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Uptake of zinc, cadmium and phosphorus by arbuscular mycorrhizal maize (*Zea mays* L.) from a low available phosphorus calcareous soil spiked with zinc and cadmium

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Abstract

In a multifactorial pot experiment, maize (*Zea mays* L.) with or without inoculation with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* BEG167 was grown in a sterilized soil spiked with three levels of zinc (0, 300 and 900 mg Zn kg^{-1} soil) and three levels of cadmium (0, 25 and 100 mg Cd kg^{-1} soil). At harvest after 8 weeks of growth, the proportion of root length of inoculated plants colonized decreased with increasing Zn or Cd additon, and was 56% in the absence of both metals and was reduced significantly to 27% in the presence of the higher levels of both metals. Mycorrhizal plants had higher biomass than non-mycorrhizal controls except at the highest soil level of Cd. Cadmium had more pronounced effects on plant biomass than did Zn at the levels studied and the two metals showed a significant interaction. The data suggest that mycorrhizal inoculation increased plant growth with enchancement of P nutrition, perhaps increasing plant tolerance to Zn and Cd by a dilution effect. AM inoculation also led to higher soil solution pH after harvest, possibly reducing the availability of the metals for plant uptake, and lowered the concentrations of soluble Zn and Cd in the soil solution, perhaps by adsorption onto the extrametrical mycelium.

Introduction

Arbuscular mycorrhizal (AM) fungi can form an association with the majority of terrestrial plant species (Smith & Smith 1997) and under certain conditions they can assist host plant uptake of relatively immobile nutrients such as phosphorus (P) (Kothari *et al.* 1991; Li *et al.* 1991a, b, 1997; Jakobsen *et al.* 1992a, b). Bradley *et al.* (1981) showed that ericoid mycorrhizal colonization led to a significant decrease in metal content of the shoots and an increase in the roots of *Calluna vulgaris*, *Vaccinium macrocarpon* and *Rhododendron ponticum* grown in sand amended with Cu or Zn. Cooper and Tinker (1978) demonstrated the accumulation and translocation of Zn by extra-radical hyphae. Turnau *et al.* (1993) showed the accumulation of heavy metals within intracellular hyphae, mainly in phosphate-rich material of vacuoles in mycorrhizal roots of *Pteridium aquilinum* from experimental forest plots treated with high doses of heavy metals. However, since AM fungi cannot be cultivated without a host plant, it is difficult to investigate the role of these

fungi in plant metal using axenic media and the potential mechanisms of interaction between mycorrhizal host plants and heavy metals are not clear (Leyval *et al*. 1997).

The effects of AM fungi on plant uptake of metals are varied. For example, some studies suggest that high concentrations of heavy metals in soil may significantly decrease root colonization by AM fungi (Gildon & Tinker 1983) or inhibit spore germination (Koomen *et al*. 1990; Weissenhorn *et al*. 1993). In some extreme conditions, AM fungal inoculation can be entirely inhibited due to heavy metal toxicity (El-Kherbawy *et al*. 1989; Weissenhorn *et al*. 1994). However, other studies have reported high levels of mycorrhizal colonization in agricultural soils contaminated with metals of different origins (Weissenhorn *et al*. 1995), and with the exception of seasonal variation, there was no significant difference in mycorrhizal root colonization among three populations of *Agrostis capillaris* growing on a sandy soil polluted by a smelter and on limestone-derived clay with or without metals of natural origin (Ietswaart *et al*. 1992). Furthermore, metal-tolerant *Oxalis acetosella* colonizing acid forest soils treated with Cd-, Zn- and Pb-containing industrial dusts showed even higher mycorrhizal colonization than on untreated soils (Turnau *et al*. 1996).

Cd-tolerant AM fungal isolates have been extracted from contaminated soils (Gildon & Tinker 1981, 1983; Weissenhorn *et al*. 1993, 1994). A strain isolated from Zn-polluted sewage sludge was found to have double-tolerance for Cd and Zn (Weissenhorn *et al*. 1994). Using ^{109}Cd as a tracer, Joner and Leyval (1997) showed that extraradical hyphae might transport Cd from soil to roots. However, because of limitations in techniques, it is difficult to elucidate the effects of AM fungi on host plant uptake of metals in heavy metal-polluted soils (Leyval *et al*. 1997). Some studies have indicated higher uptake of Zn and Cd by mycorrhizal plants growing in soil with high metal concentrations (Killham & Firestone 1983; Weissenhorn & Leyval, 1995), while many others have found depletion of Zn and Cd concentrations in plants, especially shoots, due to mycorrhizal colonization (Heggo *et al*. 1990; Li & Christie 2000; Liu *et al*. 2000; Zhu *et al*. 2001; Jamal *et al*. 2002; Bi *et al*. 2003; Chen *et al*. 2003).

Metal polluted soils seldom have elevated concentrations of one trace metal. They are usually contaminated by mixtures of metals and the metals may interact in the soil–plant system. It has been reported that mycorrhizal colonization reduced the shoot concentrations of Cd and Zn in field-grown maize and lettuce when the soil had high available concentrations of both metals (Schüepp *et al*. 1987). In the present study, we investigated the influence of AM colonization on maize growth and Zn and Cd uptake in soil artificially spiked with Zn and Cd. The aim was to evaluate the feasibility of using AM fungi for bioremediation or phytostabilization of contaminated soil.

Materials and methods

Experimental materials

Glomus mosseae BEG167 was propagated in pot culture on maize plants grown in a sandy soil for 10 weeks. Inoculum from the pot culture comprised a mixture of spores, mycelium, sandy soil and maize root fragments and contained approximately 1000 spores per 100 g.

Maize (*Zea mays* L. cv. ND108) seeds were surface sterilized with 10% v/v hydrogen peroxide for 10 min, washed with sterile water and germinated in the dark on moist filter paper at room temperature. Seedlings were selected for uniformity of size prior to planting.

The soil used was a typical sandy soil collected from Changping suburb in Beijing and had the following properties (DM basis): pH (water:soil ratio 2.5:1) 8.0, organic matter 0.62%, $NaHCO_3$-extractable P 13.2 mg kg^{-1} and DTPA extractable Zn 1.77 and Cd 0.13 mg kg^{-1}. The soil was sieved (1 mm), steam-sterilized (121°C for 30 min) and air-dried. Basal nutrients were added in solution to the dry soil at a rate of 300 mg N, 150 mg P , and 20 mg K kg^{-1} of soil.

Experimental design

The experiment was a 2×3×3 factorial design with mycorrhizal colonization (inoculated or uninoculated) combined with three levels of Zn (0, 300 and 900 mg Zn kg^{-1} soil as $ZnSO_4 \cdot 7H_20$ in aqueous solution) and three levels of Cd (0, 25 and 100 mg Cd kg^{-1} soil, as $CdSO_4 \cdot 8H_20$ in aqueous solution) with 4 replicates, giving 72 pots in total. The soils

were equilibrated for 2 weeks after the metal solutions were thoroughly mixed in. The pots were arranged in a fully randomized design.

Plants were grown in plastic pots (2.5-L) containing 300 g steam-sterilized coarse sand covered with 1.5 kg soil, 200 g mycorrhizal inoculum (steam-sterilized inoculum in the case of the non-mycorrhizal treatments) and a final surface layer of 0.5 kg soil. Three seedlings were grown in each pot. Soil moisture content was adjusted regularly to ~70% water holding capacity (WHC) with deionized water during the course of the experiment.

Harvest and chemical analysis

Shoots and roots were harvested separately. Samples were carefully washed with tap water and then deionised water. Sub-samples of fresh roots were collected for determination of root colonization rate by the AM fungus. The dry weights of shoots 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; Perkin Elmer Optima 3300DV).

Soil pH was measured with a pH meter using a soil: water ratio of 1: 2.5. Root samples for assessing root colonization were prepared using the acid fuchsin staining-grid intersect method (Kormanik & McGraw 1982).

Data analysis

Data were subjected to three-way analysis of variance with replication including linear and quadratic contrasts using Genstat Release 6.1 for Windows 2000 (Lawes Agricultural Trust, 2002).

Results

Mycorrhizal colonization

Negligible colonization of the roots uninoculated plants was found (Table 1). The mean proportion of root length of inoculated plants colonized ranged from 27% to 56%. Mycorrhizal colonization declined with increasing Zn addition and with increasing Cd addition to the soil.

Plant biomass

Mycorrhizal plants had higher shoot yields than non-mycorrhizal controls except when Cd was added to the soil at 100 mg kg^{-1} (Table 2). Zinc addition had a small effect on shoot biomass but this was not consistent across all treatments. In contrast, Cd addition led to a pronounced decrease in shoot biomass with increasing Cd application rate.

Mycorrhizal plants also had higher root biomass (again with the exception of treatments receiving 100 mg Cd kg^{-1}) and Zn addition had no effect on root biomass. There was a pronounced

Table 1. Mean percentage of inoculated and uninoculated maize root length colonized by *Glomus mosseae* in a calcareous soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Cd addition rate (mg kg^{-1})		
		0	25	100
Mycorrhizal	0	56.3	51.0	41.1
	300	54.6	46.6	36.4
	900	46.2	35.4	27.3
Non-mycorrhizal	0	0.5	1.5	0.9
	300	0.7	1.6	0.8
	900	1.4	1.7	0.4
Significance[a] due to:				
Mycorrhiza			***	
Zn level			***	
Cd level			***	
Mycorrhiza×Zn level			***	
Mycorrhiza×Cd level			***	
Zn level×Cd level			ns	
Mycorrhiza×Zn level×Cd level			ns	

[a] By analysis of variance; ***, $p < 0.001$; ns, not significant.

Table 2. Mean dry matter yield (g pot^{-1}) of mycorrhizal and non-mycorrhizal maize in a calcareous soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Shoots			Roots			Total		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100
Mycorrhizal	0	12.0	7.2	1.5	8.3	5.5	1.4	20.2	12.7	2.9
	300	11.4	9.3	2.8	7.4	5.9	2.0	18.8	15.2	4.8
	900	13.7	7.1	3.5	8.2	5.3	1.8	22.0	12.3	5.3
Non-mycorrhizal	0	8.7	1.6	1.7	6.4	1.6	1.7	15.1	3.3	3.5
	300	7.0	4.2	2.9	4.1	2.6	1.7	11.1	6.8	4.6
	900	7.8	3.7	3.0	4.2	2.2	2.2	12.0	5.9	5.1
Significance[a] due to:										
Mycorrhiza			***			***			***	
Zn level			**			ns			ns	
Cd level			***			***			***	
Mycorrhiza × Zn level			ns			ns			ns	
Mycorrhiza × Cd level			***			***			***	
Zn level × Cd level			***			**			***	
Mycorrhiza × Zn level × Cd level			*			ns			ns	

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

decrease in root biomass with increasing addition of Cd to the soil.

Plant Zn concentrations and uptake

Shoot Zn concentrations were unaffected by mycorrhizal colonization and root Zn concentrations were higher in non-mycorrhizal controls than in inoculated plants (Table 3). Shoot Zn concentration decreased with increasing Cd addition except at the higher Zn addition rate. Root Zn showed similar but more complicated trends.

Shoot Zn uptake was lower in non-mycorrhizal controls than in inoculated plants and roots showed a similar trend (Table 4). Shoot and root Zn uptake decreased with increasing Cd addition rate except for shoot uptake at the higher Zn addition rate of 900 mg kg^{-1} in which high Zn uptake occurred when Cd was added to the soil.

Plant Cd concentrations and uptake

Shoot Cd concentrations were higher in mycorrhizal plants than in controls but root Cd concentrations were unaffected by mycorrhizal colonization (Table 3). Shoot Cd concentrations were unaffected by Zn when no Cd was added to the soil but increased with increasing Zn rate when Cd was added to the soil and root Cd concentrations showed similar trends.

There was little effect of AM colonization on shoot Cd uptake, but root uptake was higher in mycorrhizal plants (Table 5). Shoot and root Cd uptake increased with Zn addition except when no Cd was added to the soil.

Plant P concentrations and uptake

Mycorrhizal colonization was associated with higher shoot and root P concentrations (Table 6). Zinc addition to the soil had no effect on plant P concentrations and Cd addition produced low shoot P concentrations at the higher Cd addition level.

Shoot and root P uptake were higher in mycorrhizal plants than in uninoculated controls (Table 7) and were unaffected by addition of Zn to the soil. Shoot and root P uptake declined with increasing Cd addition rate and the decline was very pronounced at a Cd addition rate of 100 mg kg^{-1}.

Soil pH and soluble Zn and Cd

Soil solution pH was higher in mycorrhizal treatments than in non-mycorrhizal controls (Table 8). Both metals had an acidifying effect on the soil but mean soil pH did not fall below 7.2.

There were higher concentrations of soluble Zn and soluble Cd in the soil of the non-mycorrhizal control pots than in the inoculated pots (Table 8).

Table 3. Mean Zn and Cd concentrations (mg kg^{-1}) in mycorrhizal and non-mycorrhizal maize in a calcareous soil contaminated with Zn and Cd.

Mycorrhizal Status	Zn addition rate (mg kg^{-1})	Shoot Zn concentration			Root Zn concentration			Shoot Cd concentration			Root Cd concentration		
		Cd rate (mg kg^{-1})			Cd rate (mg kg^{-1})			Cd rate (mg kg^{-1})			Cd rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100	0	25	100
Mycorrhizal	0	46	10	8	44	46	65	0.2	23.4	101.9	3	189	736
	300	369	157	150	709	441	515	0.2	38.5	167.8	3	414	1272
	900	804	15731	15436	1501	994	1366	0.2	49.8	268.6	3	592	2364
Non-mycorrhizal	0	39	8	8	42	47	68	0.2	38.7	98.5	3	135	584
	300	499	173	150	832	710	894	0.2	91.6	224.1	3	404	1189
	900	1788	16063	14838	2117	1460	1320	0.1	112.0	282.2	3	676	2350
Significance[a] due to:													
Mycorrhiza			ns			***			***			ns	
Zn level			***			***			***			***	
Cd level			***			***			***			***	
Mycorrhiza×Zn level			ns			***			***			ns	
Mycorrhiza×Cd level			ns			ns			***			ns	
Zn level×Cd level			***			***			***			***	
Mycorrhiza×Zn×Cd			ns			***			***			ns	

[a] By analysis of variance; ***, $p < 0.001$; ns, not significant.

Table 4. Mean shoot, root and total Zn uptake (mg pot^{-1}) by mycorrhizal and non-mycorrhizal maize in a soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Shoots			Roots			Total		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100
Mycorrhizal	0	0.55	0.08	0.01	0.36	0.25	0.09	0.9	0.3	0.1
	300	4.19	1.46	0.41	5.27	2.60	1.03	9.5	4.1	1.4
	900	11.07	111.32	51.61	12.39	5.17	2.37	23.5	117.0	54.0
Non-mycorrhizal	0	0.34	0.01	0.01	0.26	0.08	0.12	0.6	0.1	0.1
	300	3.51	0.72	0.43	3.40	1.86	1.54	6.9	2.6	2.0
	900	13.85	60.03	44.36	8.87	3.23	2.86	22.7	63.3	47.2
Significance[a] due to:										
Mycorrhiza			**			***			***	
Zn level			***			***			***	
Cd level			***			***			***	
Mycorrhiza×Zn level			***			*			***	
Mycorrhiza×Cd level			***			***			***	
Zn level×Cd level			***			***			***	
Mycorrhiza×Zn level×Cd level			***			ns			***	

Discussion

Complete inhibition of AM colonization has been reported following the application of heavy metals (Gildon & Tinker 1983), but numerous studies have shown the occurrence of high levels of mycorrhizal colonization in heavily contaminated mine spoils or agricultural soils contaminated with metals from different origins (Weissenhorn *et al*. 1995). In our experiment, the proportion of root length colonized

Table 5. Mean shoot, root and total Cd uptake (mg pot^{-1}) by mycorrhizal and non-mycorrhizal maize in a soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Shoots			Roots			Total		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100
Mycorrhizal	0	0.047	2.844	2.601	24	1037	1012	24	1040	1015
	300	0.040	6.037	0.777	23	2447	2549	23	2453	2550
	900	0.045	5.881	1.584	23	3117	4108	23	3123	4109
Non-mycorrhizal	0	0.037	0.999	2.904	17	220	951	17	221	953
	300	0.019	6.314	1.089	12	1050	2033	12	1057	2034
	900	0.021	7.140	1.428	11	1459	5031	11	1466	5033
Significance[a] due to:										
Mycorrhiza			ns			***			***	
Zn level			***			***			***	
Cd level			***			***			***	
Mycorrhiza×Zn level			ns			ns			ns	
Mycorrhiza×Cd level			ns			***			***	
Zn level×Cd level			***			***			***	
Mycorrhiza×Zn level×Cd level			*			ns			ns	

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

Table 6. Mean shoot and root P concentrations (%) in mycorrhizal and non-mycorrhizal maize in a calcareous soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Shoots			Roots		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100
Mycorrhizal	0	0.077	0.029	0.037	0.071	0.124	0.049
	300	0.069	0.054	0.046	0.056	0.114	0.082
	900	0.068	0.056	0.042	0.068	0.109	0.052
Non-mycorrhizal	0	0.051	0.061	0.035	0.044	0.050	0.045
	300	0.041	0.042	0.029	0.038	0.071	0.044
	900	0.038	0.035	0.032	0.047	0.053	0.049
Significance[a] due to:							
Mycorrhiza			***			***	
Zn level			ns			ns	
Cd level			***			***	
Mycorrhiza×Zn level			***			ns	
Mycorrhiza×Cd level			***			***	
Zn level×Cd level			ns			***	
Mycorrhiza×Zn level×Cd level			***			***	

[a] By analysis of variance; ***, $p < 0.001$; ns, not significant.

decreased significantly at the higher addition rates of Zn or Cd to the soil. However, even when Zn was applied at 900 mg kg^{-1} and Cd at 100 mg kg^{-1}, the mean colonization was still 27%. The interaction between the two metals was significant and this illustrates the value of studying mixtures of metals in toxicity studies.

Cadmium had more pronounced effects on plant biomass than did Zn at the application rates studied. Mycorrhizal plants had higher shoot yields than non-mycorrhizal controls except at the highest Cd addition rate (100 mg kg^{-1}). Plant yield responses to mycorrhizal colonization are difficult to interpret because, they are influenced by P

Table 7. Mean shoot, root and total P uptake (mg pot^{-1}) by mycorrhizal and non-mycorrhizal maize in a soil contaminated with Zn and Cd.

Mycorrhizal Status	Zn addition rate (mg kg^{-1})	Shoots			Roots			Total		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100
Mycorrhizal	0	9.20	2.10	0.56	5.94	6.79	0.67	15.14	8.89	1.23
	300	7.90	5.04	1.28	4.87	6.74	1.66	12.77	11.78	2.94
	900	9.34	3.97	1.50	5.64	5.76	0.91	14.99	9.72	2.41
Non-mycorrhizal	0	4.40	1.01	0.60	2.78	0.82	0.80	7.18	1.83	1.40
	300	3.02	1.77	0.84	1.70	1.93	0.74	4.72	3.70	1.58
	900	3.01	1.30	0.97	1.99	1.15	1.05	5.00	2.45	2.02
Significance[a] due to:										
Mycorrhiza			***			***			***	
Zn level			ns			ns			ns	
Cd level			***			***			***	
Mycorrhiza × Zn level			*			ns			ns	
Mycorrhiza × Cd level			***			***			***	
Zn level × Cd level			***			**			***	
Mycorrhiza × Zn level × Cd level			ns			ns			ns	

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

Table 8. Mean soil solution pH and Zn and Cd (mg kg^{-1}) of mycorrhizal and non-mycorrhizal maize in a soil contaminated with Zn and Cd.

Mycorrhizal status	Zn addition rate (mg kg^{-1})	Soil solution pH			Soil soluble Zn			Soil soluble Cd		
		Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})			Cd addition rate (mg kg^{-1})		
		0	25	100	0	25	100	0	25	100
Mycorrhizal	0	8.34	8.11	7.70	1	12	14	≤ 0.1	35	129
	300	8.01	7.92	7.49	81	81	79	≤ 0.1	34	132
	900	7.69	7.59	7.32	206	221	232	≤ 0.1	36	143
Non-mycorrhizal	0	8.07	7.80	7.70	1	13	14	≤ 0.1	37	135
	300	7.85	7.64	7.48	102	82	107	≤ 0.1	32	156
	900	7.46	7.44	7.23	248	256	251	≤ 0.1	36	154
Significance[a] due to:										
Mycorrhiza			***			***			***	
Zn level			***			***			**	
Cd level			***			**			***	
Mycorrhiza×Zn level			ns			***			ns	
Mycorrhiza×Cd level			***			ns			***	
Zn level×Cd level			***			*			***	
Mycorrhiza×Zn level×Cd level			***			*			*	

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

nutrition in addition to added Zn and Cd. In contaminated field sites, excessive heavy metals are always associated with a shortage of available mineral nutrients, especially P (Shetty *et al*. 1994a, b). Under conditions of metal contamination P deficiency may increase the severity of metal phytotoxicity. Mycorrhizas may alleviate P deficiency, thus avoiding the need to apply P fertilizers.

More acid conditions in the soil may also increase the severity of Zn or Cd toxicity. In our experiment the lower soil pH in the metal amended soil was likely due to the use of the sulphates to

spike the soil. Nevertheless, the lowest mean soil pH (in the treatment combining the higher addition rates of both metals) was 7.2, thus the confounding factor of pH may not have been significant. Clearly, the effects of the artificial application of the metal salts would have been a more serious problem using an acid soil.

It is interesting that higher soil solution pH values were found in the mycorrhizal treatments after harvest. In addition, there was less residual soil solution Zn or Cd in the mycorrhizal treatments. Thus, the AM fungus may have facilitated plant metal tolerance in a number of ways. The mycorrhiza increased plant biomass, diluting the metals in the plant tissues, increased soil pH in the rhizosphere and making the metals less available for plant uptake, reduced the concentration of soluble Zn and Cd in the soil solution possibly by adsorption of metals on the extraradical hyphae, and indirectly by enhancing P nutrition with a possible further contribution to enhanced biomass. Cadmium uptake was also increased in the roots, but not the shoots, and this may have protected the shoots to some extent from excessive Cd.

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