Chapter 16
Arsenic Uptake and Phytoremediation Potential by Arbuscular Mycorrhizal Fungi

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16.1 Introduction

Arsenic (As) contamination of soils and water is a global problem because of its impacts on ecosystems and human health. Various approaches have been attempted for As remediation, with limited success. Arbuscular mycorrhizal (AM) fungi play vital roles in the uptake of water and essential nutrients, especially phosphorus (P), and hence enhance plant performance and productivity (Smith and Read 2008). As uptake and tolerance to As toxicity in plants are also enhanced by AM fungi (Zhao et al. 2009; Smith et al. 2010; Gonzalez-Chavez et al. 2011). The use of AM fungi has thus been proposed as a potential contributor to enhance plant As uptake and accumulation and to develop plant-based As remediation. Here, we review the problem of As toxicity in terrestrial ecosystems and human health, examine the recent progress in understanding the roles of AM fungi in plant As tolerance and accumulation, and explore the promise and challenges of using AM fungi as phytoremediation approaches to tackle this environmental problem.

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Z.M. Solaiman et al. (eds.), *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration*, Soil Biology 41, DOI 10.1007/978-3-662-45370-4_16

16.2 Arsenic in the Environment and Its Toxicity

Arsenic is an odorless and tasteless semimetal element that occurs naturally in rocks (~3 mg kg^{-1} Earth crust) (Mandal and Suzuki 2002). It can be released into air, water, and soils through natural activities (volcanic action, rock and soil erosion) or agricultural and industrial practices (fertilizers, herbicides, pesticides, mining, semiconductors) (Mandal and Suzuki 2002; Adriano 2001). As accumulation, migration, and toxicity are related to its chemical speciation. Inorganic As species are the more reduced arsenite [H_3AsO_3, As(III)] and more oxidized arsenate [$HAsO_4^{2-}$, As(V)]. Generated from inorganic As via biomethylation, organic As species include mono- and di-methylarsenite [MMA(III) and DMA(III)] and mono- and di-methylarsenate [MMA(V) and DMA(V)] (Cullen and Reimer 1989). Arsenic compounds are the most notorious toxins in human history and linked to many forms of cancer, diarrhea, nausea, stomach pain, vomiting, numbness, partial paralysis, and blindness (Nriagu 2002). The toxicity order of As is as follows: MMA(III) > DMA(III) > As(III) > As(V) > MMA(V) > DMA(V) (Ali et al. 2009; Kim et al. 2009; Ralph 2008).

Arsenic exposure occurs primarily through drinking water and food. The standard for drinking water to prevent chronic effects is ≤0.01 mg As L^{-1} (0.01 ppm) (http://www.who.int/mediacentre/factsheets/fs210/en/). More than 150 million people worldwide get exposed to 0.01–0.05 mg As L^{-1} drinking water, including countries in Southeast Asia, North and South America, and Europe (Bhattacharjee 2007; Kim et al. 2009; Smith et al. 2000). At present, the World Health Organization and most countries have not established legal As limits in food, though the US FDA recommends a "tolerable daily intake" of 0.13 mg As in food (Stone 2008).

16.3 Arsenic Biogeochemistry in Soil and Its Uptake in Plants

Arsenic and P belong to the same V_A chemical group, and they thus display similar chemical properties and geochemical behaviors (Cullen and Reimer 1989; Adriano 2001). However, whereas P is an essential plant nutrient, As can be toxic to crops as well as to primary and secondary plant consumers (Stone 2008; Kim et al. 2009). Various forms of As exist in soils depending on pH and redox status. As(III) dominates in anaerobic substrates, while As(V) dominates in aerobic soils (see Wenzel et al. 2002; Raab et al. 2007; Williams et al. 2007). Typical concentrations of As(III) are 0.01–3.0 μM in contaminated soils, while As(V) concentrations are >2.3 μM in contaminated or <53 nM in uncontaminated soils (Wenzel et al. 2002). Plant roots primarily take up inorganic As(III) and As(V) and are also capable of taking up organic MMA(III) or DMA(III). The toxic limits to most plants are 5–20 mg As kg^{-1} soil (Mendez and Maier 2008), and the common symptoms of As toxicity include reduced root growth, leaf chlorosis, increased sterility, and yield

reduction (Meharg and Hartley-Whitaker 2002; Raab et al. 2007; Smith et al. 2010 and references therein).

The known plant uptake pathways for reduced and oxidized inorganic As are via silicon (Si) and phosphate (PO_4^-) transporters (Pht), respectively. As(III) enters into rice (*Oryza sativa*) roots passively by sharing a Si transport pathway through nodulin 26-like intrinsic proteins (NIPs) (Maurel et al. 2008; Ali et al. 2009; Zhao et al. 2009 and references therein). As(III) uptake was inhibited by glycerol and antimonite (Sb), but not by P (Abedin et al. 2002a, b; Meharg and Jardine 2003). In contrast, As(V) is taken up actively through PO_4^- transporters (e.g., Pht1;1 and Pht1;4) (Shin et al. 2004), which have a lower affinity for As(V) than P (Meharg and Macnair 1990; Meharg and Hartley-Whitaker 2002). The rapid reduction of As (V) to As(III) was demonstrated in tomato (*Lycopersicon esculentum*) and rice roots (Xu et al. 2007).

The uptake competition between As(V) and P was exhibited by excised roots of barley (*Hordeum vulgare*), velvet grass (*Holcus lanatus*), or mouse-ear cress (*Arabidopsis thaliana*) in solution culture (Meharg and Macnair 1990; Meharg and Hartley-Whitaker 2002; Zhao et al. 2009), but not by medic (*Medicago truncatula*) or barley in soil/sand (2:8) media (Christophersen et al. 2009a). The influx of As(III) was generally comparable to that of As(V) under low (<50 μM, high-affinity transporter range) but considerably higher under high (>100 μM, low-affinity transporter range) concentrations (Meharg and Macnair 1990; Meharg and Jardine 2003).

16.4 Arsenic Transport and Hyperaccumulation in Plants: The Basis for Phytoremediation

Arsenic is primarily accumulated in roots of most plants because its low mobility restricts its root-to-shoot translocation, except in As hyperaccumulators (Raab et al. 2007). Brooks et al. (1977) defined "hyperaccumulators" as plants that could tolerate and accumulate >1 mg metal g^{-1} (0.1 %) dry mass. An As hyperaccumulator has greater antioxidant capacity and lower reactive oxygen concentration and thus greater As tolerance than a non-As hyperaccumulator (Srivastava et al. 2005; Singh et al. 2006). After uptake, As(V) is rapidly reduced by As(V) reductases in roots to As(III), which can then be detoxified by complexation with glutathione (GSH) or phytochelatins (PCs) (Raab et al. 2005; Zhao et al. 2009; Zhu and Rosen 2009). As(III) or the complexed As(III) is transported across tonoplasts and sequestered in vacuoles, loaded into xylem, and translocated to and accumulated in shoots (Xu et al. 2007; Su et al. 2008).

High As tolerance and accumulation capacity constitute the basis of exploring plant hyperaccumulators for As phytoremediation. Candidate plants for phytoremediation must tolerate and accumulate high levels of As in their tissues and possess high biomass production potential. At present, several fern species and

Table 16.1 Potential As hyperaccumulator plant species (grouped according to De Koe 1994; Bech et al. 1997; Tu et al. 2002; Baldwin and Butcher 2007; Tripathi et al. 2007; Zhao et al. 2009)

Plant	Species
Ferns	*Pityrogramma calomelanos* (L.) Link (silverback fern), *P. austroamericana* Domin (leatherleaf goldback fern), *Pteris aspericaulis* (tricolor fern), *P. biaurita* (thinleaf brake fern), *P. cretica* var. *albolineata* (table fern), *P. cretica* var. *nervosa* (Cretan brake fern), *P. cretica* cv Mayii (moonlight fern), *P. fauriei* (Faurie's brake fern), *P. longifolia* (longleaf brake fern), *P. multifida* Poir. and *P. multifida* f. *serrulata* (spider brake fern), *P. oshimensis* Hieron. (an Asian fern), *P. quadriaurita* (striped brake fern), *P. ryukyuensis* Tagawa. (an Asian fern), *P. umbrosa* (Australian jungle brake fern), *P. vittata* (Chinese brake or ladder fern)
Grasses and forbs	*Agrostis castellana* (bentgrass or dryland browntop), *A. delicatula* (bentgrass), *Bidens cynapiifolia* (West Indian beggarticks)

a number of grasses and forbs have been identified as As hyperaccumulators (De Koe 1994; Bech et al. 1997; Tu et al. 2002; Baldwin and Butcher 2007; Tripathi et al. 2007; Zhao et al. 2009; see Table 16.1). As(III) generally accounts for 60–90 % of the total As in the shoots of As hyperaccumulator *Pteris* species, and the ratio of shoot-to-root As accumulation (translocation factor (TF)) ranges between 5 and 25 in hyperaccumulators (Tu and Ma 2002; Tu et al. 2002; Zhao et al. 2009; Leung et al. 2010a, b, 2013). This high As accumulation in plants can lead to demonstrable reductions of soil As content via phytoremediation programs (Xie et al. 2009). For instance, *Pteris vittata* (Chinese brake fern) was capable of reducing As from 190 to 140 mg kg^{-1} soil after 2 years growing in an As-contaminated field (Kertulis-Tartar et al. 2006) or from 130 to 10 μg L^{-1} after 4–6 weeks growing in an As-contaminated groundwater (Natarajan et al. 2008).

16.5 Mycorrhizal Symbiosis

The potential for mycorrhizal symbiosis to improve As tolerance and phytoremediation has been only partially explored. About 90 % of higher plants associate with mycorrhizal fungi (Wang and Qiu 2006; Smith and Read 2008; Brundrett 2009). There are about 200 AM fungal species, and all of them belong to the phylum Glomeromycota (Walker et al. 2007a, b; Palenzuela et al. 2008). AM fungi are asexual obligate symbionts, and most of them are widespread and not host specific. In AM associations, fungal hyphae penetrate inside the walls of root cortical cells to form either "little-tree-shaped" structures, called arbuscules, or hyphal coils, both of which serve as the main nutrient exchange sites between fungus and plant.

While aboveground plant structures are easily observed, mycorrhizal fungi and their activities are challenging to characterize. A single gram of soil may contain up to 50 m of AM hyphae, which can extend >9 cm beyond the roots and expand

extensively throughout the soil matrix (Nasim 2005). The small 2–10 μm diameter of mycorrhizal fungal hyphae can efficiently explore soil volume and microsites inaccessible to plant roots. One important function of mycorrhizal fungi is to enhance host plant nutrient acquisition by increasing access to inorganic N and P by hyphae extending beyond depletion zones caused by direct uptake by roots and by access to organic N and P via their extracellular protease and phosphatase activity (Smith and Read 2008).

16.5.1 Roles of Mycorrhizal Fungi in Arsenic Tolerance

There are several hypothesized mechanisms by which mycorrhizal fungi could affect host plant As tolerance (Meharg and Hartley-Whitaker 2002; Zhao et al. 2009; Smith et al. 2010; Gonzalez-Chavez et al. 2011). First, it has been hypothesized that AM fungi increase plant P nutrition and growth and thus alleviate toxic effects of As on plants due to the dilution of As uptake because P shares chemical properties with As (Adriano 2001). Second, As-tolerant fungi could provide added functional benefits over non-tolerant fungi. Numerous AM studies have addressed these hypotheses, with most studies focused on P nutrition effects.

Hypothesis 1: *P Nutrition Effects*. Consistent with the hypothesis that mycorrhizally mediated improved P nutrition enhances As tolerance, plant growth and P nutrition were simultaneously improved under As stress conditions by AM in most studies. For instance, As uptake, As tolerance, and P nutrition in both shoots and roots of maize (*Zea mays*) (Xia et al. 2007; Bai et al. 2008; Wang et al. 2008; Yu et al. 2009, 2010), lettuce (*Lactuca sativa*; Cozzolino et al. 2010), and *Eucalyptus globulus* (Arriagada et al. 2009) were concurrently enhanced by AM fungi. In addition, the activity of peroxidase, superoxide dismutase, and As(V) reductase was suppressed by *Glomus mosseae* (now *Funneliformis mosseae*), indicating that AM colonization could inhibit the reduction of As(V) to As(III) and As toxicity to plants could hence be alleviated (Yu et al. 2009). By contrast, the phytotoxicity of arsenate (AsV, $Na_2HAsO_4{\cdot}7H_2O$) led to an increase in superoxide dismutase, catalase, and peroxidase activities in a 1-month-old pea (*Pisum sativum*) (Garg and Singla 2012). Similarly, P accumulation was significantly higher under all As levels of 10, 50, 100, and 200 mg kg^{-1} soil in a 2-month-old mycorrhizal medic inoculated with *G. mosseae* BEG167 (Xu et al. 2008). Both As and P uptake were higher in 3-month-old *G. mosseae* BEG167-inoculated tomatoes growing in 25, 50, and 75 As kg^{-1} spiked soil, but similar in 150 mg As kg^{-1} spiked soil (Liu et al. 2005a). A hydroponic study with a 1-month-old *Pennisetum clandestinum* Hochst (kikuyu grass) showed that As(V) uptake was competitively inhibited by P uptake because of a higher selectivity of membrane transporters with respect to P rather than As(V) (Panuccio et al. 2012). Smith et al. (2010) and Christophersen et al. (2012) recently detailed mechanisms of direct root and/or mycorrhizal Pi/As (V) uptake pathways, summarizing the physiological basis for the observed P-mediated effects on As accumulation of both AM-responsive and

AM-nonresponsive plants. Thus, there is relatively strong support for this hypothesis.

Hypothesis 2: *Fungal As Tolerance*. Some pure culture studies suggest that the extent of As(V) toxicity to mycorrhizal fungi could vary among fungal taxa, increasing the potential for the selection of appropriate fungi for remediation efforts. There is some evidence that AM fungal populations can develop tolerance to As and that this tolerance results in improved host performance. For instance, fungal isolates of *G. mosseae* and *G. caledonium* associated with velvet grass roots from the As-contaminated site were more As(V) tolerant than those from the non-As-contaminated site (Gonzalez-Chavez et al. 2002). Root high-affinity As(V)/PO_4^- transportation was suppressed by both tolerant and non-tolerant *G. mosseae* in both tolerant and non-tolerant velvet grasses. As(V) uptake in the tolerant velvet grass growing in the As-contaminated site was reduced by inoculating with the tolerant AM isolates. The authors concluded that AM fungi had evolved As (V) tolerance and conferred enhanced As tolerance on velvet grass (Gonzalez-Chavez et al. 2002).

In summary, mycorrhizal fungi have been consistently shown to confer As tolerance on their host plants. The possible mechanism of As tolerance in mycorrhizal plants might be one or a combination of the following. First, AM fungi enhance P nutrition and plant growth, resulting in a higher P/As ratio and a relative As dilution in tissues of mycorrhizal plants (Liu et al. 2005a, b; Ahmed et al. 2006; Chen et al. 2007; Ultra et al. 2007a, b). The corresponding reasons are the induction of HvPht1;8 (*H. vulgare* phosphate transporter) and downregulation of HvPht1;1 and HvPht1;2 (Christophersen et al. 2009b) and both the upregulated and downregulated expressions of up to 130 life proteins, particularly for some glycolytic enzymes including glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and enolase (Bona et al. 2010, 2011). This could provide "protective effects" against As uptake or stress because P shares chemical properties with As. Second, As-tolerant mycorrhizal fungi enhance As(III) exudation to the external media and reduce As(V) uptake at the As-contaminated habitats and thus confer enhanced As tolerance on AM plants (Gonzalez-Chavez et al. 2002), since the induction of GiPT (*Glomus intraradices* high-affinity phosphate transporter) expression correlates with As(V) uptake in the extra-radical mycelium of *G. intraradices* (Gonzalez-Chavez et al. 2011). In addition, under 2 μM As(III), both the Lsi1 and Lsi2 As(III) transporters were significantly decreased by 0.7- and 0.5-fold in mycorrhizal than in non-mycorrhizal 2.5 month-old rice seedlings, leading to a decrease of As(III) uptake per unit of root dry mass (Chen et al. 2012).

16.5.2 Roles of Mycorrhizal Fungi in Arsenic Uptake

The chemical similarity of P and As, combined with the mycorrhizal role in P nutrition, provides the likelihood that mycorrhizal fungi may enhance As uptake. Given that mycorrhizas generally enhance P uptake, it is possible that mycorrhizal

fungi will also increase the uptake of As in their plant hosts. However, depending on mycorrhizal specificity for P vs. As uptake (Li et al. 2011), a variety of outcomes are possible. For example, if mycorrhizal fungi have more specific P uptake mechanisms than their hosts, they may reduce the proportional uptake of As relative to P (Smith et al. 2010).

Arsenic Accumulations in Shoots of Herbaceous Plants. Consistent with an enhanced As uptake via mycorrhizal fungal symbiosis, AM fungi appear to increase As accumulation in their hosts. Compared to the non-AM seedlings, As accumulation (both concentration and content) was increased in shoots and roots of 2- or 3-month-old *G. mosseae*-inoculated maize seedlings growing in 75 and 150 mg As kg^{-1} soil/sand (3:1) media (Wang et al. 2008), in 100 mg As kg^{-1} soil (Yu et al. 2009), and even in 600 mg As kg^{-1} soil/sand (2:1) media (Xia et al. 2007). A mixed inoculum of indigenous AM isolates (*Glomus* spp. and *Acaulospora* spp.) from As-contaminated soils, not the nonindigenous *G. caledonium* 90036 from non-As-contaminated soils, increased As accumulation in shoots of a 2.5-month-old maize in 185 and 290 mg As kg^{-1} soil (Bai et al. 2008). Plant total As accumulations were significantly increased in a 3-month-old *G. mosseae*-inoculated white clover (*T. repens*) and ryegrass (*Lolium perenne*) growing in 600 mg As kg^{-1} soil/sand (1:1) media (Dong et al. 2008). Shoot As and toxicity symptoms were reduced in a 6-week-old *G. aggregatum*-inoculated sunflower (*Helianthus annuus*) growing in 620 mg As kg^{-1} contaminated soil (Ultra et al. 2007a, b). Arsenic accumulation was also significantly increased in a 2-month-old *G. mosseae* BEG167-inoculated medic growing in 200 mg As kg^{-1} soil but was similar between the non-mycorrhizal and mycorrhizal plants under 10, 50, or 100 mg As kg^{-1} soil (Xu et al. 2008). In addition, P accumulation and P/As ratio of both shoots and roots were always higher in all mycorrhizal plants than in their non-mycorrhizal counterparts in almost all these studies, suggesting that AM fungi may have more specific uptake of P relative to As when compared with non-mycorrhizal plants.

Higher As Accumulations in Roots than in Shoots of Herbaceous Plants. Although the increase in As accumulation in crop plants might be of concern from a food chain perspective, interestingly, As accumulation in roots, rather than in shoots, was much more enhanced by mycorrhizal fungi in most studies with herbaceous plants, as 80–90 % of As accumulated in roots of maize, ryegrass, and clover (Dong et al. 2008; Wang et al. 2008). Grown under a range between 100 and 600 mg As kg^{-1} soil/sand media, accumulations of As contents were 10–50 times higher in roots than in shoots in tomato (Liu et al. 2005a), sunflower (Ultra et al. 2007a, b), medic (Xu et al. 2008), white clover (*Trifolium repens*), ryegrass (*L. perenne*) (Dong et al. 2008), and maize (Xia et al. 2007; Wang et al. 2008; Yu et al. 2009). In contrast, P accumulations and P/As ratio were generally higher in shoots than in roots in all these studies. In addition to the food chain implications, the enhancement of As accumulation in roots has implications for mycorrhizal plant utility in bioremediation efforts, as we shall see below. Also of relevance to As accumulation in the food chain, As concentrations in pods were reduced, while P uptake was increased in a 9-week-old nodulated AM (*G. mosseae*) lentil (*Lens*

culinaris cv. Titore) irrigated with 1, 2, 5, and 10 mg As(V) L^{-1} to the sand/terra (1:1) media (Ahmed et al. 2006). Lower As concentration in pods would most likely reduce As toxicity risk in the food chain. Further studies are required to understand if this is a general consequence of mycorrhizal colonization. In addition, the highest As accumulated in maize roots when inoculated with *Acaulospora* spp. or *Glomus* spp. and earthworm (*Eisenia foetida*) (Hua et al. 2009, 2010).

Arsenic Accumulation in Fronds of Ferns. The roles of AM fungi in As uptake and tolerance have also been investigated in the As hyperaccumulation ferns. Similar to studies summarized above, there was often an increase of As accumulation in mycorrhizal ferns (Liu et al. 2009), though there were intraspecific differences in AM fungi on As accumulation in *P. vittata* (Wu et al. 2009). In contrast, mycorrhization led to an increase in the relative proportion of As accumulated in fronds vs. roots. For example, compared to its non-mycorrhizal counterpart, the amounts of As accumulation were about five times higher in fronds, but similar in roots, in an 8-month-old mycorrhizal *P. vittata*, when grown in 100 mg As(V) with 25 or 50 mg P kg^{-1} soil and inoculated with an AM inoculum from an As-contaminated site (Al Agely et al. 2005), and in a 4-month-old *G. mosseae* BEG167-colonized *P. vittata* growing in 300 mg As kg^{-1} soil (Liu et al. 2005b). Arsenic accumulations in fronds and roots were 3.0–3.9 and 2.5–3.6 times higher, respectively, in a 2-month-old mycorrhizal (an indigenous soil inoculum) *P. vittata* than in non-inoculated plants growing in 50 or 100 mg As kg^{-1} soil (Leung et al. 2006). However, As accumulation in *P. vittata* was not affected by 2- or 3-month inoculation with either *G. mosseae*, *G. caledonium*, or *G. intraradices* growing in 106 mg As kg^{-1} soil (Chen et al. 2006). Compared to non-mycorrhizal plants, frond As accumulation was reduced, while similar in roots, in an 8-month-old AM *Pityrogramma calomelanos* (silverback fern) growing in 240 mg As kg^{-1} soil (Jankong and Visoottiviseth 2008). However, a commercial AM inoculum (a mixture of *G. mosseae*, *G. intraradices*, and *G. etunicatum*) was applied to this 8-month-old silverback fern for only 2 months, possibly reducing mycorrhizal effects on the outcome. Soil As concentration was reduced by 24 %, while tissue As accumulation was up to 0.2 % in *P. vittata* growing under a mixed inoculum [indigenous AM fungi (*G. intraradices*, *G. geosporum*, *and G. mosseae*) + nonindigenous *G. mosseae*] and the addition of phosphate rock (Leung et al. 2010a). The contrasting results may be derived from experimentation with different AM isolates, different host plants, or other experimental conditions. Further assessments of mycorrhizal effects on As accumulation are needed, particularly under field conditions. In general, most of these fern studies showed a higher ratio of frond/root As accumulation in the mycorrhizal ferns than in their non-mycorrhizal counterparts, suggesting that As translocation from root to shoot was enhanced by mycorrhizal fungi even in As hyperaccumulation ferns. The mycorrhizal-mediated enhancement of As tolerance and accumulation either in shoots of As hyperaccumulating ferns or in roots of herbaceous annuals and perennials offers potential for screening fungal species for As remediation purpose.

16.6 Potential of Mycorrhizal Fungi in Arsenic Phytoremediation

Phytoremediation is a promising alternative for As remediation from contaminated soils and water since the chemical and physical remediation technologies are quite expensive and limited to on-site applications (Mendez and Maier 2008; Mondal et al. 2006; Tripathi et al. 2007; Wenzel 2009; Garg and Singla 2011). Genetic manipulation of As hyperaccumulating traits could contribute our efforts to As phytoremediation (Zhu and Rosen 2009), though the traits and genes are largely unknown to date. Because aboveground plant parts are easier to harvest, most attention has been given to identify high shoot As accumulators for phytoextraction by aboveground harvesting, while less has been given to high root As accumulators by belowground harvesting. But all shoot As hyperaccumulation ferns require a tropical or subtropical climate and may not grow well in other habitats. As an alternative, if roots could be easily harvested, then root hyperaccumulators could be used for phytoremediation, especially in herbaceous plants with dense root systems in shallow soil profiles, though root removal technique is not available or currently impractical. Further testing of a broad suite of species is needed for screening both shoot and root hyperaccumulators, in addition to those listed in Table 16.1. The potential roles of AM fungi (Gaur and Adholeya 2004; Garg and Singla 2011) and plant-associated bacteria (Khan 2005; Weyens et al. 2009) in heavy metal phytoremediation have been respectively proposed. However, the potential for AM fungi to contribute to As (a semimetal element) tolerance and hyperaccumulation in their host plants is poorly explored, particularly under field conditions.

Can mycorrhizas potentially offer a more cost-effective, environmentally sound, and sustainable pathway to global As phytoremediation? As seen in the previous sections, mycorrhizal fungi can tolerate and perform well in high levels of As under laboratory conditions and contaminated field sites, and they also can facilitate As accumulation in host plant tissues or increase the transfer of As from roots to shoots by indigenous isolates in particular (Orlowska et al. 2012). This indicates that mycorrhizal fungi could confer both As tolerance and accumulation ability on their host plants. A range of 10 and 50 times higher As accumulations in roots than in shoots had been reported for some annuals or perennials, including lentil, maize, medic, ryegrass, sunflower, tomato, and white clover (Liu et al. 2005a, b; Ahmed et al. 2006; Xia et al. 2007; Bai et al. 2008; Dong et al. 2008; Wang et al. 2008; Xu et al. 2008; Yu et al. 2009; Ultra et al. 2007a, b; Garg and Singla 2012), or in shoots than in roots for a dozen ferns (Al Agely et al. 2005; Leung et al. 2006; Chen et al. 2006; Jankong and Visoottiviseth 2008; Zhao et al. 2009). If these phenomena are generally true, the selection of combinations of plant and fungal species with high As tolerance and accumulation ability would tap their potential for As phytoremediation, particularly for both phytoextraction and phytostabilization (Mendez and Maier 2008). At present, no one has identified either a woody As phytoremediation plant or a candidate with both high shoot and high root As accumulation capacity. Thus, the current phytoremediation

strategies are focused on herbaceous shoot hyperaccumulators, and phytostabilization is focused on herbaceous root hyperaccumulators. Furthermore, almost all current As phytoremediation practices are limited to laboratory experiments and a few very small field trials, where plants are introduced into the soil without established mycorrhizal symbioses.

Mycorrhizal diversity is high and mycorrhizal symbiosis develops well with the shoot As hyperaccumulation ferns even on As-contaminated field sites. A field investigation on both As-contaminated and As-uncontaminated fields in Central, Southern, and Southeastern China showed that the As hyperaccumulator *P. vittata* was associated with the fungal genera *Acaulospora*, *Diversispora*, *Glomus*, *Paraglomus*, and *Scutellospora*, with the common species *Glomus brohultii*, *G. geosporum*, *G. microaggregatum*, and *G. mosseae* (Wu et al. 2007). This high mycorrhizal fungal diversity may have significant ecological and physiological contributions to their host plants in contaminated sites. The known root As hyperaccumulation annuals and perennials mentioned above are mycorrhizal (Brundrett 2009; Wang and Qiu 2006). Given that indigenous AM fungi from contaminated soils performed better in both accumulation of As and plant growth (see the above section), these adapted indigenous fungi are a promising tool for As phytoremediation from the contaminated soil, particularly when large-scale on-farm production of mycorrhizal inocula becomes available (Douds et al. 2005; Ijdo et al. 2011). The introduction of As-tolerant mycorrhizal fungi to sites with no, limited, or unadapted mycorrhizal fungi could speed up not only As remediation with the establishment of mycorrhizal symbiosis between plants and fungi but also soil reclamation and vegetation restoration. Therefore, there is great potential to screen and then to integrate fungal isolates that enhance both As tolerance and hyperaccumulation with a shoot or root hyperaccumulation plant. In addition, the combination of mycorrhizal fungi with N_2-fixing microorganisms (*Rhizobia* or *Frankia*), As(V)-reducing bacteria (*Comamonas* sp., *Delftia* sp., *Rhodococcus* sp., and *Streptomyces* sp.), and dual AM and ectomycorrhizal (EM) or the tripartite AM, EM, and N_2-fixing plant (He et al. 2005, 2009; Roy et al. 2007; Yang et al. 2012) would further extend our efforts to identify plants with high As tolerance and accumulation capacity capable of functioning under nutrient-poor conditions.

Hyphae of a single fungal individual can potentially interconnect many plants of the same or different species, and a single plant can form mycorrhizas with many fungi as well. As a consequence, a common mycorrhizal network (CMN) forms within and between plant roots to link plants together (Newman 1988; He and Nara 2007; He et al. 2009). CMNs provide pathways to shuttle nutrients, such as C, N, P, and water, from one plant to another between the same and different plant species (Newman 1988; He and Nara 2007; He et al. 2009). These extensive mycorrhizal mycelia and networks could enhance As uptake and accumulation in shoots and/or roots. The transfer of As from a plant to another via a CMN has evidenced this potential. Plants were grown in two separate chambers separated by 25 μm steel mesh with a 1.0 cm air gap between chambers to restrict root growth but allow hyphal linkages. After 1 week of 0.1 % Na_2HAsO_4 application to leaves of a 50-day-old donor (either a grass of *Bromus hordeaceus*, *B. madritensis*, *Nassella*

pulchra or a forb of *Madia gracilis*, *Sanicula bipinnata*, *Trifolium microcephalum*), AM-mediated transfer of As occurred between grass donors and forb receivers, but not the other direction (Meding and Zasoski 2008). By growing plants with high biomass production but low As uptake capacity together with those having low biomass production but high As uptake capacity, As transfer between mycorrhizal plants via CMN may provide another plant-based phytoremediation strategy.

The current barriers to the adoption of mycorrhizal inoculation reside at several levels. First, there is the need to identify the best candidate fungi for both phytoremediation and phytostabilization. Inoculum sources for mycorrhizal fungi used in phytoremediation should be derived from a similar soil, climate, and geographic region as the phytoremediation site as possible. This will both increase the chances of success and minimize the likelihood of the transfer of unwanted invasive soil organisms with the fungal inoculum (Schwartz et al. 2006). In addition, screening sites with naturally high As or long-term As contamination will provide the highest likelihood for encountering As-tolerant mycorrhizal fungal populations. Given that it is likely that the best strains will be isolated from sites that have naturally high As, in these locations, mycorrhizal fungi native to the site may be sufficient as an inoculum source, greatly simplifying the process of inoculation for phytoremediation. Second, for cases where inoculation is necessary, there are existing biotechnological approaches to producing large quantities of fungal inoculum (Douds et al. 2005; Ijdo et al. 2011), but such approaches are limited at present to very few fungal strains. The magnitude of this limitation will depend on the tractability of otherwise suitable mycorrhizal fungal inocula. It may be that native soil inoculum from sites discussed above could be used when otherwise appropriate (e.g., when conforming to regulations regarding soil transportation). Third, in temperate regions, the barrier to mycorrhizal fungal use for phytoextraction is the lack of appropriate host plants, because most mycorrhizally enhanced As accumulation outside tropical ferns occurs in host roots, which are more challenging to harvest. Up to 1,400 or 1,600 mg As DW kg^{-1} was accumulated in mycorrhizal roots of tomato (Liu et al. 2005a), ryegrass, and clover (Dong et al. 2008) compared to 70 or 80 mg As kg^{-1} DW accumulated in shoots, when growing under 150 or 600 mg As kg^{-1} soil-like media. In addition, annual or perennial bentgrass (*Agrostis castellana* and *A. delicatula*) and West Indian beggarticks (*Bidens cynapiifolia*) could accumulate 1,000–1,800 mg As DW kg^{-1} in roots at As-contaminated mine sites (De Koe 1994; Bech et al. 1997), though their mycorrhizal status had not been reported. Considering that the *Agrostis* and *Bidens* genera have more than 100 or 200 species and almost all tested species are mycorrhizal (Wang and Qiu 2006), it is likely that these species are mycorrhizal. The potential range of plants for phytoremediation could thus greatly be expanded if root-harvesting technologies that are economically and environmentally appropriate could be explored in the near future. Pilot studies are urgently needed to determine whether root As accumulation is common in a magnitude sufficient to make root As harvesting feasible for those herbaceous plants with dense, sufficiently accessible root systems. Identification of such plants and appropriate root-harvesting technologies, such as those widely used for root or tuberous crops, would

greatly expand the potential range of plants for extraction of As-enriched root systems. Furthermore, if these plants can be identified, then the incorporation of mycorrhizal inoculation with appropriate strains should greatly enhance phytoremediation efforts for a broad range of host plants.

16.7 Conclusion

Chronic As exposure through drinking water or food consumption has become a major global environmental problem. Cost-effectively and environmentally sound plant-based As remediation technologies are urgently required. A number of As hyperaccumulation plants have been identified. Mycorrhizal plants display much greater tolerance to As toxicity under high As levels and exhibit enhanced As accumulation even in high As soils. These results demonstrate that mycorrhizas may offer global potential in As phytoremediation. With an appropriate combination of fungal and plant species, mycorrhizal plants with strong As tolerance and As hyperaccumulation capacity could thus be screened, particularly from naturally As-enriched sites, for As phytoextraction or phytostabilization. Biotechnological developments in the important ecological and physiological functions of mycorrhizas relevant to phytotolerance and phytoremediation will enhance the potential of mycorrhizal fungi to contribute to our efforts to curb global As contamination in a more environmentally sound, effective, practical, and sustainable manner, particularly by large-scale application of mycorrhizal inocula through on-farm production (Douds et al. 2005; Ijdo et al. 2011).

Acknowledgments Many interesting papers could not be cited due to space limitations, for which we apologize.

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