure 12.3). In spores of *G. mosseae* BEG-132, when the fungus was propagated for 2 years in polluted substrate, higher germination percentages were observed at the three highest levels of As/Cu tested. In contrast, *G. caledonium* BEG-133 showed more stable responses at the different As/Cu concentrations. In relation to the fungus isolated from NP soil, *G. claroideum* Zac-19, spore germination was drastically affected by As/Cu concentrations. Germination was lower in spores propagated for 1 year in polluted substrate in comparison to free-exposition spores, except at the highest level of As/Cu concentration. These results indicate that the *G. mosseae* BEG-132 spores germinate properly under high pollutant concentrations if continuous propagation occurs in polluted substrate in comparison with spores propagated in unpolluted substrates or alternate discontinuous cycles. Conversely, for BEG-133, absence or not of pollution during propagation does not affect importantly the germination percentage.

As a strategy of PTE tolerance, AMF and other fungi may modify structurally [111,112]. Sánchez–Viveros et al. [65] observed that spore cell wall thickness of three fungal species increased after As/Cu exposition in polluted substrate (Table 12.2). The fungi tested were isolated from PS or NPS. Spore cell wall thickness of *G. mosseae* BEG-132 (from PS) significantly increased after a 2-year, continuous polluted-substrate propagation. Higher cell wall thickness was observed in *G. caledonium* BEG-133 (from PS) and G. *claroideum* Zac-19 (from NPS).

Some species seem to be able to adapt rapidly to changing soil conditions, such as contamination. For example, *G. mosseae*, *G. fasciculatum,* and *G. claroideum* are frequently found in

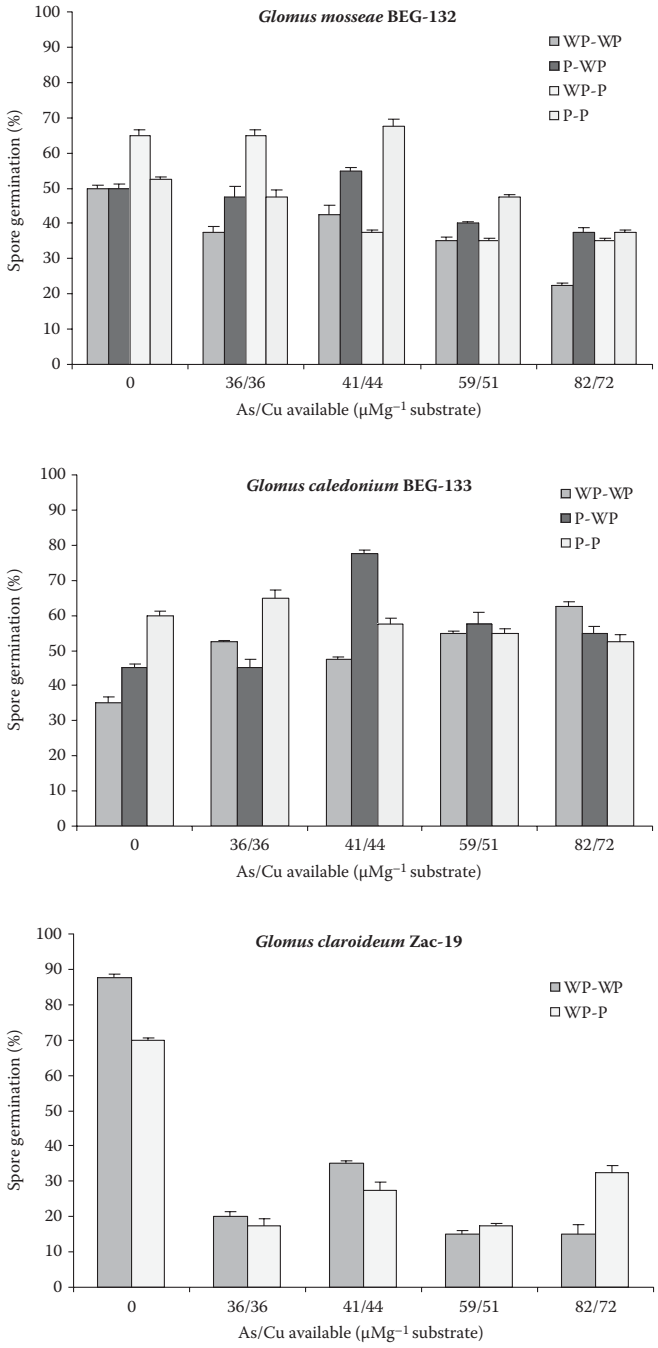

FIGURE 12.3 Spore germination of three arbuscular fungi after 2-year propagation in polluted (P) or not polluted substrate (WP). Germination tests were set on substrates with five As/Cu concentrations by open filter method (Brundett and Saito, 27, 85, 2005. *J. Soil Biochem.,*) At 25 days after germination. Treatments: WP–WP = free-pollution fungal propagation in two 1-year cycles; WP–P = free-pollution propagation first year, pollution second year; P–WP = pollution-propagation first year, free-pollution second year; P–P = pollution-propagation for two 1-year cycles. (Sánchez–Viveros, G. et al., *Rev. Int. Cont. Ambiental*, 20, 147, 2005. With permission.)

TABLE 12.2
Spore Thickness of Three Arbuscular Fungi Propagated in Polluted Substrate

Fungi	Propagation treatment	Spore thickness (μm)
G. mosseae BEG-132[a]	WP–WP	5.4b[c]
	P–P	5.9a
G. caledonium BEG-133[a]	WP–WP	3.9b
	P–P	6.0a
G. claroideum Zac-19[b]	WP–WP	5.4a
	WP–P	7.4b

[a]Fungi isolated from polluted soils and propagated for 2 years in P = polluted substrate and WP = free of pollution.
[b]Fungus isolated from nonpolluted soil propagated for one year in P = polluted substrate and WP = free of pollution.
[c]Means from 90 observations, values with the same letter within each fungus represent no significant difference (Tukey $\alpha = 0.05$).

Source: Modified from Sánchez–Viveros, G. et al., *Rev. Int. Cont. Ambiental*, 2004, in press. With permission.

diverse terrestrial systems with high adaptation to different environmental and edaphic conditions.[4,26,113]. This may be the result of phenotypic plasticity as suggested by Weissenhorn et al. [3] and Meharg and Cairney [104].

To select fungi colonizing roots growing in contaminated soil and at the same time accumulating PTEs, Turnau et al. [35] used a molecular technique and histochemical staining with rhodizoniate simultaneously. They reported that only 5% of the roots showed PTE accumulation when stained with rhodizoniate. Interestingly, *G. mosseae* was the only fungus present in approximately 75% of these stained roots. Other fungi, such as *Glomus intraradices*, *G. claroideum*, and two *Glomus* species occurred with lower frequency in roots accumulating PTEs (25%). These observations in contaminated soils support the concept of functional biodiversity, which has been observed in natural ecosystems.

Sánchez–Viveros et al. [65] showed that some isolates of AMF may have an inherent ability to tolerate elevated PTE concentrations in soil. These authors observed that spores of *G. claroideum* (Zac-19), a fungus isolated from agricultural soils, propagated for 1 year in soils contaminated with As/Cu or in noncontaminated soils, were able to tolerate elevated concentrations of these pollutants, even if its spores presented a lower percentage of germination (20 to 30%) than in NP with 90%. In contrast, two AMF (from PS) grown on an As/Cu-contaminated soil presented high germination (50 to 70%), independently of the level of As/Cu present in the soil. This result shows that *G. claroideum* Zac-19 with low spore germination may be able to colonize plants, thus assuring its survival in polluted soils. For ectomycorrhizal fungi, Blaudez et al. [114] reported a strong interspecific variation in terms of PTE tolerance. In AMF, this variation may also be observed. Adaptive metal tolerance has been reported for a population of the ectomycorrhizal basidiomycete *Suillus luteus* originating from a metal-contaminated former zinc smelter site [115].

More research is necessary in order to gain knowledge about the stability of fungal tolerance during their cultivation in unpolluted substrates, including fungi isolates from polluted and nonpolluted soils. By now, it is still difficult to state the adaptive or constitutive tolerance in AMF. One problem in estimating arbuscular mycorrhizal tolerance to PTE using only the spore germi-

nation test is that it may not reflect the potential effect of the fungus on plant tolerance, as mentioned before. Even with a low germination percentage, AMF may colonize the plant.

Additionally, often no correlation exists between root rate colonization and fungal effect on the plant. It is possible to find significant effects on plant growth, nutrients, and survival at a low colonization rate. Thus, fungal tolerance within a plant should be investigated too; however, it is very difficult to evaluate. Another important remark is that qualifying a fungus to be "tolerant" to PTE means more tolerant to an element than another fungus, and tolerant to a certain degree in particular conditions ("bioavailability" is often not discussed). Thus, it is rather a relative tolerance.

It is relevant to emphasize that fungal species selection, imposed by any stress, does not always offer the optimal AMF symbionts for the function of the soils [116]. Selection of fungal species may have strong implications in the functioning of contaminated soils. Lokke et al. [117] suggested that a loss of AMF species diversity may increase the susceptibility of the host plant to suffering from environmental stresses.

12.7 USE OF AMF IN PHYTOREMEDIATION PRACTICES

Phytoremediation of soils polluted by EPTs is based on two processes: phytostabilization and phytoextraction [118]. In the first case, plants may reduce metal availability and risks of these elements in the soil. Phytostabilization considers the role of plant roots in order to control the uptake and inactive maintenance of EPTs in the soil, which decreases environmental and human risks of these toxic elements. This alternative demands the establishment of a closed vegetation cover, preferably within a short term [119]. Very high concentrations of PTEs in the soil and consequent plant toxicity are the most important limitations of this approach. The main aim of phytostabilization is to install a sustainable ecosystem, where plant biodiversity and functional stability are required [39]; thus, grasses and other herbaceous plants, shrubs, and trees may be useful for a successful remediation of soils using plants.

On the other hand, because PTEs are persistent elements, removal is the best way to ensure minimal ecological risk [39]. Phytoextraction demands decontamination of EPTs from the soil by use of high accumulating plants; especially, hyperaccumulating plants are required. These plants are able to take up and accumulate significant amounts of EPTs (more than 100 mg Cd kg^{-1} dry weight, 1000 mg Co, Cr, Cu, and Pb kg^{-1} dry weight, or 10,000 mg Ni and Zn kg^{-1} dry weight) in their aerial parts [118,120].

In these technologies, plant roots act directly for mechanical contaminant entrapment with consequent protection of the soil surface against wind and water erosion and a reduction of leaching. Microbial activity may indirectly act on these processes [121]. When using plants for remediation, participation of symbiotic microorganisms should not be ignored because these play an important role in nutrient cycling and in the behavior of PTEs in the soil. Plant–microorganism interactions have received little attention in remediation of soils polluted by PTEs.

Despite the role that AMF play in the establishment, survival, and productivity of plants [1,2], relatively few studies have focused on the use of these fungi in the bioremediation of soils polluted with PTEs. These organisms may be crucial for revegetation efforts following plant-based containment or removal technologies. For example, phytostabilization requires plant species adapted to excessive contaminant concentrations in the soil, well developed root systems, and closed coverage of the soil surface. A protective role against PTE toxicity for plants susceptible to colonization by AMF is an important alternative when remediation using plants is needed. Any mechanism reducing plant exposure to PTEs has potential as an applied remediation technology. The use of AMF may help accumulate PTEs in a nontoxic form within the plant roots and the external mycelium.

In addition to high toxic content of PTEs, polluted soils in general also have several limitations, such as low levels or low bioavailability of essential elements and low soil aggregation, which make establishment of plants and soil remediation more difficult. The use of AMF may help to alleviate some of these limitations. Evidence shows that these fungi colonize grasses and other

plants, which are often growing in polluted and degraded soils, such as mine soils [81,104]. Some of these plants are "facultative mycorrhizal" in nonstressed conditions; this means that they are colonized by AMF, but the plants have low or no benefits from the mycorrhizal association. In contrast, under stressed conditions, these plants can profit from multiple nutritional and protection benefits from the association to AMF [81]. Some authors have suggested that AMF may help to alleviate other stresses found in PTE-polluted soils, such as drought, salinity, low availability of major nutrients, etc. [9, 104].

Although the role of AMF in remediation of polluted soils is uncertain, it could potentially help to establish a more diverse plant ecosystem [122]. Shetty et al. [123] reported that initial colonizers of PTE-polluted soils tend to be nonmycorrhizal, but the appearance of mycorrhizal plants derives successful restoration; an increase in plant community production; and the improvement of soil structure. Successful examples of the use of AMF in plant establishment on highly perturbated and polluted sites is starting to become a reality. For instance, Dodd et al. [113] showed that inoculation of different plants with AMF was a relevant strategy for soil reclamation of the chalk platforms deposited from the construction of the tunnel under the English Channel, between France and the U.K. These authors were able to promote plant establishment and revegetation in this area using the inoculation of native AMF and plants.

Many plants are obligate mycorrhizal, which means that they require the fungal symbionts to establish, grow, and survive under harsh physical and chemical soil conditions. Thus, phytoestablishment of PTEs using AMF may be an attractive, low-cost, and environmentally friendly alternative. However, some factors, such as selection of tolerant plants to EPTs and selection of the best fungal isolates for a given phytoremediation strategy, should be considered when using AMF in phytoremediation; when extremely high concentrations of PTEs are found, a combination with soil amendments in order to decrease bioavailability and toxicity of PTEs may be an important tool to realize successful soil remediation.

Selection of AMF for their use in phytoremediation practices may include fungal properties for root colonization; sequestration or accumulation of EPTs; uptake of essential nutrients; and soil stabilization properties.

Colpaert [39] suggested that inoculation of AMF suitable and adapted to polluted and climatic conditions is relevant to a successful soil remediation when native mycorrhizal fungi are absent. Plant inoculation of introduced plants in polluted soils and basic management agriculture practices increase the performance, development, and spread of AMF in these soils. For trees and shrubs colonized by AMF, inoculation in the nursery stage is the ideal method to introduce AMF in the soils, including polluted soils. When low AMF populations are present in polluted soils, proper management practices, such as multiple mycorrhizal hosts, low fertilization and pesticide levels, and no tillage, may help to increase fungal performance.

Small-scale demonstration experiments show that phytoremediation is an economical remediation alternative using different plants for slightly contaminated soils; however, for heavily contaminated soils, highly EPT-tolerant plants and hyperaccumulator plants should be used to revegetate [124]. Tolerant plants, which are mycorrhizal, have shown good performance in small-scale remediation experiments. Some examples include [39,119,125]:

- *Agrostis capillaris*
- *A. stolonifera*
- *Andropogon gerardii*
- *Festuca rubra*
- *F. arudinacea*
- *Holcus lanatus*
- *Deschampsia cespitosa*
- *D. flexuosa*
- *Dactylus glomerata*

Casuarina, *Acer*, *Salix*, and *Populus* spp. are also arbuscular mycorrhizal plants, which occur as pioneer species in early succession plant communities, and these are also used in phytoremediation of polluted soil.

Jasper [126] suggested that AMF in combination with other rhizospheric microorganisms, such as *Rhizobium* and *Frankia,* participating in N input to the soil diminish plant growth limitations and improve productivity of their hosts in polluted soils. *Rhizobium* and *Frankia* are important microbial components participating in N input to the soil.

Ernst [105] reported that few legumes may tolerate moderate PTE soil concentrations, for example, *Anthyllis vulneraria* and *Lotus corniculatus.* These plant species may be colonized by AMF and *Rhizobium*. Wu and Kruckeberg [127] reported *Lotus purshianus* and *Lupinus bicolor* as two tolerant species showing effective biological N fixation at a copper mine; however, *Lupinus* is a nonmycorrhizal plant. *Crotalaria* and *Coronilla* spp. may also be important species to recuperate polluted soils. Colpaert [39] suggested that leguminous species associated to *Rhizobium* and AMF may offer an advantage as pioneer plant colonizers in PTE-polluted soils.

It may be important to consider symbiotic microorganisms including AMF because they may assist on phytoremediation schemes. These rhizospheric microbes represent an alternative to clean or stabilize PTE-contaminated soils. Mycorrhizal plants may be of importance to build, cover, and avoid soil erosion, and at the same time stabilize PTEs in the soils.

In some plants, in an accumulator strategy, PTEs are actively concentrated within plant tissues over the full range of soil concentrations, implying a highly specialized physiology. The participation of AMF in this strategy has not been studied in detail. Davis Jr. et al. [128] showed that AMF enhance accumulation and tolerance to Cr in sunflower (*Helianthus annuus*). This plant has been reported to be able to accumulate high concentrations of PTEs, but, when colonized by AMF, higher Cr accumulation was observed without any evident toxic effect. In addition, greater mycorrhizal dependence, expressed by higher growth, was observed at higher Cr levels.

On the other hand, an extreme type of accumulation, described as hyperaccumulation, has also been identified in some plants. Many hyperaccumulators belong to the Chenopodiaceae and Brassicaceae families, which are nonmycorrhizal; however, the presence of AMF was recently demonstrated in members of Asteraceae that hyperaccumulate Ni [129]. Metallophytes in tropical and subtropical regions are potentially mycorrhizal plants, including species in the families Fabaceae, Lamiaceae, Asteraceae, and Poaceae. However, occurrence records on root colonization are lacking [39]. Knowledge about the role of AMF and soil biota in these kinds of plants is still lacking [37,38,130]. This topic needs more attention because zone bioavailability of EPTs is affected by microbial activities [131] and rhizospheric microorganisms represent an alternative to remediate soils, as mentioned by Ow [132].

Tonin et al. [37] reported that AMF from *Viola calaminaria* inoculated to clover increased eightfold and threefold the root concentration of Cd and Zn, respectively, without any significant difference in plant biomass and concentrations of metals in shoots. These AMF were efficient in sequestering metals at the root system. Hildebrandt et al. [38] reported that one *Glomus* isolated from this plant contributed to the accumulation of PTEs in plant roots in a nontoxic form.

The role of AMF in metallophyte or hyperaccumulator plants is unknown. Preliminary research showed that AMF may increase Zn accumulation in *V. calaminaria*; however, this effect depended on fungal species and Zn concentration. Fernandez–Fernandez et al. [133] found that *G. mosseae* BEG-132 (fungus from As/Cu-polluted soil) increased twofold higher Zn-shoot accumulation than *G. mosseae* BEG-25 (from NPS) and noninoculated plants of *V. calaminaria* grown in solutions containing 200 and 300 mg Zn L^{-1}. However, any significant difference was observed when plants were grown at 400 mg Zn L^{-1}.

This result opens another possibility to using AMF in phytoremediation practices into the phytoextraction alternative. All the former information shows the necessity of understanding the process involved in dealing with high concentrations of PTEs in mycorrhizal plants and fungal tolerance, which may have strong implications for the use of AMF in the biological remediation

of polluted areas. More research on AMF effects in hyperaccumulators, metallophyte, and plants growing in highly polluted soils is necessary. It should involve more fungi, PTEs, and different kinds of soils.

Chaney et al. [134] have shown evidence of effective *in situ* inactivation of PTEs, such as Pb, using a combination of technologies. They used phosphate addition and Fe biosolids compost for a 3-year test. Reduction in soil Pb bioavailability was demonstrated. Several chemicals used for inactivation also may increase the fertility of the soil and eliminate PTE toxicity to plants and soil organisms. Growing a plant cover physically stabilizes the soil and its contaminants in place; this, in turn, minimizes soil erosion and the transport of PTEs through the soil. Incorporation of amendments and plants is a natural method for restoring soil ecology compared to other techniques. Application of metal-binding soil additives in order to decrease PTE availability may be necessary. These additives may be substances with high metal-adsorption capacity, such as beringite and zeolites [135]; manures and plant residues [136]; compost; and fertilizers [137]. A combination of these chemical and physical technologies, in addition to the biological ones involving AMF, is highly recommendable for a more successful soil remediation.

12.8 FUTURE WORK

In order to use AMF into remediation technologies on a higher scale, it is still necessary to address different important aspects, such as to:

- Understand the ecology, physiology, and evolution of AMF involved in the sequestration and accumulation of PTEs and the mechanisms involved
- Learn about the biogeochemical processes involved in plant-based remediation of polluted soils
- Elucidate the role of AMF in the rhizosphere of high accumulators and hyperaccumulator plants
- Select tolerant AMF species by screening of different polluted conditions and metals (mines, sewages, and industrial sites
- Conduct more detailed studies regarding mechanisms, including genetic and molecular ones, involved in AM-mediated improvement of plant tolerance to PTE
- Increase the understanding of the extent of inter- and intraspecific variation of AMF
- Select successful plant–fungi combinations for suitable soil recuperation areas
- Compare different behavior of species alone and in consortia of native AMF in polluted soils to understand the function of the AMF species on this
- Conduct genetic and molecular studies regarding PTE tolerance in AMF, in general, because their mechanisms remain mostly unknown

12.9 CONCLUSIONS

A significant number of studies have shown the importance of mycorrhizal symbiosis for the establishment of a sustainable plant cover on soils with PTEs. However, among mycorrhizal fungi, considerable diversity in metal sensitivity is present and circumstantial evidence suggests that some mycorrhizal fungi have adapted to these adverse soil conditions. More studies are necessary to know whether PTE tolerance is indeed achieved at the population level. This includes the testing of large numbers of isolates, which can be difficult to realize. If particular tolerant isolates are used for applications, we need to better understand the stability of the trait over the long term in different conditions.

Our current knowledge of the PTE tolerance mechanisms in mycorrhizal fungi shows a very patchy picture. Several constitutive mechanisms in mycorrhizal fungi can avoid or reduce PTE

toxicity. However, it could be more interesting to investigate the adaptive mechanisms that may have evolved in some taxa colonizing very toxic soils. To be able to do so, it is necessary to obtain sufficient numbers of isolates from polluted soils — isolates that differ considerably from normal ecotypes in metal sensitivity.

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