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Influence of arbuscular mycorrhizal fungi and an improving growth bacterium on Cd uptake and maize growth in Cd-polluted soils

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Abstract

In a pot experiment, the effects of the bacterium strain *Micrococcus roseus*, native soil arbuscular mycorrhizal fungi (AMF) and the fungus *Glomus mosseae* on the growth, P, N, Fe, Mn, Zn and Cd uptake of maize in a soil polluted with Cd were investigated. A three-factor experiment was set up in a randomized complete design with three replicates of each treatment. The factors in the experiment were as follows: 1) AMF with two levels, G_1 (native AMF) and G_2 (*G. mosseae* + G_1); 2) bacterium promoting plant growth with two levels, B_0 (no inoculation) and B_1 (inoculation with *M. roseus*); and 3) cadmium with three levels (0, 100 and 200 mg Cd kg^{-1}soil). G_2 significantly increased root colonization, plant biomass, shoot nutrients and Cd uptake of plants in comparison with G_1 in Cd polluted conditions. The single presence of AMF contributed to the stabilization of Cd in roots and soil. Shoot and root dry weights, and shoot nutrients uptake of plants co-inoculated with bacterium and AMF were higher than mycorrhizal plants in the soil polluted with Cd. Plants co-inoculated with bacterium and G_2 had higher amount of shoot Cd uptake, root Cd uptake, Cd phytoextraction, translocation, and uptake efficiencies under both Cd concentrations than only mycorrhizal plants. The results showed that, most of the Cd was sequestered in roots of plants co-inoculated with bacterium and native AMF in the soil with 100 and 200 mg Cd kg^{-1}.

Additional key words: cadmium; *Glomus mosseae*; *Micrococcus roseus*; native AMF; phytoremediation; *Zea mays* L.

Resumen

Influencia de los hongos micorrícicos arbusculares y de una bacteria promotora del crecimiento en la absorción de Cd y el crecimiento del maíz en suelos contaminados con Cd

Se investigaron, en un experimento en macetas, los efectos de la bacteria *Micrococcus roseus*, hongos micorrícicos arbusculares (AMF) nativos del suelo y el hongo *Glomus mosseae* en el crecimiento, así como en la absorción de P, N, Fe, Mn, Zn y Cd por el maíz en un suelo contaminado con Cd. Se estableció un experimento trifactorial en un diseño completamente al azar con tres repeticiones por tratamiento. Los factores fueron: 1) AMF con dos niveles, G_1 (AMF nativos) y G_2 (*G. mosseae* + G_1), 2) bacterias promotoras del crecimiento de las plantas con dos niveles, B_0 (sin inoculación) y B_1 (inoculación con *M. roseus*); y 3) cadmio a tres niveles (0, 100 y 200 mg Cd kg^{-1} suelo). En condiciones de contaminación con Cd, G_2 aumentó significativamente, comparado con G_1, la colonización de las raíces, la biomasa vegetal, y la absorción de nutrientes y Cd en los brotes de las plantas. La sola presencia de AMF contribuyó a la estabilización de Cd en las raíces y el suelo. En el suelo contaminado con Cd, el peso seco de los brotes y las raíces, y la absorción de los nutrientes de los brotes en las plantas coinoculadas con la bacteria y los AMF fueron mayores que en las plantas micorrizadas. Las plantas coinoculadas con la bacteria y G_2 presentaron mayor cantidad de absorción de Cd en los brotes y raíces, fitoextracción de Cd, translocación y eficiencia de absorción con ambas concentraciones de Cd que las plantas solamente micorrizadas. Los resultados mostraron que la mayoría del Cd fue secuestrado en las raíces de las plantas coinoculadas con bacterias y AMF nativos en el suelo, con 100 y 200 mg Cd kg^{-1}.

Palabras clave adicionales: cadmio; fitorremediación; *Glomus mosseae*; hongos micorrícicos arbusculares nativos; *Micrococcus roseus*; *Zea mays* L.

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Abbreviations used: ACC (1-aminocyclopropane-1-carboxylate); AMF (arbuscular mycorrhizal fungi); HM (heavy metal); IAA (indole acetic acid); MHB (mycorrhiza helper bacteria); PGPRs (plant growth promoting rhizobacteria).

Introduction

Cadmium is an environmental contaminant and a toxic metal for living organisms. Agricultural soils in many parts of the world are moderately contaminated by Cd due to long term use of phosphoric treatments, sewage sludge application as well as smelter dust spreading (Vassilev *et al*., 2002). The remediation of heavy metals (HMs)-contaminated environments is a challenging task because these elements are not degradable and once entering the soil they can persist for a long time. Traditional methods used for the removal of HMs from the environment are in general expensive and potentially risky due to the possibility of the generation of hazardous by-products. Phytoremediation is an alternative approach and emerging low-cost and ecologically benign technology for decontamination of soils and defined as the process of utilizing plants to absorb, accumulate and detoxify contaminants in soil through physical, chemical and biological processes (Khan, 2005a,b; Abou-Shanab *et al*., 2008). The use of many hyperaccumulator plants in phytoremediation technology was restricted because of their slow growth, small size, low biomass-production and rigorous demand for growing conditions (Gamalero *et al*., 2009; Wu *et al*., 2010). Therefore, effective crops should be considered for the remediation of HM-polluted soils. The obtained results from some experiments allowed the selection of some species particularly tending to extract metals from soil, *i.e.* maize, sunflower and barley (Huang and Cunningham, 1996; Vassilev *et al*., 2002).

The success of phytoremediation may not only depend on the plant itself but also on the interaction of the plant roots with rhizospheric microbes and the speciation and concentrations of HMs in the soil (Vivas *et al*., 2003; Khan, 2005a,b). For instance, the use of plant growth promoting rhizobacteria (PGPRs) has been shown to enhance phytoremediation abilities of plants by increasing their growth and biomass (Burd *et al*., 2000; Duponnois *et al*., 2006; Abou-Shanab *et al*., 2008; Ahmad *et al*., 2008; Gamalero *et al*., 2009; Khan *et al*., 2009). Abou-Shanab *et al*. (2008) demonstrated an important role of four bacterial isolates in increasing metals availability in soil and enhancing their accumulation in maize (*Zea mays* L.) plants compared to uninoculated plants. The bacteria associated with plant roots may have profound effects on plant growth and nutrition through a number of mechanisms such as biological nitrogen fixation, synthesizing phytohormone precursors (Ahmad *et al*., 2008), vitamins, enzymes, siderophores, antibiotics (Burd *et al*., 2000) and inhibiting ethylene synthesis (Khan *et al*., 2009). In addition, the rhizobacterial strains with ability to solubilize inorganic (Khan and Zaidi, 2007) and organic P (Khan *et al*., 2007) can improve plant tolerance to metal toxicity, drought, and salinity stresses (Khan *et al*., 2009). Improvement of the interactions between plants and beneficial rhizospheric microbes can enhance biomass production and tolerance of the plants to HMs, and are considered to be an important component of phytoremediation technology (Glick, 2003; Belimov *et al*., 2005).

Arbuscular mycorrhizal fungi (AMF) provide direct links between soil and roots, and consequently may also have an essential contribution to phytoremediation by influencing HMs availability and enhancing plant tolerance (Leyval *et al*., 1997; Gaur and Adholeya, 2004). The mechanisms exerted by AMF to alleviate HMs stress in plants may include the immobilization of HMs in the mycelium, HMs adsorption to chitin in the cell walls, the improvement of plant mineral nutrition, changes in rhizosphere pH, and the regulation of gene expression under stress conditions (Joner *et al*., 2000; Li and Christie, 2001; Christie *et al*., 2004; Wang *et al*., 2007b; Upadhyaya *et al*., 2010). AMF may stimulate phytoextraction or contribute to phytostabilization. However element type, plant and fungal species may influence the effectiveness of mycorrhizal symbiosis in phytoremediation (Wang *et al*., 2007b).

The use of a combination of the PGPRs and AMF can be used to facilitate the process of phytoremediation and the growth of plants in metal-contaminated soils (Vivas *et al*., 2005; Gamalero *et al*., 2009). Synergistic effects between AMF and PGPRs in the remediation of metal contaminated soils have been reported (Vivas *et al*., 2003, 2005, 2006a,b; Duponnois *et al*., 2006; Khan, 2006). Vivas *et al*. (2003) showed that co-inoculation of *Trifolium repens* L. (white clover) with an indigenous Cd-adapted AM strain of *Glomus mosseae* and a Cd-adapted bacterium (*Brevibacillus* sp.) increased root biomass, and plant nutrient acquisition (N and P), and decreased the Cd uptake. Duponnois *et al*. (2006) showed that *Pseudomonas monteillii* not only increased AM colonization of *Sorghum bicolor* and acted as mycorrhiza helper bacteria (MHB), but also significantly increased Cd uptake by plants. These data supported the studies in which the use of indigenous AMF and MHB, inoculated together, is recommended in order to restore metal contaminated soils (reviewed by Leyval *et al*., 1997; Khan, 2004; Gamalero *et al*., 2009).

M. roseus, in addition to resistance to high concentrations of Pb, Zn, Ni and Cd (Motesharezadeh, 2008), can grow on solid medium HEPES-MES (Angle *et al*., 1992) at a concentration of 1 mM $CdCl_2.H_2O$ (Malekzadeh, 2010). The PGP activities of *M. roseus* were studied by Malekzadeh *et al*. (2010). Their results showed that this bacterium has the ability to dissolve poorly soluble organic and inorganic phosphate compounds, to produce siderophores, indole acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC)-deaminase enzyme. Since information about the interactions between *G. mosseae* and *M. roseus* and their potential for phytoremediation of Cd polluted soils is not available, this study was carried out to determine the effects of single inoculation of plants with either *G. mosseae* or *M. roseus* and co-inoculation of plants with these microorganisms on the growth and nutrient uptake of maize and accumulation of Cd in roots and shoots. The knowledge obtained may be applied for appropriate selection of microbial inoculants during phytoremediation of such contaminated areas.

Material and methods

Soil characteristics and treatments

A compound soil sample of a non-contaminated area was taken for plant cultivation from a depth of 0-30 cm in Beheshte Sakineh located in Karaj, Tehran. The physical (Page *et al*., 1982), chemical (Lindsay and Norvell, 1978; Page *et al*., 1982) and biological (Gerdemann and Nicolson, 1963; Jenkis, 1964; Zarei *et al*., 2008) properties of the selected soil are shown in Table 1. For pot experiments, air-dry soil was passed through 4 mm sieve and poured in 4 kg polyethylene plastic pots. Three Cd addition levels (0, 100 and 200 mg kg^{-1}) were applied in an analytical grade cadmium chloride ($CdCl_2.H_2O$) solution mixed thoroughly with 4 kg soil of each pot. The solutions were prepared in deionized water. The amounts of DTPA-extractable Cd after 1 month of incubation were 56.4 and 153.4 mg kg^{-1} of soil in the 100 and 200 mg Cd kg^{-1} treatments, respectively. All treatments were fertilized with 200 mg N kg^{-1} as urea (46%), 5 mg P kg^{-1} as $K_2HPO_4.3H_2O$, Fe, Zn, Cu, and Mn at a concentration of 5 mg kg^{-1} as $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$, respectively. Three-factor experiment was set up in a randomized complete design with three replicates of each treatment. The factors were as follows: 1) AMF with two levels, G_1 (native AMF) and G_2 (*G. mosseae* + G_1); 2) plant growth promoting bacterium with two levels, B_0 (no inoculation) and B_1 (inoculation with *M. roseus*); and 3) cadmium with three levels (0, 100 and 200 mg Cd kg^{-1}soil).

Table 1. Physical, chemical and biological characteristics of soil used in pot experiments

Characteristics	
Soil texture	Sandy loam
pH (1:1)	8.2
EC (dS m^{-1})	0.5
Carbonate calcium equivalent (%)	11.6
Organic carbon (%)	0.8
Field capacity (%)	20
Total Kjeldahl nitrogen (%)	0.08
Olsen-phosphorus (mg kg^{-1})	3.44
1 M NH_4OAc-extractable K (mg kg^{-1})	1093
DTPA*-extractable of Fe (mg kg^{-1})	3
DTPA*-extractable of Cu (mg kg^{-1})	2
DTPA*-extractable of Mn (mg kg^{-1})	11.28
DTPA*-extractable of Zn (mg kg^{-1})	1.64
Total microbial population (cfu g^{-1} soil)	1.3×10^8
Spore (no. g^{-1} dry soil)	7

* Diethylene triamine pentaacetic acid.

Microbial inoculation of soil and plant growth conditions

AM fungus and bacterium inoculants used in pot experiment consisted of *G. mosseae* and *M. roseus*, respectively. The *G. mosseae* fungus and *M. roseus* strain used in the current study are indigenous to HM-contaminated soils and were isolated and identified in previous studies by Zarei (2008) and Motesharezadeh (2008), respectively. Mycorrhizal inoculum was prepared through the trap culture with forage sorghum (*Sorghum bicolor* L.) with spores of *G. mosseae*. Trap culture medium was composed of autoclaved soil/quartz-sand (<1 mm) (4:1, v/v). After 4.5 months of culture, at the beginning of reproductive period, shoots were removed and contents of pot (mycorrhizal roots plus soil containing fungal spores and mycelia) were maintained in polyethylene bags at 4°C. In mycorrhizal treatments, 70 g of *G. mosseae* inoculum (containing spore numbers of 9 g^{-1} substrate and root colonization of 81%) was added to each pot at sowing time just below the maize seeds. The potential of the inoculum was measured following the methods described by Zarei *et al*. (2008), for spore extraction and counting,

and evaluation of root colonization. In bacterial treatments, each seed of maize (var. Karaj single cross 704) was inoculated with 1 mL of *M. roseus* inoculum (1×10^8 cfu mL^{-1}). The same amount of autoclaved inoculum of *G. mosseae* along with 1 mL of 1:1 filtered suspension of non-autoclaved inoculum with filter paper (Whatman, Grade 595:4-7µ pore size) was added in all control pots to provide a general microbial population free of AMF propagules. Five seeds of maize were planted. After germination, seedlings were thinned to three plants. Pots were placed in greenhouse conditions for three months (15-28˚C, with a 16/8 h light/dark period).

Plant harvesting and analyses

After a growth period of three months, shoot and root in each pot were harvested separately. Subsamples of fresh roots were taken to assess root colonization rate. Dry weight of roots and shoots after washing and drying at 65˚C for 72 h was measured. The percentage of root colonization by AMF using the grid-line intersect method was determined after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v) according to Kormanik and McGraw (1982). Cd, Fe, Zn and Mn concentrations of plant tissues were determined by the dry ash method with HCl using atomic absorption model A670 (Shimadzu, Japan) (Cottenie, 1980). Shoot P concentration was determined by colorimetry [spectrophotometer model UV-3100 (Shimadzu, Japan)] using the vanado-molybdate method and shoot N concentration was measured using Kjeldahl method (Cottenie, 1980).

Cd uptake (µg pot^{-1}) was calculated as Cd concentration (µg g^{-1} dry weight) multiplied by dry weight of plant (g pot^{-1}). Each pot consisted of three plants. Three aspects of plant Cd efficiency were assessed (Wang *et al*., 2007a). Cd uptake efficiency was calculated based on the ability of the root to take up Cd from the soil [the total amount of Cd in the plant (shoot Cd uptake + root Cd uptake) divided by root dry weight] and the Cd translocation efficiency was computed as the ability of the plant to transport the Cd to the shoot (the amount of Cd in the shoot divided by the amount of Cd in the root). Cd phytoextraction efficiency was calculated based on the ability of the root to transport Cd to shoot (the amount of Cd in the shoot divided by root dry weight).

Statistical analyses

All data were subjected to statistical analyses by three-way ANOVA using the SPSS 13.0 software. Means were calculated and compared using Duncan's multiple range tests via MSTATC software and statistical significance was determined at 5%.

Results

Root colonization

The effect of main factors and their interactions on root colonization was significant (Table 2). G_2 (*G. mosseae* + G_1) significantly increased root colonization of plants in comparison with the single presence of native AMF (G_1) in all treatments except in soil not polluted with Cd, B_1G_2 (Fig. 1A). Root colonization was significantly higher in plants inoculated with B_1G_2 than B_0G_2 in the soil with 200 mg Cd kg^{-1}.

Root colonization was not significantly different in B_0G_1 and B_1G_1 in the soil with 100 and 200 mg Cd kg^{-1}. B_1G_1 had higher root colonization than B_0G_1 in soil not polluted with Cd (Fig. 1A).

Shoot and root dry weights

The effects of main factors and their interactions on shoot and root dry weights were significant (Table 2). In all treatments, shoot and root dry weights significantly decreased, as the levels of soil Cd increased (Figs. 1B,C). Plants inoculated with G_2 had significantly higher shoot and root dry weights than plants colonized with G_1 in the soil with 100 and 200 mg Cd kg^{-1}. At these levels, B_1G_1 and B_1G_2 treatments induced higher shoot and root dry weights than B_0G_1 and B_0G_2 treatments. *M. roseus* (B_1) did not have significant effect on shoot dry weight in comparison with B_0 in soil not polluted with Cd. B_1 significantly increased root dry weight in comparison with B_0 in soil not polluted with Cd (Fig. 1C).

Cd uptake of root and shoot

The effects of main factors and their interactions on Cd uptake of root and shoot were significant (Table 2). The amount of Cd uptake in shoot of plants inoculated with G_2 significantly increased as the levels of Cd in-

creased (Fig. 1D). Plants inoculated with G_2 had higher shoot Cd uptake than plants colonized with G_1 in soil polluted with Cd, but there was not any significant difference in soil not polluted with Cd (Fig. 1D). Co-inoculation treatments of plants with B_1G_2 had significantly higher amount of shoot and root Cd uptake than B_0G_2 in Cd polluted conditions. Root Cd uptake in B_0G_1 was significantly lower than B_1G_1 in the soil with 100 and 200 mg Cd kg^{-1}, but at these levels of Cd, shoot Cd uptake in B_1G_1 and B_0G_1 did not have a significant difference (Figs. 1D,E).

P, N, Fe, Mn and Zn uptake of shoot

The effects of main factors and their interactions on P, N, Fe, Mn and Zn uptake of shoot were significant (Table 2). Shoot nutrient uptake of plants significantly decreased as the levels of soil Cd increased in all treatments (Fig. 2). Shoot P, N, Fe, Mn and Zn uptake of plants inoculated with G_2 were significantly higher than plants colonized with G_1 in the soil with 100 and 200 mg Cd kg^{-1} (Figs. 2A,B,C,D and E). B_1G_1 had higher shoot P, Mn and Zn uptake than B_0G_1 at all levels of Cd. Co-inoculation of plants with B_1G_2 significantly increased shoot P, Fe, Mn and Zn uptake at all levels of Cd compared to plants just inoculated with B_0G_2.

Uptake, translocation and phytoextraction efficiencies of Cd

The effect of main factors on uptake, phytoextraction and translocation efficiencies of Cd was significant

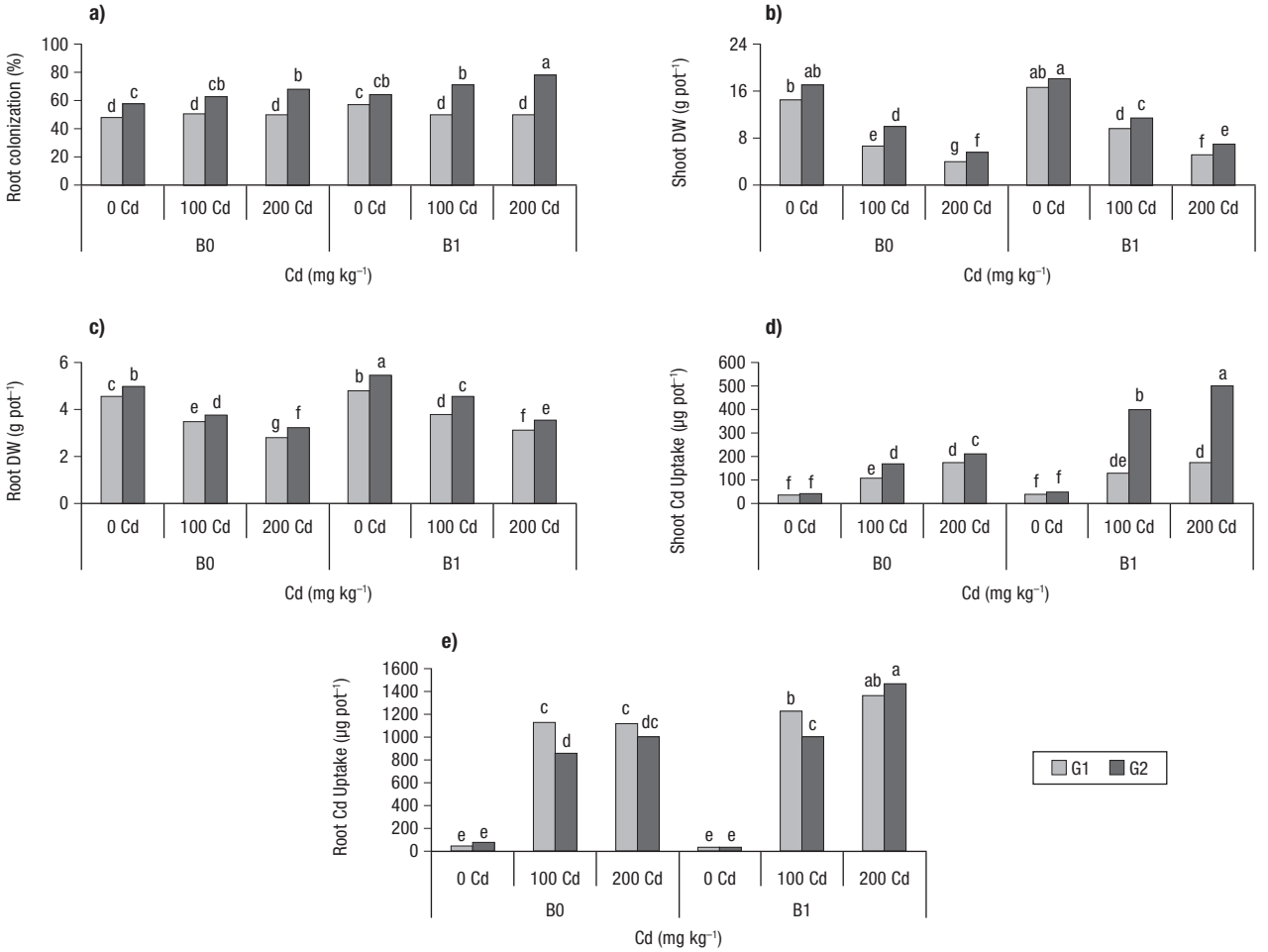

Figure 1. Effects of *Micrococcus roseus* and *Glomus mosseae* on (a) root colonization, (b) shoot dry weight, (c) root dry weight, (d) shoot Cd uptake, and (e) root Cd uptake of maize at different levels of cadmium. G_1 = native AMF; G_2= *G. mosseae* + native AMF; B_0 = no bacterium; and B_1 = *M. roseus*. Means with the same letter are not significantly different at the 0.05 level using Duncan's multiple range test.

Table 2. Significance of arbuscular mycorrhizal fungi (AMF-*Glomus mosseae*), bacterium (*Micrococcus roseus*) and Cd, and their interactions by ANOVA for all characteristics studied

Characteristics	Source of variation (F-values)						
	AMF (A)	Bacterium (B)	Cd (C)	A× B	A × C	B × C	A × B × C
Root colonization	***	*	*	**	**	**	**
Shoot dry weight	***	***	***	***	***	***	***
Root dry weight	***	***	***	***	***	***	***
Cd uptake of shoot	***	***	***	***	***	***	***
Cd uptake of root	***	**	***	***	***	***	***
P uptake of shoot	***	***	***	***	***	***	***
N uptake of shoot	**	**	**	**	**	**	**
Fe uptake of shoot	***	***	***	***	***	***	***
Mn uptake of shoot	***	***	***	**	***	**	***
Zn uptake of shoot	***	**	***	***	***	***	***
Cd phytoextraction efficiency	***	**	**	***	***	ns	***
Cd uptake efficiency	*	*	***	***	***	***	***
Cd translocation efficiency	***	***	***	***	***	***	***

ns: not significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

(Table 2). Their interactions were also significant except bacterium × Cd on phytoextraction efficiency (Table 2). In each treatment, Cd uptake and phytoextraction efficiencies increased as the levels of soil Cd increased (Table 3). In bacterial treatment, Cd phytoextraction and translocation efficiencies increased in plants inoculated with G_2 compared to plants colonized with G_1 in the soil with 100 and 200 mg Cd kg^{-1} (Table 3). The maximum Cd phytoextraction and translocation efficiencies were measured in plants co-inoculated with B_1G_2 in the soil with 100 and 200 mg Cd kg^{-1}. The minimum phytoextraction and translocation efficiencies of Cd at the levels of 100 and 200 mg Cd kg^{-1} were observed in plants inoculated with B_1G_1 (Table 3). Cd uptake efficiency was higher in plants colonized with G_1 than plants inoculated with G_2 when plants were grown in the presence of Cd. The highest Cd uptake efficiency was measured in plants inoculated with B_1G_1 in the soil with 200 mg Cd kg^{-1} (Table 3).

Discussion

Our study supported that *G. mosseae* (indigenous AM fungus to HM-contaminated area) + G_1 (*i.e.* G_2) and native AMF (*i.e.* G_1 as non-indigenous AMF isolates to HM-contaminated area) were able to colonize roots of maize under Cd polluted conditions. Cd pollution had no negative effect on root colonization of maize. These results may indicate that plants invest in AM symbiosis under soil-HM conditions (Audet and Charest, 2007). Root colonization in G_1 was not significantly different with increasing the levels of Cd from 100 to 200 mg kg^{-1}. Similar results were reported by Shetty *et al*. (1994) and Vivas *et al*. (2003) who stated that root colonization of plants inoculated with non-indigenous AMF isolates to HM-contaminated soils was not suppressed by increasing the HMs levels. These results point out that attention to non-indigenous AMF to HM-contaminated areas is also important.

G_2 increased root colonization, shoot and root dry weights, and shoot nutrients uptake of plants compared to G_1 in the soil polluted with Cd. *G. mosseae* is an indigenous AM fungus to HM-contaminated soils therefore it may help root colonization of plants. This is in agreement with findings of Weissenhorn and Leyval (1995), who indicated that the indigenous AM strain to HM-contaminated soils (as tolerant AM strain) was able to colonize more plants than the non-indigenous AM strain to HM-contaminated soils (as sensitive fungal strain) in Cd polluted conditions. *G. mosseae* isolate adapted better to Cd pollution and probably created a greater root absorption area and, due to the higher volume of soil explored by extraradical hyphae, promoted plant growth. A similar result was obtained in the studies of Vivas *et al*. (2003) who reported that *G. mosseae* isolate adapted to HM pollution was able to stimulate plant growth more than the non-indigenous isolate of *G. mosseae* BEG 119.

Shoot and root Cd contents of plants inoculated with G_2 were higher and lower, respectively than G_1. Although G_2 could increase more plant Cd phytoextraction and translocation efficiencies than G_1, the ability of colonized roots to take up Cd from the soil (*i.e*. Cd uptake efficiency) was lower. These results may indicate that G_2 prevent Cd uptake by roots and therefore most of the Cd is retained in the soil, while in G_1 Cd uptake by roots is higher and most of Cd is stabilized within the roots. The reason of plant protection against Cd toxicity in plants inoculated with AMF may occur indirectly by enhancing plant nutrition and increasing plant growth therefore resulting in a diluting effect of Cd in the plant (Chen *et al*., 2007). Also, mycorrhizal plants might actively reduce heavy metal uptake from soils by mediating metal solubility via soil pH changes, which may be a defense strategy adopted by mycorrhizae to avoid or escape the negative impacts of high soil metal concentrations (Wang *et al.*, 2007a). Furthermore, chelation/immobilization of metals by extraradical mycelium, glomalin, or exudates can also sequester metals (Joner *et al*., 2000; Wang *et al.*, 2007a); thus contributing to a reduced metal uptake by mycorrhizae (Wang *et al.*, 2007a). In the current study, the single presence of AMF especially G_1 may contribute more to the retention of Cd in roots and also to soil stabilization.

Co-inoculation of plants with *M. roseus* and AMF improved growth and shoot P, N, Fe, Mn and Zn uptake of plants in soil polluted with Cd in comparison with only mycorrhizal plants. The synergistic effects of co-inoculation of plants with *M. roseus* and AM fungi on nutrients uptake may act as a protection mechanism

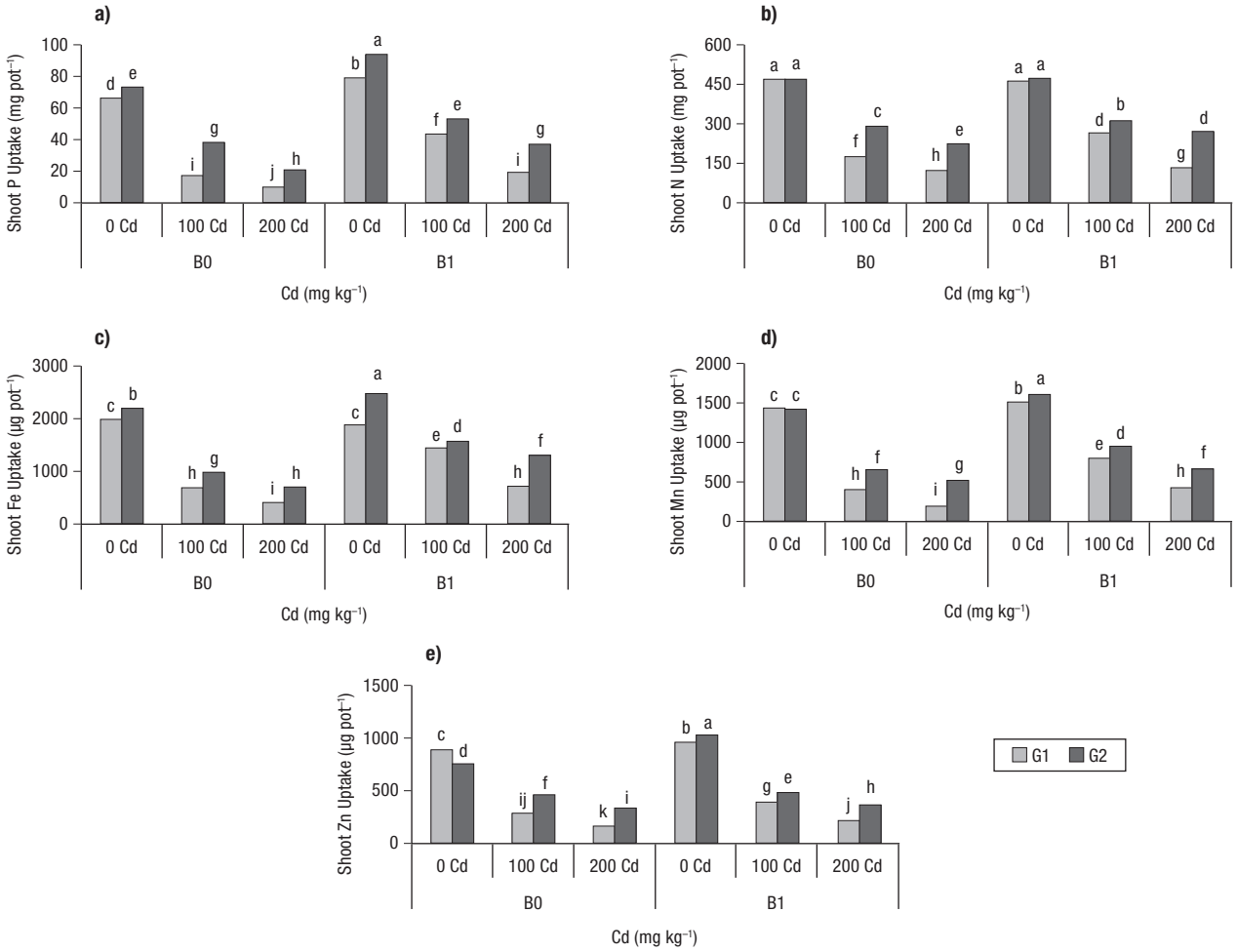

Figure 2. Effects of *Micrococcus roseus* and *Glomus mosseae* on shoot P (a), N (b), Fe (c), Mn (d) and Zn (e) uptake of maize at different levels of cadmium. G_1 = native AMF; G_2 = *G. mosseae* + native AMF; B_0 = no bacterium; and B_1 = *M. roseus*. Means with the same letter are not significantly different at the 0.05 level using Duncan's multiple range test.

Table 3. Effects of *Micrococcus roseus* and *Glomus mosseae* on Cd phytoextraction, uptake and translocation efficiencies of maize at different levels of cadmium

Treatments*	Cd level (mg kg^{-1})	Phytoextraction efficiency (µg g^{-1})	Uptake efficiency (µg g^{-1})	Translocation efficiency
B_0G_1	0	7.58g	16.92g	0.812c
B_0G_2	0	7.63g	22.63g	0.508d
B_1G_1	0	7.60g	12.88g	1.439b
B_1G_2	0	8.15g	12.96g	1.689a
B_0G_1	100	39.06e	352.06d	0.144i
B_0G_2	100	50.30d	267.76f	0.198h
B_1G_1	100	29.04f	356.03d	0.090j
B_1G_2	100	87.18b	307.04e	0.396e
B_0G_1	200	63.46c	462.75c	0.169h
B_0G_2	200	65.70c	377.23d	0.211g
B_1G_1	200	54.93d	555.17a	0.123i
B_1G_2	200	140.96a	496.55b	0.340f

*G_1= native AMF, G_2 = *Glomus mosseae* + native AMF, B_0 = no bacterium and B_1 = *M. roseus*. Means with the same letter are not significantly different at the 0.05 level using Duncan's multiple range test.

that decreases Cd toxicity (Yan-De *et al*., 2007). Siderophore production by *M. roseus* (Malekzadeh *et al*., 2010) may increase shoot Fe uptake. In addition to siderophore production, several studies have stated that the production of IAA also enhances Fe absorption and other elements by a mechanism different from that involving siderophores in plant roots (Patten and Glick, 1996; Khan *et al*., 2009). Metabolically related precursors of IAA can solubilize soil Fe (III) reductively and therefore increase its availability (Khan *et al*., 2009). Iron in shoots positively affects formation of chlorophyll and plant photosynthesis (Gamalero *et al*., 2009), this may be the reason for prevention of chlorosis formation and leaf yellowing in the presence of high levels of Cd. In the present study, positive effects of bacterium on the plant growth could also occur through their ability to solubilize phosphate (Malekzadeh *et al*., 2010), synthesize ACC deaminase (Malekzadeh *et al*., 2010) and by the higher development of AMF, which enhances supply of essential nutrients from the soil to the host plant (Hetrick *et al*., 1994; Hildebrandt *et al*., 1999; Chen *et al*., 2003; Medina *et al*., 2003) and therefore reduces phytotoxic effects. Heavy metal stress may induce ACC production and then ethylene synthesis. ACC deaminase synthesized by bacterium metabolizes ACC of roots to ketobutyrate and ammonia. The bacteria utilize the ammonia evolved from ACC as a source of N and thereby restrict the accumulation of ethylene within the plant (Khan *et al*., 2009) and increase N content and growth of plants. Medina *et al*. (2003) demonstrated that the role of AM symbiosis to plant growth and efficient use of nutrients may be increased in association with selected rhizosphere bacteria. They also stated that the microbial communities associated to the root system regulate the availability of plant nutrients and are key factors in the context of sustainable systems.

In the presence of bacterium, shoot and root Cd uptake, Cd phytoextraction, translocation and uptake efficiencies of plants inoculated with G_2 significantly increased compared to only mycorrhizal plants in soil polluted with Cd. This may show that the roots of plants inoculated with B_1G_2 had high ability to take up Cd from the soil and transfer to aerial parts of plants. This treatment may help in phytoextraction and decontamination of Cd. Microorganisms could modify the soil environment to increase the Cd bio-availability and plant Cd uptake (Duponnois *et al*., 2006; Denton, 2007). In plants inoculated with G_2, *M. roseus* significantly increased root colonization when compared to mycorrhizal plants only at the level of 200 mg Cd kg^{-1}. The bacterium also increased root colonization of plants colonized with native AMF in the soil not polluted with Cd. Similarly, an enhanced AM fungal colonization level in roots in the presence of PGPRs has been previously demonstrated (Vivas *et al*., 2003; Radziah *et al*., 2007). It was also described that the stimulation of plant susceptibility to AM infection and growth of AM extraradical mycelium could be due to growth promoting compounds produced by bacteria (Azcón, 1993). *M. roseus,* due to production of IAA and ACC-deaminase enzyme (Malekzadeh *et al*., 2010), may develop

AMF hyphae and also reduce the level of ethylene in plant roots at high concentration of Cd. This bacterium acted as mycorrhizal helper bacterium (MHB) at high Cd concentrations. It has been previously reported that a MHB, isolate of *Pseudomonas monteillii* (isolate HR 13), stimulated AM colonization of *Acacia holosericea* seedlings by *Glomus intraradices* (Duponnois and Plenchette, 2003).

In the presence of bacterium, Cd phytoextraction and translocation efficiencies of plants colonized with G_1 significantly decreased, while root Cd content and Cd uptake efficiency of plants increased compared to only mycorrhizal plants in soil polluted with Cd. These results indicate that although the roots of plants inoculated with B_1G_1 had high ability to take up Cd from the soil, most of the Cd was in roots and translocation to the shoot decreased. This treatment may assist in phytostabilization of Cd. The PGPRs and AMF can change the HMs speciation from available to non available form by changing the oxidation state of the metal. For example, they can bind metals inside the cell wall, to proteins and extracellular polymers, or to sulfide compounds forming insoluble metal sulfides (Göhre and Paszkowski, 2006). These bonds alter the toxic chemical properties of the metals and reduce their bioavailability. The increasing concentration of H^+ by PGPRs may be the mechanism that transfers HMs into storage vacuoles (Zhuang *et al*., 2007). Consequently, the storage in vacuoles separated the HMs from their biological active sites in the cell and reduced their toxicity (Denton, 2007).

Del Val *et al*. (1999) demonstrated that AMF and bacterium isolated from HM-contaminated soils were often more resistant to metals and effective in plants HM uptake than those collected from non-contaminated environments. Therefore, co-inoculation of HM-contaminated soils with indigenous microorganisms of these soils such as *M. roseus* and *G. mosseae* seems to be a strategy which can be recommended for promoting plant growth in soil polluted with Cd. In this case, isolation of efficient metal-adapted microorganisms may be an interesting biotechnological tool for inoculation purposes in contaminated soils (Dodd and Thomson, 1994). However, these effects should be further validated in greenhouse experiments and under field conditions to gain better understanding of the microsymbiont-plant interactions and their role in Cd uptake.

In conclusion, the results show that AMF can colonize plant roots in Cd polluted conditions and *G. mosseae*, an indigenous AM fungus to HM-contaminated soils, improved root colonization of plants. Only in mycorrhizal plants, AMF could possibly help phytostabilization. The synergistic relationships of *M. roseus* and AM fungi on biomass production and nutrient uptake may act as a protection mechanism that decreases Cd toxicity. *M. roseus,* due to plant growth promoting activities, may alleviate Cd phytotoxic effects and improve root colonization of plants in Cd polluted conditions. Co-inoculation of plants with B_1G_2 may assist in phytoextraction, while the presence of bacterium together with native AMF (B_1G_1) may help in phytostabilization of Cd-contaminated sites.

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