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Impact of Mycorrhizae Formation on the Phosphorus and Heavy-Metal Uptake of Alfalfa

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*Three pot experiments were set up to determine how efficiently mycorrhizal fungi affect the uptake, translocation, and distribution of labeled phosphorus (^{32}P), phosphorus (P), and heavy metals in alfalfa (*Medicago sativa *L.). In experiments 1 and 2, the efficiencies of different arbuscular mycorrhizal fungi (AMF) species including* Glomus mosseae, G. etunicatum, G. intraradices *and a mixed strain (*G. mosseae, Gigaspora hartiga, *and* G. fasciculatum*) on uptake, translocation, and distribution of ^{32}P and P in alfalfa were investigated, respectively. In a third experiment, the efficiency of* G. mosseae *on uptake and distribution of heavy metals [cadmium (Cd), cobalt (Co), lead (Pb), and combinations] was tested. Results of experiments 1 and 2 suggest that* G. mosseae *was the most effective at increasing the uptake of ^{32}P and P. Experiment 3 result showed that in the triple-metal-contaminated soil, inoculated plants had greater Co (32.56 mg kg^{-1}) and Pb (289.50 mg kg^{-1}) concentration and* G. mosseae *enhanced the translocation of heavy metals to shoot. Hence, mycorrhizal alfalfa in symbiosis with* G. mosseae *can be used for remediation of heavy metals polluted soils with high efficiency.*

Keywords Alfalfa, arbuscular mycorrhizal fungi (AMF), heavy metals, ^{32}P

Introduction

Arbuscular mycorrhizal fungi (AMF) are commonly occurring soil microbes that have a symbiotic association with their host plant and hence significantly affect its activities and growth (Klironomos 2003). They usually affect plant growth, nutrient uptake, community structure, and biodiversity (Callaway et al. 2003; O'Connor, Smith, and Smith 2002; Zhou et al. 2001).

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The function of AMF depends on the efficiency with which the fungal symbiont absorbs inorganic and/or organic available nutrients from soil (Marschner and Dell 1994). Arbuscular mycorrhizal fungi are important because of their great capability to increase plant growth and yields under certain conditions. The major reason for this increase is the enhanced ability of the host plants to take up nutrients such as phosphorus (P) efficiently (Podila and Douds 2001).

Mycorrhizoremediation technology has implications in reducing a plant's exposure to potentially toxic elements. Some reports showed that external mycelium of AMF is the main place for heavy-metal localization (Kaldorf et al. 1999; Turnau 1998). However, other researchers have indicated the role of chitinous cell walls in selective absorbing and hence removing toxic and nontoxic elements (Zhou 1999) or the roles of extracellular lycoprotein, glomalin (Wright and Upadhyaya 1998; Rajkumar et al. 2009), and intracellular precipitation (Khan 2006).

Extraradical hyphae of the AMF can develop up to 8 cm beyond the root-growing zone (Rhodes and Gerdemann 1975) and act as extensions of the root system in acquiring nutrients from the soil (Douds and Millner 1999). As a result of this symbiotic association between AMF and host plants, and subsequent enhancement of P content, plant physiological parameters are also affected (Paradi, Bratek, and Lang 2003). Phosphorus nutrition is a critical factor in increasing plant tolerance to excessive levels of metals in the soil, and mycorrhizal plants may have better opportunities to grow in soils with high levels of lead (Pb) (Andrade et al. 2004). Phosphorus plays a key role in detoxification of heavy metals in plants through the following functions: (1) production of phytates that neutralize excess metals, (2) provision of metabolic energy indirectly as ATP for possible compartmentalization within the cell vacuoles, and (3) sequestration of heavy metals (Davies, Davies, and Francis 1991; Mehrag et al. 1994; Yang, Feng, and Stoffella 2005). Andrade et al. (2004) showed that with increased Pb concentration, AMF were still able to provide sufficient P for soybean growth. They concluded that the mycorrhizal effects on P uptake probably increased plant growth and indirectly alleviated the stress caused by excess Pb in the soil by maintaining greater P/Pb ratios in the shoots. Vivas, Barea, and Azcón (2005) reported that phosphatase and dehydrogenase activities were all increased by AMF inoculation in cadmium (Cd)–polluted soil. These results are similar to results by Varma (1998), Mar Vázquez et al. (2000), and Rao and Tak (2001).

The populations of AMF have potential roles in phytoremediation of heavy metal–contaminated soil (Wu et al. 2009). Therefore, it is necessary to have a precise understanding of the ecological details of plant–microbe–soil interactions as well as their remediation strategies employed for polluted soil (Jadia and Fulekar 2009). The study of plant rhizosphere and the diversity of soil microbes is important for understanding the ecological complexities between diverse plants, microbes, soils, and climates and their role in phytoremediation of contaminated soils. Soil microorganisms inhabiting the rhizosphere environment interact with plant roots and mediate nutrient availability, for example, those forming useful symbiotic associations with the roots contribute to plant nutrition (Khan 2006). Such root–microbial interactions must be investigated in greater detail using molecular, biochemical, and physiological techniques (Khan 2006). Hence a comprehensive molecular physiological understanding of the mycorrhizosphere, dissecting the role of plant and root microbe gene interaction in the process, would be important to decipher plant tolerance mechanisms under heavy-metal stress especially with respect to phytoremediation (Upadhyaya et al. 2010).

There is support for the idea that AMF plants may have an important role in enhanced P uptake and dry-matter production in plants. The present study was undertaken to evaluate

the response of *Medicago sativa* L. (alfalfa) to different AMF species. Alfalfa is one of the perennial Leguminosae. Alfalfa has rapid growth rate, high biomass production, and deep root system distribution in soil. Moreover, alfalfa is a perennial plant that establishes permanent coverage on the soil surface. Establishment of such continuous soil coverage can be effective in permanent cleanup of contaminated soil. We were looking to differentiate among different AMF species with regard to their P uptake and biomass allocation. Furthermore, we tested the effects of selected AMF species on the ability to absorb P and partition of heavy metals in alfalfa plants growing on contaminated soil.

There are large areas of contaminated soils with Pb, Cd, cobalt (Co), chromium (Cr), nickel (Ni), and arsenic (As) in Iran, resulting from activities associated with agriculture, industry, and domestic effluents. Lead is a common problem in tea fields located near the roads, with a high correlation between road distance and Pb concentration.

Materials and Methods

Arbuscular Mycorrhizal Fungi Inoculum Production

Arbuscular mycorrhizal fungi inoculum was produced using the trap culture method (Norris, Read, and Varma 1994). Pots were washed with ethanol (70%), filled with a sand and clay mixture (4:1), and autoclaved (at 120 °C and 182.4 kPa for 1 h). Sorghum [*Sorghum bicolor* (L.) Moench.] seeds were surface sterilized for 30 s with ethanol (95%) and immediately submerged in mercuric chloride ($HgCl_2$) (15%) for 5 min. Seeds were then rinsed and washed with distilled and tap water, respectively, and incubated in Hoagland's medium for 48 h. Arbuscular mycorrhizal fungi isolates included *Glomus mosseae*, *G. etunicatum*, and *G. intraradices* originally from the northeast of Iran that were produced by Soil and Water Research Institute, Tehran, Iran. Also, a mixed strain including an equal mixture of *G. mosseae*, *Gigaspora hartiga*, and *G. fasciculatum* (a commercial inoculum originally from India) was used. Sorghum germinated seeds were planted and inoculated with 25 g of different AMF inoculums in the sterilized pots. Pots were irrigated with distilled water twice during the week. Also, the plant nutrient requirements were supplied using Hoagland's solution [nitrogen (N) / P / potassium (K) as 1:0.85:3.35] (25% v/v) (Hoagland and Boyer 1936). Plants were cut before flowering. When the roots dried completely, the roots and soil were mixed and ground for use as inoculum in the next experiments (mixture of soil, root segments, mycelium, and spores) (Miransari et al. 2007, 2008).

Experiment 1: Radioactive Trial with Labeled Phosphorus (^{32}P) Supply

An experiment with ^{32}P was set up to evaluate the symbiotic efficiency of mycorrhizal alfalfa using five treatments in a completely randomized design (CRD). Treatments including the species *Glomus mosseae*, *G. etunicatum*, and *G. intraradices*, a mixed treatment (including an equal mixture of *G. mosseae*, *Gigaspora hartiga*, and *G. fasciculatum*), and uninoculated control were tested in four replications. The inoculums produced in the previous stage (AMF inoculum production) were used.

A soil with the following properties was used for the experiment: 35% clay, 40% silt, 25% sand, pH 7.91, and 1.48% organic carbon (C). The unsterilized soil was air dried, passed through a 2-mm sieve, and used in pots 30 cm high and 30 cm in diameter (10 kg soil for each pot). The AMF species were applied at 50 g of inoculum mixed with 5 cm of the soil upper surface. Alfalfa seeds were treated with *Sinorhizobium meliloti* (prepared

in the Soil and Water Research Institute, Tehran, Iran) before planting. After germination, plants were thinned to maintain a plant density of five plants per pot and watered with tap water as required. Plants were grown in a greenhouse with natural light, a day/night temperature of 32/20 °C, and a relative humidity of 40–60%.

At the stage of maximum vegetation growth, nearly 85 days after planting, 111 GBq ^{32}P as orthophosphoric acid was diluted and 1 mL of solution was used to treat each pot. Radioactivity of each treated pot was 127.6 kBq (IAEA 1990). Pots were irrigated right after application to ensure even distribution of ^{32}P. In the early stages of flowering (135 days after planting), plants were harvested and separated into stems and leaves. Samples were dried in an oven at 70 °C for 48 h and then weighed, ground, and sieved.

Labeled P activity in plant samples was counted by β counter (multi-low-level counter FHT770-Eberline Company, Franklin, MA) for 1000 s. After calibration of the β counter applied in the trial according to the efficiency of standardized ^{32}P source on the basis of a gas proportional system, the value of E was considered as 0.36. The amount of activity in each sample (1 g dry matter) was expressed by Bq (IAEA 1990).

Plant labeled P is an indicator of AMF efficiency in uptake and translocation of P to the plant. In other words, ^{32}P radioactivity in the plant indicates the amount of absorbed ^{32}P by plant and AMF symbiosis. Specific activity of each sample was calculated based on the radioactivity of ^{32}P per unit weight/total amount of P (both active and stable isotopes) (IAEA 1990). Accordingly, the specific activity of labeled fertilizer was calculated at 217.09 Bq g^{-1}.

Experiment 2: Nonradioactive Trial with P Supply

This experiment was designed and carried out same as experiment 1, except that ^{32}P was not applied. Also, the environmental condition during growth was the same as experiment 1. Plants were uprooted from each pot and then separated into shoot and root. Aboveground materials were oven dried at 70 °C for 48 h. The P concentration of shoots was measured by an inductively coupled plasma optical emission spectrometer (ICP-OES) (Varian-Liberty 150AX Turbo; Varian Inc., Palo Alto, Calif.).

Roots were washed with tap water, and subsamples were taken for mycorrhizal colonization evaluation. Mycorrhizal root colonization (on the basis of root length percentage) was estimated on 30 root pieces of each treatment by the grid-line intersect method (Giovannetti and Mosse 1980) after clearing the root systems with 25 gl^{-1} potassium hydroxide (KOH) and staining with trypan blue (Phillips and Haymann 1970).

Experiment 3: The Interaction Effects between Heavy Metals and Plant P Uptake

The third experiment was set up as a 2×8 factorial on the basis of a completely randomized design, with four replicates. The first factor was inoculation (I) or uninoculation (I0) with *G. mosseae.* According to results of experiments 1 and 2 *G. mosseae* was the most effective strain. The second factor was level of heavy-metal contaminants [seven levels of Co, Cd, Pb, CoCd, CdPb, PbCo, and PbCoCd plus a control treatment (C)]. The same experimental soil was also used for this experiment. The concentration of heavy metals on the basis of soil dry weight was as follows: Co 51.91 mg kg^{-1}, Cd 8.5 mg kg^{-1}, and Pb 436 g kg^{-1} dried soil. The heavy-metal salts cobalt sulfate ($CoSO_4$), cadmium chloride ($CdCl_2$), and lead nitrate [$Pb(NO_3)_2$] were used for the experiment.

Heavy-metal salts were dissolved in distilled water and used for the contamination of soil before planting while thoroughly mixed with the experimental soil. After 15 days,

which was necessary for the equilibrium of heavy metals in the soil, *G. mosseae* inoculum at 50 g was mixed with 5 cm of soil upper surface, and alfalfa seeds were planted as previously mentioned. After germination, plants were thinned to maintain a plant density of five plants per pot. Environmental condition during growth was the same as experiments 1 and 2. During the trial, pots were irrigated with tap water.

Harvest and Chemical Analysis of Plant Samples

Plants were cut from the soil surface at the early flowering stage. Roots were also harvested from each pot. Aboveground materials, separated into the stems and leaves, were washed by distilled water. Plant material was dried at 70 °C for 48 h.

Plant samples were digested with a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$), and P and heavy-metal concentrations were determined by ICP-OES (Varian-Liberty 150AX Turbo; Varian Inc., Palo Alto, Calif.).

Statistical Analysis

Statistical analysis of data was performed using SAS (SAS Institute Inc. 1988). The data were analyzed by one-way analysis of variance (ANOVA), and comparisons between means were performed using Duncan's multiple-range test at the significance level of $P < 0.05$ (Steel and Torrie 1980).

Results and Discussion

Experiment 1

There were significant differences among different AMF species affecting shoot biomass. Alfalfa inoculated with *G. mosseae* had the greatest shoot dry weight relative to the other treatments (Table 1), and *G. mosseae* resulted in the greatest rate of shoot specific activity (Table 1) ($P < 0.05$). The uptake and transfer of ^{32}P to the plant via the AMF was studied by Rhodes and Gerdemann (1975). They demonstrated the ability of AMF hyphae to

Table 1
Comparison of effects of different AMF species on some characteristics of afalfa determined by Duncan's multiple-range test

	Experiment 1		Experiment 2		
Strains	Shoot dry weight (g)	Shoot specific activity (Bq g^{-1})	Shoot dry weight (g)	Mycorrhizal colonisation (%)	Shoot P concentration
Uninoculated	27.82b	41.10d	23.20b	21c	1243.04c
Glomus etunicatum	27.00b	37.36e	24.72b	46a	1287.52b
G. intraradices	28.85ab	45.93c	24.48b	42ab	1287.52b
mixed species	26.70b	53.00b	24.54b	36b	1284.01b
G. mosseae	32.15a	101.47a	28.69a	48a	1451.95a

Note. Same letters within the same column indicate nonsignificant differences ($P < 0.05$).

take up ^{32}P from some distance away from the roots. Pearson and Jakobsen (1993) showed different efficiencies among fungus in ^{32}P uptake and transportation to plant. The underlying reasons for these differences could involve different colonization patterns by different strains (Olsson et al. 2005). Moreover, there may be some variations in symbiotic efficiency of strains at the level of rates of transfer across the interfaces or differences in metabolic activity (Olsson et al. 2005). A difference among the AMF strain in ^{32}P uptake in barley (*Hordeum vulgare* L.) was confirmed by Ardakani et al. (2009).

Experiment 2

G. mosseae resulted in greater shoot biomass and colonization index (Table 1) ($P < 0.05$). Even uninoculated plants were colonized (about 21%) by the soil native AMF. The greatest P concentration in the plant shoot was also a result of *G. mosseae* (Table 1) ($P < 0.05$).

Enhancement of P uptake and growth of many plants is a key role of these microorganisms. Evidence shows that AM roots can be more efficient in P uptake than nonmycorrhizal roots. Also, AMF plants were both larger and contain greater concentrations of P in their tissues than uninoculated ones (Smith and Read 2008). Ardakani et al.'s 2009 study on barley showed that *G. mosseae* had more efficiency in uptake of P and also translocation to shoot than other strains and uninoculated plants. These results are also in agreement with the results of other researchers (Safir, Boyer, and Gerdmann 1971; Tarafdar and Marschner 1993; Smith and Read 2008; Entry et al. 2002).

Experiment 3

Plant Biomass. Results of experiment 3 showed that the treatment with inoculation by *G. mosseae* and without contaminated soil (IC) produced the maximum amount of plant biomass, but soil contamination reduced plant biomass. The I0PbCoCd treatment resulted in the minimum dry weight. In all treatments, inoculated plants produced greater biomass than uninoculated ones (Figure 1) ($P < 0.05$). The beneficial effect of plant mycorrhization on plant dry-matter production in heavy metal–contaminated soil was observed in this study. This effect of mycorrhizae was confirmed by Davies et al. (2002), Anderade et al. (2004), and Smith and Read (2008). In barley, Ardakani et al. (2009) observed increasing

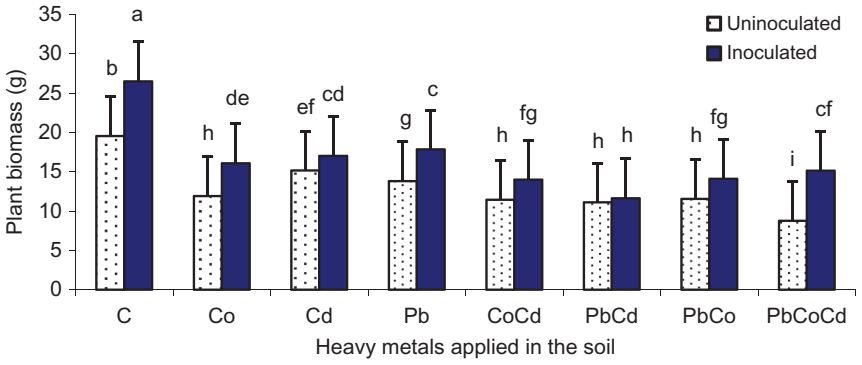

Figure 1. Mean comparisons of interaction effect between *Glomus mosseae* and heavy metals on plant biomass by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

plant biomass by *G. mosseae* in heavy metal–contaminated soil. Thus, the increased plant biomass by *G. mosseae*, which is necessary for efficient bioremediation, is the most important reason, significantly contributing to the bioremediation of heavy metals by alfalfa. These results are also similar to the results by Yang, Yang, and Römheld (2002) and Song et al. (2004). This ability of AMF in contaminated soil can be defined in terms of improvement of P nutrition (Smith and Read 2008).

Phosphorus Concentration in Plant Tissues. The IC treatment resulted in significantly greater ($P < 0.05$) P concentration in the leaves relative to the other treatments. For the ICo, ICd, IPb, and ICoCd treatments, *G. mosseae* resulted in greater P concentration in the leaf relative to the control treatment; however, in the treatments including all heavy metals *G. mosseae* did not increase P concentration over the control treatment (Figure 2) ($P < 0.05$). Generally, *G. mosseae* enhanced P uptake and translocation to the leaves in comparison with the uninoculated plants. The P concentration in the plant stem in the uncontaminated pots without inoculation was greater than other treatments. In mono-metal-treated pots inoculated plants had greater P concentration than uninoculated ones. In double-metal-contaminated treatments, just CoCd treatment resulted in greater P concentration. In soils treated with three heavy metals uninoculated plants had a greater P concentration than inoculated plants (Figure 3) ($P < 0.05$). Inoculated plant roots with *G. mosseae* for control, Cd, PbCd, and PbCoCd treatments had significantly ($P < 0.05$) greater P concentrations in roots in comparison with other treatments (Figure 4). In our experiment, the concentrations of Co, Cd, and Pb in plant root were greater for IPbCoCd treatment than for I0PbCoCd (data not shown).

Arbuscular mycorrhizal fungi species differ in nutrient transfer processes, host–fungus specificity, etc. Plant properties can also affect the process of bioremediation by affecting the allocation of heavy metals to different parts of the plant and the root/shoot ratio (Niu, Sun, and Sun 2009). Results of Ardakani et al. (2009) suggested that efficiency of barley–*G. mosseae* association in uptake and sequestration of heavy metals, particularly in multi-metal-contaminated soils, depended on contribution of P uptake of mycorrhizal plants roots. They showed greater P concentration of barley inoculated plants roots than uninoculated ones in three-metal-contaminated soil. In the present study in alfalfa, P

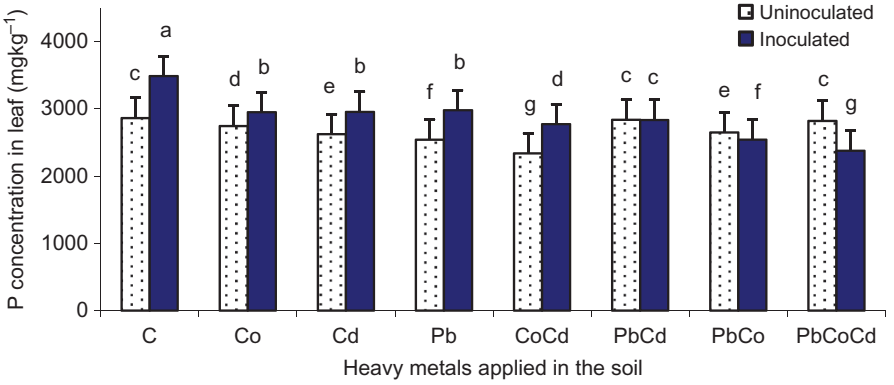

Figure 2. Mean comparisons of interaction effect between *Glomus mosseae* and heavy metals on P concentration in leaf by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

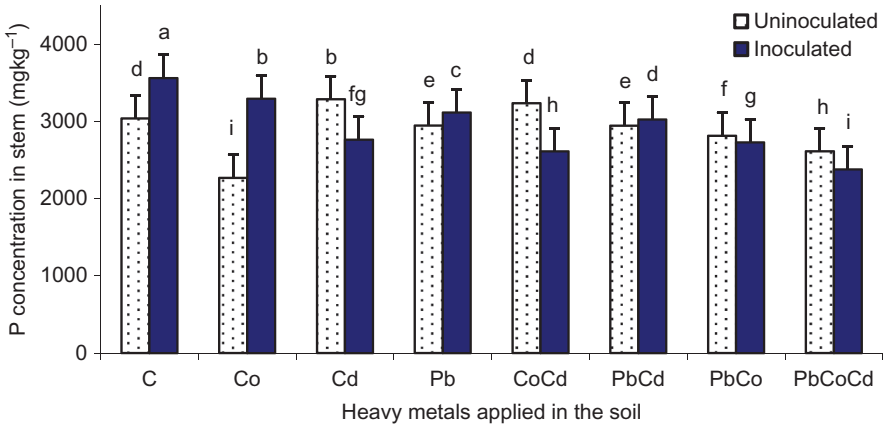

Figure 3. Mean comparisons of interaction effect between *Glomus mosseae* and heavy metals on P concentration in stem by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

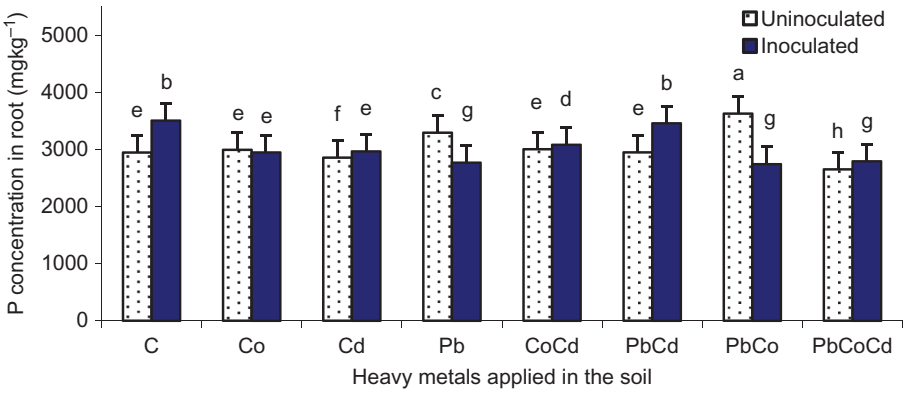

Figure 4. Mean comparisons of interaction effect between *Glomus mosseae* and heavy metals on P concentration in root by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

concentration of inoculated plants root was more than that in the uninoculated ones in three-metal-contaminated soil. These results support by the hypothesis that AMF, by increasing P content of root in heavy metal–contaminated soil, can enhance plant tolerant to multiple metal stress (Turnau, Kottke, and Oberwinkler 1993).

Heavy Metal Concentration in Plant Tissues. Arbuscular mycorrhizal fungi species and different contaminants significantly affected Cd concentrations in plant. The Cd concentration in the I0CoCd treatment was remarkably greater than in the other treatments. In all treatments, the uninoculated plants resulted in greater Cd uptake than inoculated plants with *G. mosseae* (Figure 5) ($P < 0.05$). The results of this experiment showed that the greatest rate of Co uptake by plant was related to I0Co treatment. With the exception of the IPbCoCd treatment, the uninoculated plants increased plant Co uptake for all the treatments (Figure 6) ($P < 0.05$). The maximum Pb uptake was related to I0PbCd treatment. Only in

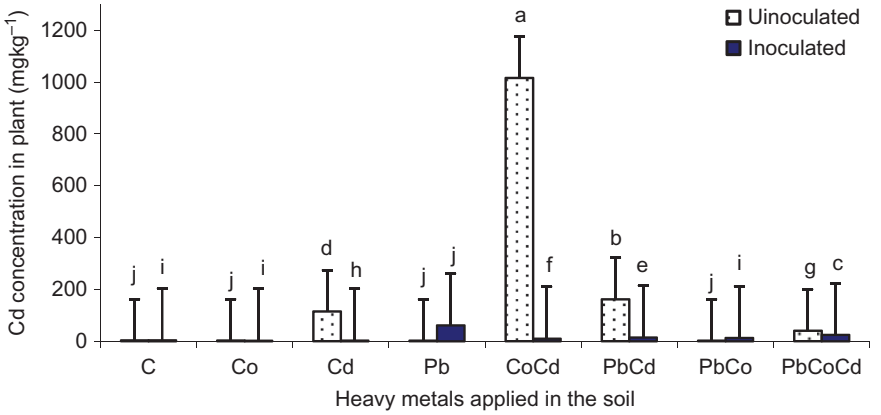

Figure 5. Mean comparisons of interaction effect between *Glomus mosseae* and contaminations on Cd concentration in plant by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

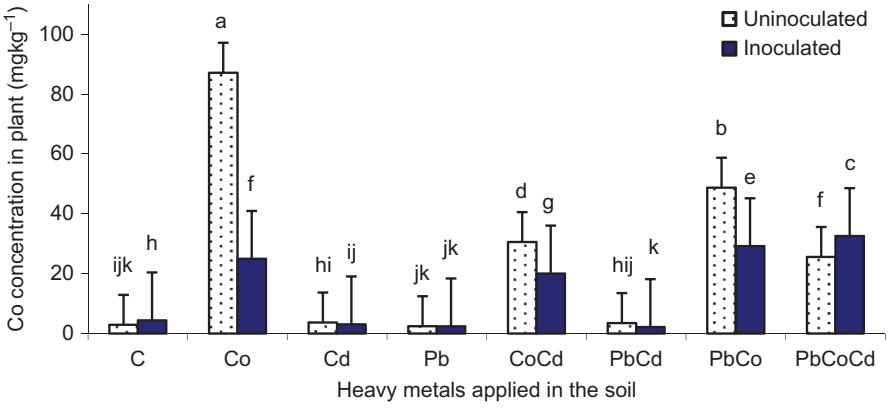

Figure 6. Mean comparisons of interaction effect between *Glomus mosseae* and contaminations on Co concentration in plant by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

the triple-metal-contaminated soil did the mycorrhizal plants have greater Pb concentration than in uninoculated ones (IPbCoCd > I0PbCoCd) (Figure 7) ($P < 0.05$).

Lead and cobalt contents in the uninoculated plant tissues were greater than inoculated ones, except in the IPbCoCd treatment. Inoculation with *G. mosseae* in the three-metal-contaminated pots (PbCoCd treatment) increased total uptake of Pb and Co into the upper biomass of plants by about 62% and 27%, respectively. Arbuscular mycorrhizal fungi supply plants with essential nutrients from the soil through uptake by extraradical hyphae. Some elements may also be transported by hyphae (Guo, George, and Marschner 1996) but the AMF may constitute a biological barrier against translocation of heavy metals to shoots (Joner and Leyval 1997). Thus, for AMF the reports are conflicting. In some reports, AMF reduce excess plant uptake of heavy metals such as Zn, Cd, and Mn (Heggo, Angle, and Chaney 1990; Li and Christie 2000), although in some cases enhanced uptake of heavy metals was observed (Jamal et al. 2002; Liao et al. 2003).

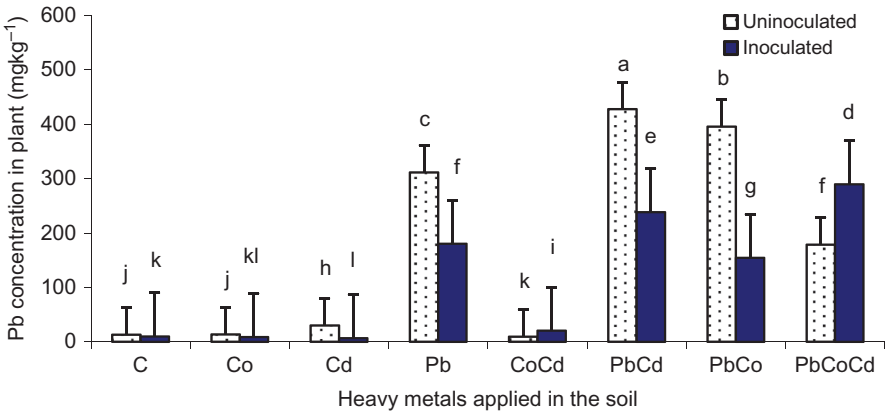

Figure 7. Mean comparisons of interaction effect between *Glomus mosseae* and contaminations on Pb concentration in plant by Duncan's multiple-range test ($P < 0.05$). Error bars represent SD (color figure available online).

In the three-metal-contaminated soil, *G. mosseae* increased translocation of Pb and Co to plants, which is in contrast to mycorrhizoremediation (Wright and Upadhyaya 1998; Zhou 1999; Khan 2006). Inconsistent results on the effects of AMF on heavy metal uptake may be a consequence of a wide range of factors such as the inherent heavy metal uptake capacity of plants, plant root density, corresponding fungal properties and soil adsorption/desorption characteristics (Leyval, Turnau, and Haselwandter 1997; Joner and Leyval. 2001). According to our results *G. mosseae* inocula developed typical and significant associations with alfalfa and increase its growth in three-metal-contaminated soils. These results also showed that kind and concentration of contamination affected the response of *G. mosseae* to added heavy metals.

In the control treatments where plants absorbed more Pb, Cd, and Co than inoculated ones, AMF might have selectively contributed to the exclusion of toxic and nontoxic elements (i.e., mycorrhizoremediation). Metals may be sequestered in the hyphae and not translocated to the plants. These results are in agreement with those of other authors (Zhou 1999; Kaldorf et al. 1999; Turnau 1998; Khan 2006). Accordingly, the sequestration of metals by polyphosphate in the fungus is important to reduce their translocation to the plant (Turnau, Kottke, and Oberwinkler 1993).

Heavy metal uptake by plants from artificially contaminated soils often differs from the uptake in geogenic contaminated soils with the same level of contamination. This can significantly affect the efficiency of mycorrhizae inoculum in the field use. Mycorrhizae have been found in plants growing on naturally heavy metal–contaminated lands (Chaudhry et al. 1998, 1999), indicating that these fungi have evolved a tolerance for heavy metals and that they may play a role in the phytoremediation of the site. It is important to use indigenous AMF strains, which are best adapted to actual soil and climatic conditions, to produce site-specific AMF inocula (Khan 2005).

Conclusions

We may conclude that AMF are able to enhance plant bioremediation ability and tolerance to heavy metal contamination by increasing plant growth and P uptake and heavy metal sequestration by increasing P uptake, affecting heavy metal compartmentation in the plant,

and altering root exudates. Arbuscular mycorrhizal fungi effects on mycorrhizoremediation differ and probably depend on the kind and concentration of heavy metals in the soil. Accordingly, AMF can be efficiently used for cleaning up multi-metal-contaminated soil, indicating practical applications in phytoremediation of such soils.

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