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Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (*Zea mays* L.) in soil experimentally contaminated with zinc

Baodong Chen^{1,2}, Hong Shen¹, Xiaolin Li^{1,4}, Gu Feng¹ & Peter Christie^{1,3}

¹Department of Plant Nutrition, China Agricultural University, Beijing, 100094, China. ²Present address: Department of Soil Environmental Science, Research Center for Eco-Environmental Sciences, CAS, Beijing, 100085, China. ³Agricultural and Environmental Science Department, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK. ⁴Corresponding author*

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Abstract

A pot experiment was conducted to investigate the uptake of Zn from experimentally contaminated calcareous soil of low nutrient status by maize inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus caledonium*. EDTA was applied to the soil to mobilize Zn and thus maximize plant Zn uptake. The highest plant dry matter (DM) yields were obtained with a moderate Zn addition level of 300 mg kg⁻¹. Plant growth was enhanced by mycorrhizal colonization when no Zn was added and under the highest Zn addition level of 600 mg kg⁻¹, while application of EDTA to the soil generally inhibited plant growth. EDTA application also increased plant Zn concentration, and Zn accumulation in the roots increased with increasing EDTA addition level. The effects of inoculation with *G. caledonium* on plant Zn uptake varied with Zn addition level. When no Zn was added, Zn translocation from roots to shoots was enhanced by mycorrhizal colonization. In contrast, when Zn was added to the soil, mycorrhizal colonization resulted in lower shoot Zn concentrations in mycorrhizal plants. The P nutrition of the maize was greatly affected by AM inoculation, with mycorrhizal plants showing higher P concentrations and P uptake. The results indicate that application of EDTA mobilized soil Zn, leading to increased Zn accumulation by the roots and subsequent plant toxicity and growth inhibition. Mycorrhizal colonization alleviated both Zn deficiency and Zn contamination, and also increased host plant growth by influencing mineral nutrition. However, neither EDTA application nor arbuscular mycorrhiza stimulated Zn translocation from roots to shoots or metal phytoextraction under the experimental conditions. The results are discussed in relation to the environmental risk associated with chelate-enhanced phytoextraction and the potential role of arbuscular mycorrhiza in soil remediation.

Introduction

Contamination of agricultural soils with trace metals of geological origin or from human activities has compromised soil environmental quality and poses a potential threat to sustainable agriculture in many parts of the world. There is therefore considerable interest in the development of remediation strategies for contaminated soils, and phytoremediation has emerged as a developing technology. The various remediation

strategies available have been described in several review papers (Cunningham and Ow, 1996; Luo, 1999; McGrath et al., 2002; Salt et al., 1995). One strategy is to grow hyperaccumulator plant species that can accumulate large concentrations of metals in their aerial parts, but which typically have low yields that limit the quantities of metals that they can remove from soils. A second strategy is to grow high-biomass crop plants with lower capacity to accumulate metals, and to optimize metal removal of soil metals by chelate-enhanced phytoextraction (Luo, 2000).

* FAX No.: +86 10 62891016. E-mail: lixl@cau.edu.cn

In chelate-enhanced phytoremediation, a chelating agent is applied to the soil to release adsorbed heavy metals from soil solids and thus increase the mobility of the metals in the soil so that certain plants can take up larger quantities of metal. Some of the crop species that could be used for this are typically mycorrhizal, but there is little published information on the effects of chelating agents on mycorrhizas or on the effects of mycorrhizas on plant uptake of heavy metals mobilized using chelating agents.

It is well known that the extraradical mycelium of AM fungi can increase the volume of soil explored by plant roots for the uptake of mineral nutrients (Bolan, 1991; Marschner, 1998). When nutrients of low mobility such as Zn, Cu or Fe are in short supply, uptake of these nutrients by plant roots can often be increased by the formation of a mycorrhizal association (Faber et al., 1990; Kothari et al., 1991; Li et al., 1991; Liu et al., 2000). On the other hand, when excessive concentrations of trace metals are present in soils, mycorrhizal plants can contain lower shoot metal concentrations than uninoculated plants growing under similar conditions (Dueck et al., 1986; Leyval et al., 1991; Liu et al., 2000; Tao, 1997). The lower metal concentrations in mycorrhizal plants has often been attributed to larger plant biomass with a resultant 'growth dilution' effect (Nielsen and Jensen, 1983). However, dilution effects cannot explain all the experimental results. In some investigations mycorrhizal plants have absorbed more metals with increasing supply of Cu, Ni, Pb and Zn, or with decreasing soil pH value, and adverse mycorrhizal effects have sometimes been recorded (Jamal et al., 2002; Killham and Firestone, 1983; Weissenhorn and Leyval, 1995). The effects of AM colonization on metal uptake by host plants are therefore complicated and will depend on the exact conditions in any experimental study.

The significance of arbuscular mycorrhiza in plant uptake of metals from metal-contaminated soils merits systematic studies (Leyval et al., 1997). The wide distribution of AM fungi on metal contaminated sites has shown general adaptation and tolerance of these symbionts to heavy metals (Pawlowska et al., 1996; Shetty et al., 1994a,b), and metal-tolerant fungal strains have been isolated by several research groups (Griffioen, 1994; Hildebrandt et al., 1999; Raman et al., 1993; Weissenhorn et al., 1993, 1994). Recent research has focused on the role of AM fungi in the stabilization, rather than the remediation, of metal polluted ecosystems (Vangronsveld et al., 1996; Leyval et al., 1997; Khan et al., 2000).

This paper describes an experiment that was designed to investigate the potential for chelate-induced remediation of Zn-contaminated soil using crop plants colonized by an AM fungus. Maize has a high biomass and rapid growth and was adopted as the test plant. We investigated the effects of EDTA application and AM colonization on Zn uptake and mineral nutrition by maize grown in soil experimentally contaminated with Zn. Our hypothesis was that Zn uptake, especially shoot accumulation, would be increased by the mobilization of soil Zn by the chelate EDTA and the metal tolerance of the host plant would be increased by the protective effects of mycorrhizal colonization. As a result, the combination of EDTA application and mycorrhizal colonization might facilitate soil remediation.

Materials and methods

Mycorrhizal inoculum

The AM fungus *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann was originally isolated from a sandy soil in Fengqiu County, Henan Province, and was kindly provided by Professor X.G. Lin of the Institute of Soil Science, Chinese Academy of Sciences. The fungus was propagated in pot culture on maize plants grown in a sandy soil for 10 weeks. Inoculum from pot culture was a mixture of spores, mycelium, sandy soil and maize root fragments containing approximately 1000 spores per 100 g.

Host plants

Seeds of maize (*Zea mays* L. cv. ND108) were surface sterilized in a 10% (v/v) solution of hydrogen peroxide for 10 min and immersed in deionized water for 6 h. They were then germinated on moist filter paper for about 24 h until the radicles appeared. They were selected for uniformity before sowing.

Soil mixture

A calcareous sandy soil of low nutrient status was employed, but was mixed in a ratio of 3:1 with a loam soil to improve its water retention characteristics and to alleviate any potential negative effects of EDTA on plant growth that might perhaps conceal other treatment effects. The sandy soil was collected from the vicinity of Lugouqiao, Fengtai district, Beijing and had the

following properties (DM basis): pH (soil: water ratio 1:2.5) 8.67, organic matter 0.30%, total N 0.018%, $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ -extractable P 3.4 mg kg^{-1} , and $1 \text{ mol L}^{-1} \text{ NH}_4\text{OAc}$ -exchangeable K 33.6 mg kg^{-1} . The soil extractable trace element concentrations were: Fe 4.96, Mn 4.15, Cu 0.28 and Zn 0.48 mg kg^{-1} . Soil metals were extracted with a 0.005 mol L^{-1} DTPA (diethylene triamine pentaacetic acid) - $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ - 0.1 mol L^{-1} TEA (triethanolamine) solution at pH 7.30. The calcareous loam soil was collected from Yizishan village, Zhenzi town, Tangshan City, Hebei Province and had the following properties (DM basis): pH 7.60, organic matter 0.83%, total N 0.027%, $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ -extractable P 9.7 mg kg^{-1} , and $1 \text{ mol L}^{-1} \text{ NH}_4\text{OAc}$ -exchangeable K 60.0 mg kg^{-1} . Extractable trace element concentrations were: Fe 2.60, Mn 8.35, Cu 0.45 and Zn 1.36 mg kg^{-1} . The soils were passed through a 1-mm sieve, sterilized by autoclaving at 120°C for 2 h and then air-dried. Prior to the experiment the sandy soil and loamy soil were mixed in a ratio of 3:1 and the soil mixture was used as the cultivation medium. The soil mixture had a pH value (soil: water ratio, 1:2.5) of 8.18, DTPA-extractable Zn 0.44 mg kg^{-1} , and $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ -extractable P 3.96 mg kg^{-1} . In this paper, the term 'soil' in the context of the present study refers to the mixture of the two soils.

Experimental procedure

Three Zn addition levels (0, 300 and 600 mg kg^{-1}) were applied as analytical grade ZnSO_4 solution mixed thoroughly with the soil. The solutions were prepared in deionized water and mixed with the soil manually. There were three EDTA application levels of 0, 2.5 and 10 mmol kg^{-1} (E0, E2.5 and E10), and maize plants were either inoculated with (+M) the AM fungus *G. caledonium* or uninoculated controls (-M) under all Zn addition and EDTA application levels. Thus, there were 18 treatments in total with 3 replicates, giving a total of 54 pots in a randomized block design. All treatments received a basal application of mineral nutrients added in solution to each compartment at rates of $300 \text{ mg N (NH}_4\text{NO}_3)$, $20 \text{ mg P (KH}_2\text{PO}_4)$, and $150 \text{ mg K (K}_2\text{SO}_4) \text{ kg}^{-1}$ soil.

Round plastic pots were used, each containing 2.0 kg of soil. Firstly, 1.5 kg soil was placed in the pot, then mycorrhizal inoculum, and finally the remainder of the soil. Inoculum was placed about 2 cm below the soil surface. Mycorrhizal treatments received 100 g of inoculum, while non-inoculated plants received

an equivalent amount of sterilized inoculum together with an aqueous filtrate ($0.25 \mu\text{m}$ pore size) of unsterilized soil to provide a similar microflora except for the absence of the mycorrhizal fungus. Five germinated seeds (carefully selected for uniformity) were sown in each pot and 7 days after seedling emergence these were thinned to 3 seedlings. During the experimental period deionized water was used to maintain a moisture content of 15% (DM basis) by regular weighing.

The experiment was conducted in the glasshouse of the Department of Plant Nutrition, China Agricultural University, Beijing, and the plants grew for 7 weeks from 25 April 2001 to 6 June 2001 under natural light conditions with no supplementary illumination. During the period of plant growth the day temperature ranged from 20°C to 30°C and the night temperature from 15°C to 20°C . At the start of the experiment, there were Zn addition and AM inoculation treatments only (6 treatments and 9 replicates each). EDTA was applied in solution 1 week before harvest (7 weeks after seedling emergence) at 3 application levels as described above for each Zn addition level and AM inoculation treatment. After a further growth period of 1 week the maize plants were harvested.

Leaves, stems (including leaf bases) and roots were harvested separately after growth for 8 weeks. Samples were carefully washed with tap water and then deionized water. Sub-samples of fresh roots were collected for determination of AM colonization rate. The dry weights of leaves, stems and roots were determined after oven drying at 70°C for 48 h. Oven-dried sub-samples were milled to pass through a 0.5-mm sieve and dry ashed at 560°C for multi-element analysis by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Sun et al., 2002; Sahrawat et al., 2000). Soil P was determined colorimetrically by the vanadomolybdate method, and Zn and other trace metals by atomic absorption spectrophotometry (AAS) using standard methods. Samples for assessing root colonization were prepared using the acid fuchsin staining-grid intersect method (Giovannetti and Mosse, 1980; Kormanik et al., 1979). Data were tested by three-way analysis of variance and means were compared by LSD at the 5% level.

Table 1. Proportion of maize root length colonized by the mycorrhizal fungus

Zn addition level (mg kg ⁻¹)	Inoculation treatment	Root infection rate (%)		
		E 0 ¹	E 2.5	E 10
0	-M ²	0.0c ³	0.0d	0.0c
	+M	76.9a	72.9a	76.1a
300	-M	0.0c	0.0d	0.0c
	+M	49.6b	51.0b	55.5b
600	-M	0.0c	0.0d	0.0c
	+M	46.4b	43.0c	50.3b

Significance⁴ of:

Zn addition level

Inoculation

EDTA application rate

Zn × inoculation

Zn × EDTA

Inoculation × EDTA ns

ns

ns

¹E0, E2.5, E10: EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹.

²-M, +M: uninoculated control, inoculation with the mycorrhizal fungus *G. caledonium*.

³In each column, means followed by the same letter are not significantly different by LSD at the 5% level.

⁴By analysis of variance; ***, $p < 0.001$; ns, not significant.

Results

Mycorrhizal colonization

No root infection was detected in the non-inoculated plants, while inoculated plants had relatively high infection rates (Table 1). When no Zn was added the rate of root colonization exceeded 70%, while mycorrhizal colonization was markedly inhibited by Zn addition as the proportion of root length colonized decreased to around 50%. There was no significant difference in root colonization rate between the two Zn addition levels, and EDTA application had no detectable influence on root colonization rate.

Plant growth

The highest dry biomass of maize plants occurred under the Zn addition level of 300 mg kg⁻¹, followed by the zero-Zn controls (except for foliar P contents), and the lowest dry biomass occurred under the highest Zn addition level of 600 mg kg⁻¹ (Table 2). Actually, mild symptoms of Zn phytotoxicity were detected under the highest Zn addition level with the plants showing stunted growth and the young leaves especially appearing etiolated, but no symptoms were

Figure 1. Shoot (A) and root (B) dry matter yield of maize plants under different treatments. -M and +M represent non-inoculation treatment and inoculation with mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹. Different letters above the columns indicate significant difference between means by LSD at the 5% level. By analysis of variance, both Zn addition level and EDTA application rate were highly significant ($p < 0.001$) and inoculation had a significant effect ($p < 0.05$) on root yield but not shoot yield. The interaction between Zn and EDTA rates was significant for both shoot ($p < 0.05$) and root ($p < 0.001$) yields.

Table 2. Dry matter yield of maize under different Zn addition levels¹

Zn addition levels (mg kg ⁻¹)	Dry weight (g pot ⁻¹)		
	Leaves	Stems	Roots
0	17.67b	10.89b	15.05a
300	20.98a	12.37a	16.07a
600	15.57c	7.92c	11.85b

Significance² of:

Zn addition level

Inoculation

EDTA application rate

Zn × inoculation

Zn × EDTA

Inoculation × EDTA

ns

*

ns

¹ Means of 18 replicates of all EDTA application and inoculation treatments under the same Zn addition level.

² By analysis of variance; ***, $p < 0.001$; *, $p < 0.05$; ns, not significant.

observed under the Zn addition level of 300 mg kg⁻¹. Under all Zn addition levels, plant dry weight tended to decrease with increasing EDTA application rate, and at the EDTA application rate of 10 mmol kg⁻¹, maize plants showed significantly lower shoot and root dry weights compared with plants receiving no EDTA (Figure 1A, B). The EDTA application rate of 10 mmol kg⁻¹ also led to obvious plant withering under the zero-zinc treatment, but somewhat surprisingly this was not the case when Zn was added.

Plant growth was generally enhanced by mycorrhizal colonization, especially under the highest Zn addition level of 600 mg kg⁻¹ under which mycorrhizal treatments produced significantly larger root dry weights than did uninoculated controls (Figure 1A, B). However, with a few specific exceptions, there were no significant differences in shoot dry weight (sum of leaf dry weight and stem dry weight) between mycorrhizal and non-inoculated treatments. Mycorrhizal colonization also showed no marked effects on root growth when no Zn was added and under the Zn addition level of 300 mg kg⁻¹.

Zn uptake and partitioning

Figure 2 shows that Zn concentrations of maize plants increased markedly with increasing Zn addition levels. Compared with the controls, Zn concentrations of maize leaves, stems and roots under the Zn addition level of 600 mg kg⁻¹ increased 30-, 40- and 50-fold, respectively. Under all Zn addition levels, Zn concentrations of roots and leaves also increased with increasing EDTA application rate, but stem Zn concentrations were not greatly affected by EDTA applications.

Mycorrhizal colonization showed diverse effects on Zn concentrations of maize plants depending on the experimental treatments. When no Zn was added, foliar Zn concentrations generally increased, stem Zn concentrations were slightly affected, and root Zn concentrations tended to decrease with mycorrhizal colonization. An exception was the highest EDTA application rate under which foliar Zn concentrations showed no significant difference between mycorrhizal and non-inoculated treatments, but stem Zn concentrations of mycorrhizal plants were significantly higher than those of uninoculated plants. Under the Zn addition level of 300 mg kg⁻¹ and with no EDTA applied (or at the EDTA application rate of 2.5 mmol kg⁻¹), mycorrhizal colonization tended to decrease foliar Zn concentrations and increase stem and root Zn concentrations, but no significant differences were

Figure 2. Zn concentration of maize leaves (A), stems (B) and roots (C) under different treatments. -M and +M represent non-inoculation and inoculation with the mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹. Different letters above the columns indicate significant difference between means by LSD at the 5% level. By analysis of variance, Zn addition level was highly significant ($p < 0.001$) in all three plant components, and EDTA application rate and the interaction between Zn addition rate and EDTA application rate were also highly significant ($p < 0.001$) in leaves and roots.

found between mycorrhizal and uninoculated treatments. However, at the EDTA application rate of 10 mmol kg⁻¹, foliar, stem and root Zn concentrations were all significantly increased by mycorrhizal colonization. Under the highest Zn addition level of 600 mg kg⁻¹, mycorrhizal colonization decreased plant Zn concentrations overall, and especially foliar Zn concentrations. Under the 10 mmol kg⁻¹ EDTA application rate, foliar Zn concentrations of mycor-

Figure 3. Zn uptake by maize shoots (A) and roots (B) under different treatments. -M and +M represent non-inoculation treatment and inoculation with the mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹. Different letters above the columns indicate significant difference between means by LSD at the 5% level. By analysis of variance, shoot Zn uptake was highly significantly affected by Zn addition level ($p < 0.001$) and root Zn uptake by Zn addition level, inoculation and EDTA application rate (all $p < 0.001$). In root Zn uptake there were significant interactions between Zn addition level and EDTA application rate ($p < 0.001$) and between Zn addition level and inoculation ($p < 0.01$).

rhizal plants were significantly lower than those of corresponding uninoculated plants.

Zn uptake by maize plants also markedly increased with increasing Zn addition level, while EDTA application generally had no discernible effect on shoot Zn uptake, and the same held for root Zn accumulation in the zero-Zn treatment and the Zn addition level of 300 mg kg⁻¹ (Figure 3A, B). However, EDTA application significantly increased Zn uptake by roots under the Zn addition level of 600 mg kg⁻¹.

Mycorrhizal effects on Zn uptake by maize plants varied with Zn addition level, and were influenced by EDTA application. When no Zn was added, shoot Zn uptake was significantly increased by mycorrhizal colonization. On the other hand, root Zn uptake of mycorrhizal plants was significantly lower than controls when no EDTA was applied, but this difference disappeared under EDTA applications. With Zn addition, shoot Zn uptake was not affected by the inoculation

Figure 4. Zn partitioning in maize plants under different treatments. -M and +M represent uninoculated treatments and inoculation with the mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹.

treatments, but root Zn accumulation was increased by mycorrhizal colonization. At the 2.5 mmol kg⁻¹ EDTA application rate, Zn uptake by mycorrhizal roots was significantly higher than that in uninoculated roots.

Leaf-to-stem and shoot-to-root ratios of Zn uptake reflected Zn partitioning at the plant organ level. Figure 4 shows that mycorrhizal plants generally took up higher leaf-to-stem and shoot-to-root ratios of Zn than did corresponding controls when no Zn was added. Mycorrhizal colonization had clearly facilitated Zn transport from the roots to the aerial parts and also translocation to the leaves. With increasing EDTA application rates shoot-to-root ratio of Zn uptake in uninoculated plants tended to increase, with the opposite trend in mycorrhizal treatments. Under Zn addition, the mycorrhizal effects on Zn partitioning in the plants were reversed, with mycorrhizal plants generally showing lower leaf-to-stem and shoot-to-root ratios of Zn uptake than controls. This may be indicative of mycorrhizal inhibition of Zn transport from roots to shoots and translocation to leaves. With increasing EDTA application rate, leaf-to-stem ratio of

Figure 5. P concentration of maize leaves (A), stems (B) and roots (C) under different treatments. -M and +M represent non-inoculation and inoculation with the mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹. Different letters above the columns indicate significant difference between means by LSD at the 5% level. For results of three-way ANOVA, see Table 3.

Zn uptake tended to increase, and the opposite trend occurred in shoot-to-root ratio, indicating that EDTA had enhanced Zn accumulation in plant roots and Zn partitioning to leaves in the aerial parts of the plants.

Plant phosphorus nutrition

P concentrations in the leaves, stems and roots of the maize plants were generally increased by mycorrhizal colonization (Figure 5), and also tended to increase with increasing EDTA application rate. One exception was stem P concentration, which decreased with increasing EDTA application rate under the Zn addition level of 600 mg kg⁻¹, and under the Zn addition level of 300 mg kg⁻¹, leaf and stem P concentrations in non-inoculated plants were unaffected by EDTA

Figure 6. Soil DTPA-extractable Zn (A) and 0.5 M NaHCO₃-extractable P (B) of different treatments after harvest. -M and +M represent non-inoculation and inoculation with the mycorrhizal fungus *G. caledonium*, and E0, E2.5 and E10 represent EDTA application rate of 0, 2.5 and 10 mmol kg⁻¹. Different letters above the columns indicate significant difference between means by LSD at the 5% level. By analysis of variance, Zn addition level and EDTA application rate were highly significant for extractable P and Zn and inoculation for extractable P (all $p < 0.001$). The interaction between Zn level and EDTA rate was significant for extractable Zn ($p < 0.001$) and that between inoculation and Zn level for extractable P ($p < 0.01$).

application. Maize plants contained the highest P concentrations under the Zn addition level of 600 mg kg⁻¹ (Table 3).

Mycorrhizal plants generally took up more P than corresponding uninoculated plants. Zinc addition and EDTA application had similar effects on plant P uptake to those on plant growth: plant P uptake decreased with increasing EDTA application rate (data not shown), and the highest P uptake by leaves, stems and roots occurred under the Zn addition level of 300 mg kg⁻¹, followed by those receiving no Zn addition (except for foliar P concentration), and the lowest P uptake under the highest Zn addition level of 600 mg kg⁻¹ (Table 3).

Table 3. P concentration and P uptake of maize leaves, stems and roots under different Zn addition levels¹

Zn addition levels (mg kg ⁻¹)	P concentration (%)			P uptake (mg pot ⁻¹)		
	Leaves	Stems	Roots	Leaves	Stems	Roots
0	0.12b	0.12b	0.12b	21.08b	12.74b	16.66b
300	0.12b	0.12b	0.11b	25.09a	14.04a	18.05a
600	0.14a	0.15a	0.13a	22.17b	11.41c	15.25c
Significance ² of:						
Zn addition level	***	***	***	***	***	***
Inoculation	***	***	***	***	***	***
EDTA application rate	***	***	***	ns	ns	**
Zn × inoculation	ns	ns	ns	*	ns	*
Zn × EDTA	ns	***	***	***	**	*
Inoculation × EDTA	ns	ns	ns	ns	*	ns

¹ Means of 18 replicates of all EDTA application and inoculation treatments under the same Zn addition level.

² By analysis of variance; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Soil extractable Zn and P concentrations after harvest

After harvest, concentrations of extractable soil Zn tended to be higher with increasing Zn addition level and EDTA application rate (Figure 6A). Soil Zn concentration in mycorrhizal treatments was generally lower than corresponding uninoculated treatments, but significant differences were only occasionally found (Figure 6A).

Concentrations of residual soil available P were greatly affected by mycorrhizal treatment, with soil P concentration in all mycorrhizal treatments significantly lower than in corresponding uninoculated controls (Figure 6B). With increasing EDTA application rate, soil P concentrations increased markedly (Figure 6B), and the highest residual P concentrations occurred under the 600 mg kg⁻¹ Zn addition level (data not shown).

Discussion

Although in some cases application of metals may entirely eliminate AM colonization (Gildon and Tinker, 1983), high levels of mycorrhizal colonization have been reported in both heavily contaminated mine spoils and agricultural soils contaminated with metals of different origins (Diaz and Honrubia, 1993; Weissenhorn et al., 1995a,b). It can therefore be concluded that adaptation to metal contamination could occur in these soil microorganisms under field conditions. The results of the present study also indicate some

tolerance to Zn contamination in our fungal strain. Although mycorrhizal colonization was markedly inhibited by Zn addition to the soil, root colonization rates higher than 50% were still observed under Zn contamination. It would be interesting to carry out further investigations on the behaviour of this fungal strain in naturally contaminated soil, together with the responses of fungal isolates from naturally contaminated sites to experimental Zn additions.

In the present study, growth of maize was not inhibited by Zn addition of 300 mg kg⁻¹. In fact, this treatment produced significantly larger plant dry matter compared with pots to which no Zn additions were made. Plant dry weight showed a clear decrease only under the highest Zn addition level of 600 mg kg⁻¹. One explanation is that the soil supply of Zn could not meet the demand for maximum growth when no Zn was added (Marschner, 1993). The Zn supply under the Zn addition level of 300 mg kg⁻¹ may have been excessive for the maize plants but below a level that would have led to zinc phytotoxicity (Hinsely et al. 1977; MacNicol and Beckett, 1985).

In addition to direct toxicity, a possible reason for the general and marked inhibition on plant growth by EDTA may have been an imbalance in mineral nutrition. Enhanced Zn accumulation in roots due to Zn mobilization by EDTA could inhibit root growth under Zn contamination and other metals could also be mobilized by EDTA, as indicated by increased Fe, Mn and Cu concentrations with increasing EDTA application levels (data not shown). Physiological activities of roots, including nutrient and water transport and

metabolism could suffer severe inhibition from metal toxicity. When no Zn was added, EDTA increased Zn uptake by the roots only, with no increase in Zn transport to the shoots. In contrast, when Zn was added, although EDTA application did not increase shoot Zn uptake compared with corresponding treatments with no EDTA application, Zn was partitioned to the leaves, resulting in Zn phytotoxicity, despite the occurrence of only mild symptoms in the present study. However, while foliar Fe concentrations were unaffected, foliar Mn and Cu were increased by EDTA application (data not shown). The resulting imbalances among mineral nutrients may have contributed to the observed decrease in plant growth. It is true that under the Zn addition level of 600 mg kg^{-1} Fe and P concentrations in the stems decreased with increasing EDTA application level, but this effect would have exacerbated any deficiency of Fe and P in plants under high Zn additions.

Our data indicate that EDTA made little contribution to Zn phytoextraction, especially by increasing shoot Zn uptake. Moreover, the negative effects of EDTA on plant growth, in addition to potential environmental risks, such as metal leaching to the ground water (Madrda et al., 2003; Romkens et al., 2002), would need to be considered in practical phytoremediation. Certainly, we cannot make the general conclusion from the present study that metal chelates would not be useful in phytoextraction of soil metals, as some chelating agents could have higher mobilizing capacity for different metals. Other investigations have indicated that EDTA is a powerful mobilizing agent for increasing Pb concentrations in the soil solution and in plant shoots, and Pb uptake by plant shoots showed high correlations with soil solution Pb concentration (Blaylock et al., 1997; Huang et al., 1997). Furthermore, the partitioning pattern of metals in plants has been found to differ among plant species (Guo and Marschner, 1995). It would be more important to choose a suitable plant species with the potential of efficient uptake and translocation of mobilized metals for the purpose of phytoextraction.

Under our experimental conditions, when no Zn was added or under the 300 mg kg^{-1} Zn addition level, mycorrhizal colonization generally showed no marked growth benefit to the host plants, with even the mycorrhizal plants showing lower shoot dry weight than corresponding controls when high doses of EDTA were applied to the soil. The apparent decrease in plant biomass by mycorrhizal colonization may have been due to immobilization of excessive metal in

mycorrhizal structures (mycorrhizal roots contained much higher metal concentrations than uninoculated roots), with resulting high metal concentrations exerting direct phytotoxic effects on the host plant roots. In addition, the soil mixture may have provided adequate available P for plant growth in the absence of mycorrhizal colonization, which may have lowered the mycorrhizal dependency of the maize plants.

However, the protective effects of arbuscular mycorrhiza on plant growth under the Zn addition level of 600 mg kg^{-1} were still notable. Under such severe Zn contamination, the mycorrhizal effect may have resulted from direct binding of metal by fungal mycelium (Joner et al., 2000), resulting in reduced Zn partitioning to the shoots, together with lower shoot (especially leaf) Zn concentrations due to growth dilution effects (as total Zn uptake showed no decrease). The strong metal binding capacity of arbuscular mycorrhiza (which tended to be more pronounced with the increasing Zn addition levels) may have played an important role in alleviation of Zn phytotoxicity. Furthermore, improved P nutrition of the host plants by mycorrhiza may have had some effect under conditions of Zn contamination. Phosphate in the roots may have immobilized metals, while in the shoots increased P concentration may have helped to maintain the balance of mineral nutrients. In contaminated field sites excessive heavy metals are often associated with a shortage of available mineral nutrients, especially P (Shetty et al., 1994a,b). Thus, arbuscular mycorrhiza might improve the survival of plants on contaminated soils through improvement of plant P nutrition.

In conditions where mycorrhizal colonization had no significant effect on plant growth (under the zero-Zn treatment and the Zn addition level of 300 mg kg^{-1}), the direct involvement of AM fungi in plant metal uptake can be evaluated because there is no growth dilution effect on metal concentrations in mycorrhizal plants to mask the direct effects. In the present study, when no Zn was added shoot Zn uptake was increased and root Zn accumulation decreased with mycorrhizal colonization. Zn transport from roots to shoots clearly increased. On the other hand, when Zn was added mycorrhizal colonization resulted in higher Zn uptake by the roots but had no marked effect on shoot Zn uptake. Therefore, enhanced binding of Zn by mycorrhiza may have played an important role in metal tolerance of the host plants under Zn contamination. Mycorrhizal colonization showed different effects on Zn uptake by the host plants under different Zn addition levels. There may have been a

critical plant tissue or soil available Zn concentration below which Zn uptake by plants and partitioning to shoots could be increased by mycorrhizal colonization, and above which the protective effects of the mycorrhiza would appear. Chen et al. (2003) suggested a threshold for Zn of 50 mg kg⁻¹ soil based on their experimental data. Clearly, this value is likely to differ according to soil type, plant species and cultivar, and would need to be determined on a site-specific basis.

Soil extractable P concentrations tended to increase with increasing EDTA application rate. The chelation of soil metals by EDTA may have facilitated the release of P from inorganic compounds. Soil extractable Zn concentration together with Zn accumulation in roots also increased due to EDTA application, but most of the mobilized Zn was not transported to the aerial parts. Furthermore, mycorrhizal colonization decreased available soil Zn concentrations and large amounts of Zn were retained in the mycorrhiza. The data lead to the general conclusion that the combined effects of EDTA and AM colonization did not promote metal removal from the soil. Shoot metal uptake was negligible compared with the application rates of Zn studied in the experiment. However the results provided further evidence for the alleviation of heavy metal phytotoxicity by arbuscular mycorrhiza. More detailed studies are required to elucidate fully the potential role of AM fungi in phytoremediation of metal contaminated soils.

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