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Potential contribution of arbuscular mycorrhiza to cadmium immobilisation in soil

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Abstract

The contribution of arbuscular mycorrhiza (AM) to immobilisation of Cd in substrate was studied in two experiments. In the first experiment, substrates prepared by cultivating tobacco, either non-mycorrhizal or inoculated with the AM fungus *Glomus intraradices* were enriched with a range of Cd concentrations, and Cd toxicity in the substrates was assessed using root growth tests with lettuce as a test plant. The tests revealed lower Cd toxicity in the mycorrhizal than in the non-mycorrhizal substrate, and the difference increased with increasing total Cd concentration in the substrates. In the second experiment, extraradical mycelium (ERM) of *G. intraradices* exposed in vivo to Cd was collected and analysed on Cd concentration. The ERM accumulated 10–20 times more Cd per unit of biomass than tobacco roots. While Cd concentrations were lower in the biomass of mycorrhizal plants than of non-mycorrhizal plants, Cd immobilisation by ERM did not affect the total Cd content in mycorrhizal tobacco.

It is concluded that mycorrhiza may decrease Cd toxicity to plants by enhancing Cd immobilisation in soil. The results therefore suggest a potential role of AM symbiosis in the phytostabilisation of contaminated soils, where high Cd availability inhibits plant growth.

Keywords: Extraradical mycelium; *Glomus*; Heavy metals; Rhizosphere; Tobacco

1. Introduction

Anthropogenic soil contamination with Cd represents an important environmental problem in view of the relatively high solubility of Cd in soils and Cd toxicity to plants and animals (Schachtschabel et al., 1992). Plants growing on Cd-contaminated soils are an important factor influencing the fate of soil Cd. Root uptake and translocation to the aerial parts of plants increase the risk of heavy metals (HMs) entering the food chain. Vegetation cover, however, also decreases the danger of HM dispersal by water and wind erosion and is therefore desirable on contaminated soils. Plant uptake and the toxic effects of soil Cd depend on its bioavailability, which is determined by many soil characteristics such as pH, concentration of carbonates, clay and organic matter content (Adriano, 2001). Additionally, the bioavailability of Cd and other HMs is modified by root exudates and associated microorganisms in the plant rhizosphere (Wenzel et al., 1999). Cd is effectively immobilised by soil organic matter and microorganisms (Kurek et al., 1982; Prokop et al., 2003), but organic acids released by roots can also mobilise Cd by the formation of soluble complexes (Nigam et al., 2001).

The growth and physiology of most herbs in natural habitats and field-grown crops is influenced by arbuscular mycorrhiza (AM), a symbiosis formed with fungi of the order Glomeromycota. AM symbiosis may improve plant growth and affect HM uptake of plants growing on soils with high HM concentrations (as reviewed by Leyval et al., 1997). It is, however, unclear whether these effects are mediated by the same benefits of mycorrhiza to their host plants as in uncontaminated environments, mainly by improved nutrient acquisition, or whether mycorrhiza

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confers some additional resistance by specific mechanisms (Meharg and Cairney, 2000). Nevertheless, utilization of AM symbiosis in the revegetation or phytoremediation of HM-contaminated soils is being discussed (Khan et al., 2000; Gaur and Adholeya, 2004) and there is a need to determine the mechanisms of mycorrhizal effects on plant growth, HM tolerance and uptake.

In the ectomycorrhizal association, immobilisation of HMs by mycelium has been proposed to protect plants against HM exposure (Denny and Wilkins, 1987; Frey et al., 2000) thus reducing the contact of sensitive plant structures with active Cd forms. AM fungi produce abundant ERM, which reaches densities of several meters per gram of soil and spreads to the distance of about 10 cm from roots (Jakobsen et al., 1992). Joner et al. (2000) suggested Cd immobilisation in soil by ERM based on the finding that excised ERM accumulated up to 0.5 mg Cd mg^{-1} of dry biomass. However, Cd immobilisation by ERM has not yet been studied in vivo and related to the Cd uptake by the host plant. In other studies, enhanced Cd immobilisation in the rhizosphere of AM plants has been concluded based on indirect indications only such as lower Cd concentrations in the biomass of mycorrhizal plants (e.g. Vivas et al., 2003), which is not an appropriate indicator of HM bioavailability in soil (Jentschke and Godbold, 2000).

The study described in this paper focused on the contribution of AM symbiosis to Cd immobilisation in substrate. In the first experiment, Cd toxicity was tested in mycorrhizal and non-mycorrhizal substrate using root growth tests, which represent a simple and sensitive method (An, 2004). In the second experiment, Cd concentration was determined in intact ERM and related to Cd concentrations and uptake by the host plant.

2. Materials and methods

2.1. Experiment 1: Cd toxicity in mycorrhizal and non-mycorrhizal substrates

2.1.1. Preparation of substrates

Pots containing 3 kg of dry river sand, washed with deionised water and sterilised by heating at 120 °C for 8 h on 2 consecutive days, were planted with tobacco, *Nicotiana tabacum* L., variety Wisconsin 38. The tobacco plants were either left non-inoculated (NM) or inoculated with the AM fungus *Glomus intraradices* Schenck and Smith, isolate PH5 (M), originating from a Pb-contaminated waste disposal site of a Pb smelter near Příbram, Czech Republic. Each treatment was established in 10 replicates. The selection of the plant and AM fungal isolate was based on previous results that inoculation with *G. intraradices* PH5 alleviates Cd-induced growth inhibition and decreases Cd concentrations in the biomass of this tobacco variety (Janoušková et al., 2005a,b).

Each M plant was inoculated with 10 ml of suspension containing colonised root segments, ERM and spores derived from a 4-months-old sand-zeolite culture of the PH5 isolate on maize. Each NM plant received the same amount of autoclaved inoculum suspension. In order to equalise the microbial community in all inoculation treatments, 5 ml of filtrate from the non-sterile inoculum was added to each pot. The filtrate was obtained by passing soil suspension from the culture of origin through a filter paper. The plants were supplied three times a week with the modified White's nutrient solution P2N3 (Gryndler et al., 1992): 60 ml in the 1st to 3rd weeks of the cultivation, 90 ml in the 4th to 6th weeks, 240 ml from the 7th week until harvest. From the 7th week, the nutrient solution was enriched with P as KH_2PO_4 to total 5 mg l^{-1}.

After 12 weeks of cultivation in a greenhouse with light supplement (12 h, metalhalide-lamps, 400 W), each plant was carefully lifted from the pot, loosely adhering sand was removed from roots, and the substrate was air-dried and homogenised. The substrates contained fragments of fine tobacco roots, but no sieving was performed to remove the roots, because this would have also removed aggregates in the M substrate, formed probably by sand adhering to ERM. Equal amounts of sand from all 10 pots per treatment were mixed and this mixture served as the substrate for the subsequent root growth tests. In addition to the pre-cultivated M and NM substrates, clean sand (S), washed and sterilised as for the tobacco cultivation, was included in the tests as a control.

2.1.2. Root growth tests

Cd was added to the tested substrates in defined concentrations and its toxicity was estimated by measuring the root growth of lettuce seedlings (*Lactuca sativa* cv. Safír, Veleliby). The method was modified from ISO 11269 (1993), but only one test plant species that is known to sensitively react to HMs in substrate was used.

The tested substrate (250 g) was mixed with 40 ml of distilled water or Cd solution (as $Cd(NO_3)_2$) in deionised water and filled into a plastic vessel (10 × 7 × 10 cm) with a lid. Ten lettuce seeds were placed on the substrate surface at regular distances and the vessels were sealed with plastic tape. Germination potential and root length of the lettuce seedlings were recorded after incubation at 22 °C in darkness for 5 days. Average root length per vessel was calculated excluding non-germinated seeds and regarded as one replicate. Relative root length (RL%), i.e., the percentage of the average root length in the corresponding substrate without Cd addition, was used to compare Cd-induced inhibition of root growth in the different substrates.

Three consecutive tests were performed, each with the M, NM and S substrates. The following Cd concentrations were tested (mg kg^{-1} substrate): test 1 – 0, 1.2, 2.4 and 4.8 (4 replicates per treatment); test 2 – 0, 3.2 and 6.4 (5 replicates); test 3 – 2.4, 4.8 and 7.2 (5 replicates).

2.2. Experiment 2: Cd concentrations in tobacco and ERM

Tobacco (var. Wisconsin 38) was cultivated in pots containing 7.2 kg of river sand, washed and sterilised as

described above, either left non-inoculated or inoculated with the AM fungus *Glomus intraradices* PH5 (7 replicates per treatment). To isolate clean ERM, a nylon mesh (pore size 42 μm), permeable for ERM but not for roots, separated the pots into two equally sized compartments (3.6 kg of sand): a central compartment with the tobacco plant (root compartment) and a peripheral compartment containing only ERM radiating from the plant if inoculated (hyphal compartment). The establishment of the experiment and fertilisation were carried out as described above. After 12 weeks of growth, the plants received 500 ml of Cd solution weekly in a concentration of 5 mg l^{-1} for an additional 3 weeks, i.e. the total Cd added to the substrate corresponded to a concentration of 1 mg kg^{-1} sand. This mode of Cd application was selected to avoid contact between Cd and the plant before the plant is embedded into an ERM network. A potential effect of AM symbiosis should have been therefore more pronounced under these conditions of Cd application. ERM was extracted from the hyphal compartment by wet-sieving and decanting the whole volume of sand with subsequent vacuum filtration of the mycelial suspension through a membrane filter (24 mm diameter, 0.4 μm pore size). The mycelium formed a pellet on the filter, which was dried at 80 °C, weighed and transferred to Eppendorf tubes.

2.3. Experiments 1 and 2: Determination of parameters at harvest

The dry weights of shoots and roots were recorded after drying at 80 °C. Root length was estimated by counting intersections on a grid (Newman, 1966) of one root subsample of defined dry weight (30–50 mg) per pot. Percentage of root colonisation by *G. intraradices* was determined after staining with 0.05% trypan blue in lactoglycerol (Koske and Gemma, 1989) using the grid-line intersect method (Giovannetti and Mosse, 1980). The ERM length in the inoculated treatments was estimated on a homogenised subsample from each pot using the modified membrane filtration technique (Jakobsen et al., 1992). The values were calculated to meters of ERM g^{-1} of air-dried substrate. The average background length of mycelium in the corresponding non-mycorrhizal pots was subtracted from all values obtained in the inoculated treatments. In the compartmented pots (Experiment 2), ERM length was estimated separately for each compartment.

In Experiment 2, roots and shoots were grinded and the plant material and the mycelial pellets were decomposed in a dry ashing procedure performed in a mixture of oxidising gases (O_2+O_3+NO_x) using Apion Dry Mode Mineralizer (Tessek, CZ). The ash was dissolved in 1.5% HNO_3 (Miholová et al., 1993). Varian SpectrAA-400 (Australia) atomic absorption spectrometer with a GTA-96 graphite tube atomizer was applied for the cadmium determinations. A pyrolytically coated tube with a L'vov platform was used for all the measurements.

The pH of the substrates was measured in supernatant after shaking a sample of homogenised substrate with deionised water (1:2.5 w:v) for 2 h.

2.4. Experiments 1 and 2: Statistical treatment

Differences between the M and NM treatments were evaluated by *t*-test in both experiments. In the root growth tests, data for RL% were arcsine-transformed, and the effects of the factors Cd concentration (continuous variable) and mycorrhiza (fixed factor) were evaluated by ANOVA for each test separately.

3. Results

Inoculation of tobacco plants in Experiment 1 resulted in high levels of root colonisation and the establishment of a dense ERM network in the substrate (Table 1). NM plants produced higher shoot biomass and higher root density than M plants. The M and NM substrates differed in pH: while cultivation of M tobacco did not change the original pH of the river sand (6.7), cultivation of NM tobacco resulted in its significant decrease to pH 6.2 (Table 1).

In the root growth tests, the germination potential of lettuce seeds was 79% (s.d. = 15) and was not affected by Cd or mycorrhiza within each test at $P = 0.05$ (data not shown). Root growth was generally better in the S substrate than in the NM and M substrates when no Cd was added. However, it was better in both NM and M substrate in all treatments with Cd addition (Fig. 1a). Growth inhibition by Cd, expressed as RL%, was thus most pronounced in the S substrate, following a non-linear relationship (Fig. 1b), while the relationship between Cd concentration and RL% was linear for the M and NM substrates. The lower negative slope of the linear model for the M substrate indicated that Cd toxicity was lower in the M than in the NM substrate, especially at higher Cd concentrations. This was confirmed by the effects of the Cd x mycorrhiza interaction on root growth as determined by ANOVA (Table 2). The interaction

Table 1
Characteristics of mycorrhizal (M) and non-mycorrhizal (NM) substrate prepared in Experiment 1 to be compared in root growth tests: development of mycorrhiza, tobacco growth and substrate pH

Parameter	M	NM	Significance (*t*-value)
Root colonisation (%)	95 (4)	0	n.d.
ERM length (cm g^{-1})	114 (23)	0	n.d.
Dry weight shoots (g)	7.2 (1.3)	9.0 (1.2)	**(3.1)
Dry weight roots (g)	2.9 (0.8)	3.3 (1.1)	n.s. (0.9)
Specific root length (cm g^{-1})	283 (59)	479 (84)	*(2.7)
Substrate pH	6.7 (0.02)	6.2 (0.03)	***(3.6)

The values are means (s.d.) of 10 replicates. Significant differences between M and NM according to *t*-test: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. non-significant effect at $P = 0.05$, n.d. not determined. ERM = extraradical mycelium.

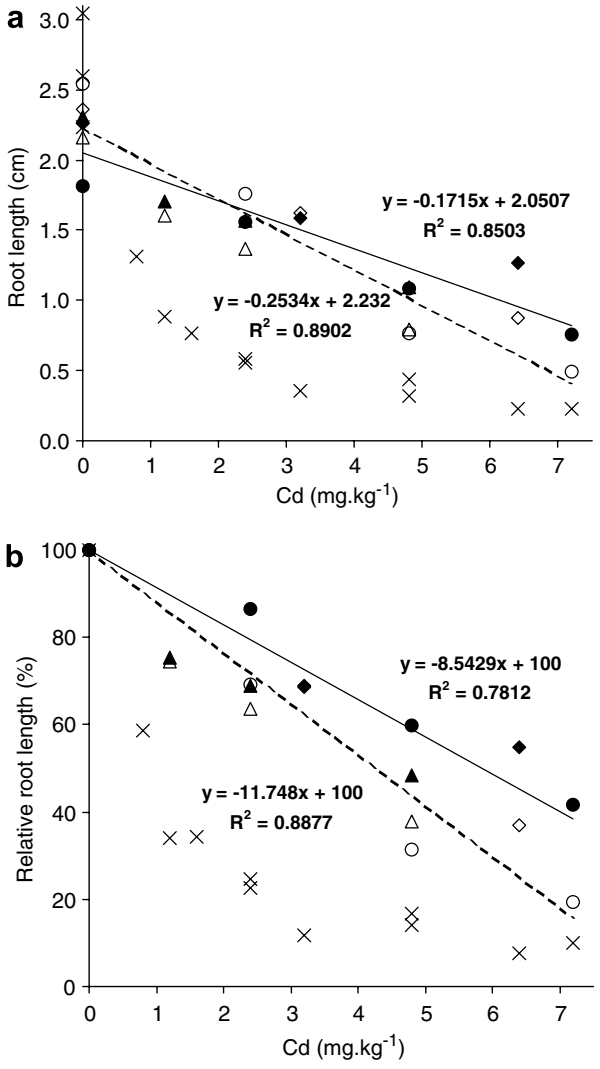

Fig. 1. Root length (a) and relative root length (b) of lettuce seedlings as affected by Cd in mycorrhizal substrate, non-mycorrhizal substrate and clean sand in 3 root growth tests of Experiment 1. Marks represent mean values per treatment (substrate × Cd concentration × test). Full symbols (full line) = mycorrhizal substrate, empty symbols (dashed line) = non-mycorrhizal substrate; triangle = Test 1, rhombus = Test 2, circle = Test 3; crosses = clean sand (values for Test 1, 2 and 3 are not differentiated by distinct symbols within the clean sand treatment).

was significant in tests 2 and test 3, where Cd was applied in higher concentrations, but not in test 1, where the tested Cd concentrations were lower.

Table 2
Significant effects (*F*-value) of the factors Cd concentration and mycorrhiza on the relative root length of lettuce seedlings in 3 root growth tests according to ANOVA

	Test 1	Test 2	Test 3
Cd (A)	***(221.5)	***(189.5)	***(553.7)
Mycorrhiza (B)	n.s. (0.0)	n.s. (0.3)	n.s. (2.0)
A×B	n.s. (2.3)	*(5.2)	***(14.5)

Significant effects: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. non-significant effect at $P = 0.05$.

Table 3
Comparison of the mycorrhizal (M) and non-mycorrhizal (NM) treatment in Experiment 2: mycorrhizal parameters; biomass, Cd concentrations and Cd contents of tobacco and extraradical mycelium (ERM); substrate pH

Parameter	M	NM	Significance (*t*-value)
Root colonisation (%)	96 (3)	0	n.d.
ERM length root comp. (cm g^{-1})	130 (47)	0	n.d.
ERM length hyphal comp. (cm g^{-1})	124 (30)	0	n.d.
Dry weight (g)			
Shoots	4.8 (0.6)	3.3 (1.1)	**(3.17)
Roots	1.4 (0.2)	0.8 (0.5)	**(3.25)
ERM hyphal comp.	0.024 (0.008)	0	n.d.
Cd concentration (μg g^{-1})			
Shoots	65 (8)	104 (33)	*(3.07)
Roots	120 (22)	247 (83)	*(2.86)
ERM hyphal comp.	2592 (592)	n.d.	n.d.
Cd content (μg pot^{-1})			
Shoots	315 (59)	328 (84)	n.s.(0.33)
Roots	166 (21)	180 (96)	n.s.(0.37)
ERM	134 (64)	n.d.	n.d.
Substrate pH			
Root comp.	7.2 (0.04)	7.0 (0.02)	***(9.99)
Hyphal comp.	7.2 (0.02)	7.1 (0.02)	**(4.61)

The values are means (s.d.) of 7 replicates. Significant differences between M and NM according to *t*-test: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, n.s. non-significant effect at $P = 0.05$, n.d. not determined.

In Experiment 2, inoculation resulted in the successful establishment of mycorrhiza similarly to Experiment 1, and mycorrhiza significantly improved tobacco growth (Table 3). The substrate of NM plants had lower pH than the substrate of M plants similarly as in Experiment 1, but the substrate pH was generally higher and the difference smaller than in Experiment 1. NM plants had significantly higher Cd concentrations in shoots and roots than M plants, but the total Cd content did not significantly differ between NM and M plants (Table 3). The Cd concentration in extracted ERM was about 10 times higher than in NM roots and about 20 times higher than in M roots. If we assume the same Cd concentration in the ERM of the root and hyphal compartment and multiply the ERM biomass obtained in the hyphal compartments by the factor two (substrate volume and ERM density were equal in both compartments), the Cd content in ERM pot^{-1} was comparable to that in the roots of mycorrhizal plants (Table 3). However, the amount of Cd pot^{-1} immobilised by mycorrhizal plants (shoots + roots + ERM) was not significantly higher than the Cd content of non-mycorrhizal plants (shoots + roots).

4. Discussion

Lower Cd toxicity in sand collected after tobacco cultivation as compared to clean sand shows the importance of soil organic matter in Cd immobilisation as previously

reported, e.g. by Prokop et al. (2003). While the clean sand did not contain any organic matter to immobilise Cd it was enriched with soluble and insoluble organic substances after the tobacco cultivation. However, Cd toxicity was also lower in the mycorrhizal than in the non-mycorrhizal substrate which strongly suggests that AM fungi could protect plants against HM toxicity by immobilising HMs in the soil.

The hypothesis that mycorrhiza could enhance Cd immobilisation in substrate was mainly based on studies indicating a high HM sorption capacity of AM fungal ERM (Joner et al., 2000; Chen et al., 2001; Gonzalez-Chavez et al., 2002). The experimental approach of the present study consisted in amending Cd to air-dried and homogenised substrate, which enabled determining Cd toxicity in a series of tests and excluded the effects of spacial heterogeneities in intact rooting zones. Heat-inactivated fungal biomass seems to maintain a similar Cd sorption capacity as live mycelium (Gurisik et al., 2004) and thus, Cd immobilisation in the mycorrhizal substrate was probably not overestimated as effect of drying. In contrast, Joner et al. (2000) showed that drying considerably increases the cation exchange capacity (CEC) of roots while it does not affect that of AM fungal ERM. Drying may therefore increase the Cd sorption capacity of roots relatively to that of ERM, because Cd is mostly bound to organic matter by non-specific cation exchange processes (Ross, 1994). This could result in an underestimation of the contribution of ERM, if cation exchange processes on roots ad ERM are involved in the Cd immobilisation in substrate. Furthermore, the interaction of ERM with Cd was limited to non-metabolic biosorption in the air-dried substrate, which may also have decreased the amount of Cd accumulated by the mycelium (Blaudez et al., 2000).

However, not only direct interaction of the AM fungal hyphae with Cd, but also plant-mediated effects of mycorrhiza may have contributed to the observed lower Cd toxicity in mycorrhizal substrate. The substrate collected after cultivation of non-mycorrhizal tobacco had significantly lower pH than that of mycorrhizal tobacco. A similar effect of mycorrhiza on the rhizosphere pH has been previously described (Li and Christie, 2001; Marschner and Baumann, 2003). Soil pH is the most important single soil property that determines Cd bioavailability to plants (Adriano, 2001); reduced pH generally decreases the rate and extent of metal biosorption (Gadd, 1990). The results of Marschner and Baumann (2003) suggested that the mycorrhizal effects on rhizosphere pH are plant-mediated. They could be related to the different P nutrition of mycorrhizal and non-mycorrhizal plants as plants respond to P-shortage by enhanced proton release (Hinsinger et al., 2003). Mycorrhiza consistently improved the P-nutrition of the tobacco variety used for the preparation of the substrates in previous studies (Janoušková et al., 2005a,b). Moreover, it has been shown that root exudates of tobacco effectively mobilise Cd in soil (Mench and Martin, 1991) with low molecular weight organic acids being probably the most efficient component (Nigam et al., 2001). Both the P status of a plant and AM symbiosis influence the amount and composition of the soluble organic matter released by roots (Jones et al., 2004) and it can be speculated that Cd may be better immobilised in mycorrhizal rhizosphere also due to the differential release of Cd-chelating organic compounds by mycorrhizal and non-mycorrhizal plants. However, the contribution of mycorrhizal effects (direct or plant-mediated) to Cd immobilisation should be also tested in soils with a higher sorption capacity for Cd or buffering capacity to pH changes.

The Cd concentration in ERM found in Experiment 2 and the calculated Cd content in ERM pot^{-1} support the suggestion of Joner et al. (2000) that ERM could play a significant role in Cd immobilisation in soil. The difference in Cd accumulation per unit of biomass between ERM and roots was about one order of magnitude, much higher than between Cd sorption by excised ERM and roots as determined by Joner et al. (2000). However, it corresponds with reported differences in Zn concentration between ERM and roots after exposition to Zn in vivo (Chen et al., 2001) and is also comparable with differences in CEC between ERM and roots (Marschner et al., 1998; Joner et al., 2000). Further studies should address whether active Cd sequestration inside hyphae contributes to the determined high Cd concentration in the ERM. The mycelium of ectomycorrhizal fungi seems to bind HMs mainly in cell walls (Galli et al., 1994), but localisation of significant amounts of Cd in the vacuoles has been also demonstrated (Turnau et al., 1994; Blaudez et al., 2000).

It remains to be elucidated to which extent the observed interaction of AM symbiosis with Cd can affect the Cd uptake and tolerance of AM plants in comparison with non-mycorrhizal plants. Experiment 2 failed to demonstrate lower Cd availability to mycorrhizal than to non-mycorrhizal tobacco. The lower Cd concentrations in the biomass of mycorrhizal plants can be ascribed to a dilution effect, when the same amount of an element is diluted in greater plant biomass (Jarrell and Beverly, 1981). However, the Cd content of the plant and AM fungus, amounting to about 10% of the total added Cd in the mycorrhizal treatment, did probably not represent all available Cd in the system. In a previous study, tobacco extracted about 70% of the added Cd from the same growth substrate at comparable total Cd concentrations (Janoušková et al., 2005b).

The relationship between total Cd concentration and root growth inhibition in mycorrhizal and non-mycorrhizal substrates indicates that AM symbiosis may significantly contribute to Cd immobilisation in soil only at higher total Cd concentrations. Because various AM fungal isolates were shown to be relatively tolerant to Cd (Rivera-Becerril et al., 2002; Janoušková et al., 2005a), this finding may not necessarily limit the significance of the effects observed in Experiment 1 due to inhibition of fungal growth by high Cd concentrations. It may, however, explain why Cd accumulation in ERM did not decrease tobacco's Cd uptake in Experiment 2, where Cd concentration added to the

substrate was lower than the concentration range with significant differences between mycorrhizal and non-mycorrhizal substrates in the root growth tests.

In conclusion, it could be demonstrated by the root growth tests that Cd is more effectively immobilised in mycorrhizal than in non-mycorrhizal rhizosphere at higher soil Cd concentrations. Both direct interaction of AM fungal structures with Cd as well as plant-mediated effects on the rhizospheric properties may have contributed to the effect. ERM exposed to Cd in vivo accumulated Cd concentrations about one order of magnitude higher than tobacco roots. The Cd accumulation in ERM, however, did not significantly decrease Cd uptake by tobacco in the experiment, which may have been related to the relatively low Cd concentration applied. The results indicate that AM symbiosis could contribute to Cd immobilisation in soils decreasing Cd toxicity to plants, which can positively affect the phytostabilisation of highly Cd-contaminated sites.

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References

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. Springer-Verlag, New York, Berlin, Heidelberg.

An, Y.J., 2004. Soil ecotoxicity assessment using cadmium sensitive plants. Environ. Pollut. 127, 21–26.

Blaudez, D., Botton, B., Chalot, M., 2000. Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiology 146, 1109–1117.

Chen, B., Christie, P., Li, X., 2001. A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. Chemosphere 42, 185–192.

Denny, H.J., Wilkins, D.A., 1987. Zinc tolerance in *Betula* spp. 4. The mechanism of ectomycorrhizal amelioration of zinc toxicity. New Phytol. 106, 545–553.

Frey, B., Zierold, K., Brunner, I., 2000. Extracellular complexation of Cd in the Hartig net and cytosolic Zn sequestration in the fungal mantle of *Picea abies – Hebeloma cristuliniforme* ectomycorrhizas. Plant Cell Environ. 23, 1257–1265.

Gadd, G.M., 1990. Heavy metal accumulation by bacteria and other microorganisms. Experientia 46, 834–840.

Galli, U., Schuepp, H., Brunold, C., 1994. Heavy-metal binding by mycorrhizal fungi. Physiol. Plantarum 92, 364–368.

Gaur, A., Adholeya, A., 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr. Sci. India 86, 528–534.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques to measure vesicular–arbuscular infection in roots. New Phytol. 84, 489–500.

Gonzalez-Chavez, C., D'Haen, J., Vangronsveld, J., Dodd, J.C., 2002. Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240, 287–297.

Gryndler, M., Vejsadová, H., Vančura, V., 1992. The effect of magnesium ions on the vesicular–arbuscular mycorrhizal infection of maize roots. New Phytol. 122, 455–460.

Gurisik, E., Arica, M.Y., Bektas, S., Genc, O., 2004. Comparison of the heavy metal biosorption capacity of active, heat-inactivated and NaOH-treated phanerochaete chrysosporium biosorbents. Eng. Live Sci. 4, 86–89.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248, 43–59.

ISO, 11269-1, 1993. Soil Quality – Determination of the Effects of Pollutants on Soil Flora. Part 1: Method for the Measurement of Inhibition of Root Growth.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytol. 120, 509–516.

Janoušková, M., Pavlíková, D., Macek, T., Vosátka, M., 2005a. Arbuscular mycorrhiza decreases cadmium phytoextraction by transgenic tobacco with inserted metallothionein. Plant Soil 272, 29–40.

Janoušková, M., Pavlíková, D., Macek, T., Vosátka, M., 2005b. Influence of arbuscular mycorrhiza on the growth and cadmium uptake of tobacco with inserted metallothionein gene. Appl. Soil. Ecol. 29, 209–214.

Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34, 197–224.

Jentschke, G., Godbold, D.L., 2000. Metal toxicity and ectomycorrhizas. Physiol. Plantarum 109, 107–116.

Joner, E.J., Briones, R., Leyval, C., 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226, 227–234.

Jones, D.L., Godge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163, 459–480.

Khan, A.G., Keuk, C., Chaudhry, T.M., Khoo, C.S., Hayes, W.J., 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41, 197–207.

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486–505.

Kurek, E., Czaban, J., Bollag, J.M., 1982. Sorption of cadmium by microorganisms in competition with other soil constituents. Appl. Environ. Microbiol. 43, 1011–1015.

Leyval, C., Turnau, K., Haselwandter, K., 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7, 139–153.

Li, X.L., Christie, P., 2001. Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42, 201–207.

Marschner, P., Baumann, K., 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant Soil 251, 279–289.

Marschner, P., Jentschke, G., Godbold, D.L., 1998. Cation exchange capacity and lead sorption in ectomycorrhizal fungi. Plant Soil 205, 93–98.

Meharg, A.A., Cairney, J.W.G., 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv. Ecol. Res. 30, 69–112.

Mench, M., Martin, E., 1991. Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L. Plant Soil 132, 187–196.

Miholová, D., Mader, P., Száková, J., Slámová, A., Svatoš, Z., 1993. Czechoslovak biological certified reference materials and their use in the analytical quality assurance system in a trace element laboratory. Fresen. J. Anal. Chem. 345, 256–260.

Newman, E.I., 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3, 139–145.

Nigam, R., Srivastava, S., Prakash, S., Srivastava, M.M., 2001. Cadmium mobilisation and plant availability – the impact of organic acids commonly exuded from roots. Plant Soil 230, 107–113.

Prokop, Z., Cupr, P., Zlevorova-Zlamalikova, V., Komarek, J., Dusek, L., Holoubek, I., 2003. Mobility, bioavailability, and toxic effects of cadmium in soil samples. Environ. Res. 91, 119–126.

Rivera-Becerril, F., Calantzis, C., Turnau, K., Caussanel, J.-P., Belimov, A.A., Gianinazzi, S., Strasser, R.J., Gianinazzi-Pearson, V., 2002. Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. J. Exp. Bot. 53, 1177–1185.

Ross, S.M., 1994. Retention, transformation and mobility of toxic metals in soils. In: Ross, S.M. (Ed.), Toxic Metals in Soil–Plant Systems. John Wiley & Sons, Chichester, pp. 63–152.

Schachtschabel, P., Blume, H.-P., Brümmer, G., Hartge, K.-H., Schwertmann, U., 1992. Lehrbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart.

Turnau, K., Kottke, I., Dexheimer, J., Botton, B., 1994. Element distribution in mycelium of *Pisolithus arrhizus* treated with cadmium dust. Ann. Bot.-London 74, 137–142.

Vivas, A., Vörös, A., Biró, B., Barea, J.M., Ruiz-Lozano, J.M., Azcón, R., 2003. Beneficial effects of indigenous Cd-tolerant and Cd-sensitive *Glomus mosseae* associated with a Cd-adapted strain of *Brevibacillus* sp. in improving plant tolerance to Cd contamination. Appl. Soil Ecol. 24, 177–186.

Wenzel, W.W., Lombi, E., Adriano, D.C., 1999. Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants. Springer-Verlag, Berlin, Heidelberg, pp. 273–303.