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Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L.

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Abstract

An inhibitory effect of lead (Pb) on hyphal growth of *Glomus intraradices* was observed after the incubation of colonised root segments in the media containing Pb concentrations higher than 0.01 mM. The comparison of two *G. intraradices* isolates that originated either in Pb-contaminated or non-contaminated soil suggests higher Pb tolerance of the native isolate from Pb-contaminated soil in comparison to the reference isolate from non-contaminated soil.

The role of both isolates in Pb accumulation by two plant species with different tolerance to heavy metals—common bent grass (*Agrostis capillaris*) from a Pb-contaminated site and maize (*Zea mays*)—was investigated in a sand-based hydroponic experiment with simulated Pb stress. Increased Pb concentrations influenced neither the growth of host plants nor the development of AM fungi (root colonisation, ERM length and NADH-diaphorase activity). In spite of low Pb toxicity, mycorrhizal inoculation substantially influenced Pb accumulation by the plants and the translocation into their shoots, but its effect differed considerably between plant species.

When maize plants were treated with 0.01 mM Pb, mycorrhizal inoculation with both *G. intraradices* isolates resulted in decreased Pb concentrations in both shoots and roots in comparison with non-inoculated plants. At a higher Pb level (0.1 mM), the inoculation decreased Pb concentrations in maize roots, but not shoots, when compared to non-inoculated plants. For the common bent, shoot Pb concentrations were not influenced by the inoculation in either Pb treatment. At a lower Pb level, the *G. intraradices* isolate from contaminated soil increased Pb concentrations in the roots of *Agrostis* plants in comparison with control plants or plants inoculated with the isolate from non-contaminated soil. No significant differences in Pb concentrations in *Agrostis* roots were found between the inoculation treatments at the higher Pb level.

Keywords: Arbuscular mycorrhizal fungi; Soil contamination; Heavy metals; Pb stress

1. Introduction

High concentrations of heavy metals (HM) have an adverse effect on soil microorganisms including arbuscular mycorrhizal (AM) fungi (McGrath et al., 1995; Kandeler et al., 1996) that provide the interface between roots of host plants and soil. Nevertheless, AM fungi are able to colonise plants growing even on highly contaminated sites, as has been demonstrated in field studies performed on mine spoils or in the vicinity of metal smelters (Ietswaart et al., 1992; Griffioen, 1994; Pawlowska et al., 1996). AM fungi isolated from contaminated sites have been reported to possess higher HM tolerance in comparison with

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those collected from non-contaminated substrates (Gildon and Tinker, 1983; Weissenhorn et al., 1994; Weissenhorn and Leyval, 1995; del Val et al., 1999).

Studies dealing with the effect of AM fungi on HM uptake by host plants have provided conflicting results. Some authors have demonstrated higher concentrations of heavy metals in host plant tissues due to AM, whereas others have shown reduced HM concentrations in mycorrhizal plants in comparison with non-mycorrhizal plants (reviewed by Leyval et al., 1997; Meharg and Cairney, 2000). Controversial data indicate that the effect of AM fungi on HM uptake by plants depends on the type and concentration of heavy metal, on physico-chemical properties of the substrate, on the combination of the AM fungal isolate and the host plant, and on cultivation conditions (Leyval et al., 1997). For example, Schüepp et al. (1987) observed lower shoot concentrations of Cd and Zn with high concentrations of metals in the substrate, while decreased Cd and increased Zn uptake were found with low metal concentrations in the substrate. Similarly, Heggo et al. (1990) reported lower Zn, Cd and Mn concentrations in the shoots of mycorrhizal plants growing in highly contaminated soil but higher metal concentrations when the plants were grown in soil with relatively low HM content. El-Kherbawy et al. (1989) reported increased metal concentrations in the shoots of mycorrhizal plants at higher pH level causing lower metal availability and decreased metal uptake at lower pH level. AM fungi affect not only the total uptake of HM by host plants, but also their translocation from roots to shoots and HM distribution patterns appear to be dependent on HM concentration in the soil. Loth and Höfner (1995) observed a higher metal concentration in the roots of mycorrhizal oats but a reduced translocation to the shoots in comparison with the non-mycorrhizal control, when the plants were grown in highly contaminated soil. In contrast, higher metal concentrations in both the roots and the shoots of mycorrhizal plants were found at lower HM contamination level.

Díaz et al. (1996) compared the effects of AM fungi isolated from contaminated and non-contaminated soil on HM accumulation in two plant species naturally growing in heavy metal-contaminated soils. At low Pb and Zn amendments, mycorrhizal plants showed equal or higher concentrations of Zn or Pb than non-mycorrhizal ones. At higher concentrations, however, metal accumulation was lower in the shoots of plants inoculated with *Glomus mosseae* (isolated from a Pb- and Zn-contaminated soil) whereas *G. macrocarpum* (isolated from a non-contaminated site) either did not affect or increased (depending on host plant species) Zn and Pb concentrations in the shoots. Differences between native and non-native AM isolates were also reported by Weissenhorn et al. (1995), who found higher copper concentrations in the shoots of plants inoculated with native *G. mosseae* than in non-inoculated plants and plants inoculated with a non-native isolate.

The effect of inoculation with AM fungi on HM uptake by host plants can vary considerably between plant species (Díaz et al., 1996; Joner and Leyval, 2001). The question remains whether or not AM fungi of different origins (either from a contaminated or a non-contaminated area) have different effects on metal uptake by plants of different HM tolerance. In our present study, the effect of Pb on two *G. intraradices* strains (isolated either from Pb-contaminated or non-contaminated soil) was investigated in vitro by measuring the growth of hyphae from colonised root segments. In a subsequent hydroponic experiment, the influence of these two AM isolates on Pb accumulation by two host plants—*Zea mays* and *Agrostis capillaris*—was studied.

2. Materials and methods

2.1. AM fungi

Two AM isolates of *G. intraradices* Schenck and Smith were chosen. The isolate, referred to as PH5, originated in a Pb-contaminated waste disposal site in the proximity of the Příbram lead smelter (Czech Republic). The pH (H_2O) value of the original substrate is 5.0–5.7 and the Pb content in aqua regia-digested substrate reaches 25,150 mg kg^{-1}. For comparison, the total background concentrations of Pb in soils away from human activity range from 10 to 30 mg kg^{-1} (Adriano, 2001). Using four different extractants, the following Pb contents were determined: 1 M ammonium acetate–0.1 M EDTA extractable Pb—14,776 mg kg^{-1}, 1 M ammonium acetate extractable Pb—2333 mg kg^{-1}, 0.005 M DTPA–0.01 M $CaCl_2$–0.1 M triethanolamine

extractable Pb—573 mg kg^{-1} and 0.1N $Ca(NO_3)_2$ extractable Pb—34 mg kg^{-1}. Using a method after Weissenhorn et al. (1993), Pb concentration in water soil extract was determined as 3.5 mg l^{-1}. The isolate PH5 was maintained and multiplied exclusively in the original Pb-contaminated soil, sterilised by autoclaving. The reference isolate of the same species, *G. intraradices* BEG75, originated in a non-polluted agricultural area (Switzerland).

2.2. In vitro experiments

The preparation of root segments for experiments followed the method described by Gryndler et al. (1998). Yellow-coloured mycorrhizal roots were collected from 5-week-old maize (*Z. mays* L.) plants inoculated with *G. intraradices* PH5 or *G. intraradices* BEG75. The selected roots were shaken five times with sterile distilled water for 1 min to remove retained particles of the substrate. Subsequently, the roots were surface-disinfected for 4 h with a mixture of the antibiotics streptomycin (500 mg l^{-1}), polymyxin B (500 mg l^{-1}), penicillin G (500 mg l^{-1}), neomycin (500 mg l^{-1}) and rolitetracycline (250 mg l^{-1}). The roots were then immersed in a 1:50 solution of sodium hypochlorite (commercial bleach Savo) for 3 min, washed with 1 l of sterile distilled water, cut into segments 1–2 mm long and washed with sterile deionised water. The segments were incubated in 30 μl drops of filter-sterilised solutions on the inside of lids of polystyrene Petri dishes. Each treatment involved seven Petri dishes as replicates. The incubation solutions contained different concentrations of Pb applied in the form of $Pb(NO_3)_2$; nitrate concentrations were equalised in all treatments using magnesium nitrate $Mg(NO_3)_2$ (magnesium cations in the concentration range used did not influence hyphal growth and did not operate as antagonists to Pb cations, data not shown). One millimole BIS–TRIS was used as a buffering substance in the incubation solutions and the pH was adjusted to 6.3. The dishes, each containing 16 hanging drops of the medium with the root segments, were incubated in the dark in a humid chamber for 5 days at 25 °C. After the incubation, the proliferation of hyphae from root segments was observed under a microscope (magnification 63×). One Petri dish was taken as an experimental unit. The length of AM hyphae per root segment was estimated using a grid-line intersect method and the mean percentage of segments bearing proliferating hyphae was calculated. One intersection corresponded to a hyphal length of 0.059 mm. Only non-contaminated root segments were taken into account because of the possible interference of AM hyphal growth with contaminating saprophytic microorganisms.

To determine the Pb concentration inhibiting the growth of intraradical hyphae, the effect of the Pb concentration range (0, 0.001, 0.05, 0.01, 0.05, 0.1 and 0.5 mM) on hyphal proliferation of *G. intraradices* PH5 was studied in Experiment 1. In Experiment 2, both *G. intraradices* isolates were compared with respect to their Pb tolerance at three Pb concentrations (0, 0.01 and 0.1 mM). To verify the results, the response of both *G. intraradices* isolates to higher Pb concentrations (0, 0.1 and 0.2 mM) was studied in Experiment 3.

2.3. Cultivation experiment

2.3.1. AM fungi, plants, experimental design

The effect of both *G. intraradices* isolates on Pb accumulation by two plant species—maize (*Z. mays* L.) and common bent (*A. capillaris* L.) was studied in Experiment 4, a sand-based hydropony. The seeds of common bent were collected from a tolerant population growing in close proximity to a Pb smelter, strongly influenced by air deposition. Maize was selected as an example of a plant with lower HM tolerance that has been used in a number of previous studies focused on the interaction of AM fungi with heavy metals. For each host plant, the experiment was designed as a two factorial with the following factors: (a) Pb treatment (0, 0.01 and 0.1 mM), (b) inoculation treatment (non-inoculated plants, inoculated with *G. intraradices* PH5 and inoculated with *G. intraradices* BEG75). The seeds of host plants were surface-disinfected and pre-germinated on filter papers saturated with distilled water. After 1 week, each seedling was planted in a 300-ml plastic tube filled with autoclaved sand. Both mycorrhizal treatments (*G. intraradices* PH5 and BEG75) received 10 ml of an inoculum suspension (involving colonised root segments, ERM and spores) per plant. The non-mycorrhizal treatments received the same amount of autoclaved inoculum. To equalise the conditions, all inoculation treatments were irrigated with a filtrate

from non-sterile mycorrhizal inoculum. The filtrate was obtained by passing a 1:10 suspension of the soil inoculum through a filter paper to remove AM fungal spores and mycelia. Each treatment involved 12 tubes inserted into a plastic hydroponic vessel. For the first 2 weeks, the plants were supplied with only distilled water (2 l per vessel). After 2 weeks, the plants were supplied with P2N3 nutrient solution weekly (Gryndler et al., 1992) for 8 weeks. The solution contained the following concentrations of minerals ($mg\,l^{-1}$): 12.2 KH_2PO_4, 295 $Ca(NO_3)_2{\cdot}4H_2O$, 240 KNO_3, 13.5 KCl, 720 $MgSO_4{\cdot}7H_2O$, 0.75 $MnCl_2{\cdot}4H_2O$, 0.75 KI, 0.75 $ZnSO_4{\cdot}7H_2O$, 1.5 H_3BO_3, 3.2 $FeCl_3{\cdot}6H_2O$, 0.001 $CuSO_4{\cdot}5H_2O$, 0.00017 $Na_2MoO_4{\cdot}2H_2O$. During the first 10 weeks, the development of AM symbiosis was not disturbed by Pb addition. Then, the application of P2N3 solution was finished and Pb treatment was initiated. The plants were supplied with 2 l of distilled water containing a corresponding concentration of Pb three times a week. Pb was added as $Pb(NO_3)_2$ and the pH of the solution was adjusted to 4.8 prior to the application. The mode of Pb application in a single salt solution was chosen in order to avoid precipitation as Pb phosphate and/or hydroxide. The plants were grown in a greenhouse for 20 weeks. During the growth period, the vessels with plants of different treatments were randomly moved within the greenhouse once a week in order to minimise a variance in cultivation conditions.

2.3.2. Harvest, plant analyses, mycorrhizal parameters

After 20 weeks, all of the plants were harvested and the dry weights of the shoots and roots were recorded after drying at 80 °C. Phosphorus and Pb concentrations in the shoots and the roots were determined for six randomly selected plants per treatment. The samples were ground, digested in HNO_3 and H_2O_2 and Pb concentrations were determined by atomic absorption spectrometry (Unicam 9200X, Allen et al., 1986). P concentrations in biomass were assessed spectrophotometrically (630 nm, Unicam UV4-100, Olsen et al., 1954). Root samples for an evaluation of mycorrhizal colonisation were stained with 0.05% trypan blue in lactoglycerol (Koske and Gemma, 1989) and root colonisation was assessed using the grid-line intersect method (Giovannetti and Mosse, 1980). The substratum from each tube was homogenised and small aliquot samples were taken to estimate the length of ERM using a modified membrane filtration technique (Jakobsen et al., 1992) and NADH-diaphorase activity of ERM (Sylvia, 1988; Hamel et al., 1990). A small sample of the substratum was placed in a household blender with 500 ml of distilled water and blended for 30 s. One millilitre of supernatant was pipetted onto a membrane filter (24 mm diameter and 0.40 μm pore size) and vacuum filtered. ERM retained on the surface of the filters was stained with 0.05% trypan blue in lactoglycerol. The total length of the ERM was assessed using the grid-line intersect method under a microscope, using an ocular grid at 100× magnification, and expressed in meters of hyphae in 1 g of air-dried substrate. The background length of mycelium found in non-inoculated treatments was subtracted from all values in mycorrhizal treatments. The NADH-diaphorase activity of ERM was stained in the remaining ERM extracted from the substrate by wet sieving. Three hundred microlitres of NADH-diaphorase staining solution were mixed in Eppendorf tubes with the mycelium sample. The Eppendorf tubes were incubated at 28 °C for 14 h in the dark. The proportion of ERM length that contained red precipitate (NADH-diaphorase activity) was estimated under a microscope at 400× magnification.

2.4. Statistical treatment

The results of the experiments were analysed using one-way (Experiment 1) or two-way (Experiments 2–4) analysis of variance (ANOVA). The effects of inoculation treatments on Pb accumulation by host plants were analysed separately for each Pb concentration using one-way ANOVA. Comparisons between means were carried out using the Duncan's multiple range test at a significance level of $P < 0.05$.

3. Results

In Experiment 1, a significant inhibitory effect of Pb on the percentage of proliferating hyphae and hyphal growth of *G. intraradices* PH5 was observed at concentrations higher than 0.01 mM (Table 1). However, hyphal growth was not completely eliminated, even at the concentration of 0.5 mM Pb, where 10% of root

Table 1
The effect of lead (Pb) treatment on the percentage of root segments with proliferating hyphae and on the length of proliferating hyphae of *G. intraradices* PH5

Pb (mM)	Proliferation of hyphae (% root segments)	Mean length of hyphae (mm per root segment)
0	80 (±4) a	8.0 (±0.8) a
0.001	71 (±2) ab	7.4 (±0.8) a
0.005	79 (±6) a	7.8 (±0.9) a
0.01	65 (±6) b	4.0 (±0.4) b
0.05	58 (±5) b	3.6 (±0.6) b
0.1	38 (±5) c	1.5 (±0.2) c
0.5	8 (±3) d	0.6 (±0.2) c
F-value/ significance	30.4***	24.6***

The values are given as means (±S.E.) of seven replicates. Significances according to one-way ANOVA. Means followed by the same letters are not significantly different according to Duncan's multiple range test at the level $P < 0.05$.
*** Significant effect at the level $P < 0.001$.

segments showed proliferating hyphae, compared to the treatment without Pb addition. In Experiment 2, a Pb concentration of 0.1 mM decreased the proportion of segments with proliferating hyphae by 55 and 82% for *G. intraradices* PH5 and BEG75, respectively (Table 2). The drop in the length of proliferating hyphae at this Pb concentration was 40 and 91% for *G. intraradices* PH5 and BEG75, respectively, in comparison to 0 mM Pb treatments. The interaction between the effects of the fungal isolate and Pb concentration was non-significant for both parameters studied. In Experiment 3, with higher Pb concentrations applied, the proliferation and hyphal growth were significantly affected by the Pb concentration and the fungal isolate as well as by their interaction (Table 3). The native isolate *G. intraradices* PH5 was more tolerant to higher Pb concentrations than the non-native isolate *G. intraradices* BEG75.

In the cultivation experiment, the two plant species responded differently to the inoculation with AM fungi. In a control treatment without Pb application, maize growth was improved by inoculation with *G. intraradices* PH5 and not influenced by the isolate BEG75, when compared to non-inoculated plants (Table 4). In contrast to maize, inoculation with either *G. intraradices* isolate significantly decreased shoot and root dry weights of *Agrostis* plants (Table 5). The addition of Pb into the hydroponic solution did not influence shoot dry weights of either plant species or the root dry weights of maize plants. Root dry weight of *Agrostis* plants was significantly decreased when treated with 0.1 mM Pb.

Inoculation with both *G. intraradices* isolates increased P concentration in the roots of maize plants treated with higher Pb concentrations, whereas the root P concentrations of control plants were not

Table 2
The effect of lead (Pb) treatment on the percentage of root segments with proliferating hyphae and on the length of proliferating hyphae of *G. intraradices* PH5 and *G. intraradices* BEG75

Pb (mM)	Isolate	Proliferation of hyphae (% root segments)	Mean length of hyphae (mm per root segment)
0	*G. i.* PH5	92 (±4) a	15.1 (±2.8) a
0.01	*G. i.* PH5	77 (±11) a	13.5 (±3.2) a
0.1	*G. i.* PH5	41 (±11) b	9.1 (±2.6) a
0	*G. i.* BEG75	72 (±4) x	15.2 (±1.0) x
0.01	*G. i.* BEG75	68 (±5) x	13.0 (±1.6) x
0.1	*G. i.* BEG75	13 (±3) y	1.4 (±0.4) y
F-value/significance			
Pb (A)		32.8***	11.8***
Isolate (B)		10.3**	2.3 (n.s.)
A × B		0.9 (n.s.)	2.0 (n.s.)

The values are given as means (±S.E.) of seven replicates. Significances according to two-way ANOVA. Means followed by the same letters are not significantly different within each inoculation treatment according to Duncan's multiple range test at the level $P < 0.05$. *G. i.*: *G. intraradices*; n.s.: non-significant effect.
** Significant effect at the level $P < 0.01$.
*** Significant effect at the level $P < 0.001$.

Table 3
The effect of lead (Pb) treatment on the percentage of root segments with proliferating hyphae and on the length of proliferating hyphae of *G. intraradices* PH5 and *G. intraradices* BEG75

Pb (mM)	Isolate	Proliferation of hyphae (% root segments)	Mean length of hyphae (mm per root segment)
0	*G. i.* PH5	92 (±4) a	28.1 (±2.3) a
0.1	*G. i.* PH5	49 (±7) b	12.6 (±1.8) b
0.2	*G. i.* PH5	17 (±4) c	3.2 (±1.4) c
0	*G. i.* BEG75	48 (±6) x	13.7 (±3.4) x
0.1	*G. i.* BEG75	17 (±4) y	4.9 (±2.2) y
0.2	*G. i.* BEG75	3 (±2) z	0.2 (±0.2) y
F-value/significance			
Pb (A)		75.6***	23.7***
Isolate (B)		57.4***	42.4***
A × B		4.9*	3.7*

The values are given as means (±S.E.) of seven replicates. Significances according to two-way ANOVA. Means followed by the same letters are not significantly different within each inoculation treatment according to Duncan's multiple range test at the level $P < 0.05$. *G. i.*: *G. intraradices*.

* Significant effect at the level $P < 0.05$.

*** Significant effect at the level $P < 0.001$.

affected by inoculation (Table 4). Significantly higher P concentrations were found in the roots and shoots of maize plants irrigated with higher Pb concentrations compared to the control. In spite of higher P concentration in the biomass of maize plants treated with the higher Pb level, a negative correlation between Pb and P concentrations in maize shoots ($r = -0.5330$, $P < 0.001$) and roots ($r = -0.4276$, $P < 0.01$) was found. In the case of *Agrostis*, inoculation with either *G. intraradices* isolate and 0.01 mM Pb treatment elevated shoot and root P concentrations (Table 5). Pb and P concentrations were significantly correlated only in roots of *Agrostis* plants cultivated at 0.1 mM Pb ($r = 0.7483$, $P < 0.001$).

The development of AM symbiosis varied between host plants (Tables 4 and 5). While mycorrhizal colonisation in maize roots reached approximately 70%, it was only about 40% in the roots of *Agrostis* plants. Similarly, the length of the ERM was found to be more than three times greater for maize in comparison to *Agrostis*. Root colonisation, ERM length and NADH-diaphorase activity of ERM were not influenced by higher Pb concentrations in either inoculation treatment or plant species. Root colonisation did not differ between AM isolates, but the length of ERM formed by *G. intraradices* PH5 in symbiosis with maize was significantly higher in comparison to the isolate BEG75 (Table 4). For both plant species, NADH-diaphorase activities were considerably lower in the ERM of *G. intraradices* PH5 than in the mycelium of *G. intraradices* BEG75.

In comparison with non-inoculated plants, Pb concentrations in the roots of inoculated maize plants were significantly decreased in both Pb treatments (Fig. 1). When exposed to 0.01 mM Pb, maize plants inoculated with either *G. intraradices* isolate also had significantly lower Pb concentrations in the shoots than non-inoculated plants. In contrast, no significant difference in shoot Pb concentrations was found between the inoculated and non-inoculated maize plants treated with 0.1 mM Pb. No difference was observed between the two *G. intraradices* isolates regarding Pb accumulation by maize plants. When Pb concentrations in maize were converted to total Pb content per plant, the effects of inoculation remained similar (Table 6).

Pb concentrations in *Agrostis* shoots were not influenced by inoculation in either of the Pb treatments (Fig. 2). In the 0.01 mM Pb treatment, higher root Pb concentrations were found for *Agrostis* plants inoculated with *G. intraradices* PH5 compared to non-inoculated plants and plants inoculated with the isolate BEG75. At 0.1 mM Pb treatment, no significant difference in root Pb concentration between inoculation treatments was detected although a trend of

Table 4
The effects of lead (Pb) and inoculation treatment on the growth of *Z. mays* plants and on the development of arbuscular mycorrhizal (AM) symbiosis

Pb (mM)	Inoculation	Shoot DW (g)	Root DW (g)	Shoot P concentration ($\mu g\,g^{-1}$)	Root P concentration ($\mu g\,g^{-1}$)	Root colonisation (%)	ERM length ($m\,g^{-1}$)	NADH-diaphorase activity (%)
0	Non-inoculated	2.97 (±0.16) b	1.33 (±0.10) b	639 (±52) a	446 (±37) a			
0	*G. i.* PH5	5.04 (±0.39) a	2.28 (±0.28) a	721 (±75) a	472 (±100) a	71 (±3) a	13.2 (±0.9) a	35 (±5) a
0	*G. i.* BEG75	3.18 (±0.35) b	1.15 (±0.20) b	436 (±21) b	474 (±58) a	67 (±3) a	9.2 (±1.6) a	44 (±4) a
0.01	Non-inoculated	4.81 (±0.34) m	1.58 (±0.13) m	724 (±61) n	586 (±34) n			
0.01	*G. i.* PH5	3.57 (±0.39) n	1.66 (±0.37) m	908 (±73) mn	733 (±89) n	72 (±1) m	14.4 (±1.5) m	26 (±5) n
0.01	*G. i.* BEG75	3.88 (±0.33) mn	1.19 (±0.12) m	1057 (±67) m	1101 (±192) m	69 (±5) m	7.9 (±0.9) n	46 (±4) m
0.1	Non-inoculated	2.72 (±0.17) y	1.38 (±0.09) xy	753 (±60) x	519 (±25) y			
0.1	*G. i.* PH5	4.56 (±0.30) x	1.83 (±0.24) x	820 (±86) x	723 (±43) x	68 (±2) x	12.0 (±0.8) m	24 (±4) y
0.1	*G. i.* BEG75	3.43 (±0.35) y	1.22 (±0.12) y	882 (±50) x	770 (±57) x	69 (±3) x	7.8 (±0.6) n	38 (±4) x
F-value/significance								
Pb (A)		2.1 (n.s.)	0.2 (n.s.)	17.2***	17.7***	0.2 (n.s.)	0.9 (n.s.)	1.8 (n.s.)
Inoculation (B)		7.8***	8.7***	2.4 (n.s.)	6.7**	1.0 (n.s.)	28.6***	15.7***
A × B		8.7***	1.6 (n.s.)	5.2**	1.8 (n.s.)	0.5 (n.s.)	0.7 (n.s.)	0.6 (n.s.)

The values are given as means (±S.E.) of 12 replicates (P concentrations—6 replicates). Significances according to two-way ANOVA. Means followed by the same letters are not significantly different within each Pb treatment according to Duncan's multiple range test at the level $P < 0.05$. *G. i.*: *G. intraradices*; n.s.: non-significant effect.

** Significant effect at the level $P < 0.01$.

*** Significant effect at the level $P < 0.001$.

Table 5
The effects of lead (Pb) and inoculation treatment on the growth of *A. capillaris* plants and on the development of arbuscular mycorrhizal (AM) symbiosis

Pb (mM)	Inoculation	Shoot DW (g)	Root DW (g)	Shoot P concentration ($\mu g\,g^{-1}$)	Root P concentration ($\mu g\,g^{-1}$)	Root colonisation (%)	ERM length ($m\,g^{-1}$)	NADH-diaphorase activity (%)
0	Non-inoculated	1.32 (±0.21) a	2.74 (±0.25) a	586 (±72) c	495 (±29) c			
0	*G. i.* PH5	0.62 (±0.07) b	1.72 (±0.21) b	979 (±93) b	746 (±76) b	41 (±4) a	2.1 (±0.3) a	21 (±3) b
0	*G. i.* BEG75	0.29 (±0.07) c	0.91 (±0.30) c	1520 (±277) a	1307 (±142) a	35 (±4) a	3.0 (±0.8) a	40 (±4) a
0.01	Non-inoculated	1.01 (±0.21) m	2.67 (±0.33) m	857 (±85) n	697 (±56) o			
0.01	*G. i.* PH5	0.65 (±0.07) m	1.28 (±0.32) n	1150 (±49) n	1106 (±93) n	38 (±3) m	2.7 (±0.4) m	15 (±3) n
0.01	*G. i.* BEG75	0.07 (±0.02) n	0.15 (±0.03) o	2629 (±771) m	2991 (±196) m	38 (±7) m	3.0 (±0.7) m	40 (±5) m
0.1	Non-inoculated	0.87 (±0.07) x	1.65 (±0.35) x	956 (±73) y	615 (±73) y			
0.1	*G. i.* PH5	0.76 (±0.08) x	1.38 (±0.27) x	1118 (±113) xy	1026 (±53) x	31 (±3) x	3.1 (±0.5) x	16 (±2) y
0.1	*G. i.* BEG75	0.21 (±0.07) y	0.47 (±0.20) y	1486 (±233) x	1088 (±143) x	42 (±5) x	3.6 (±0.3) x	37 (±1) x
F-value/significance								
Pb (A)		1.7 (n.s.)	4.2*	5.3**	24.8***	0.0 (n.s.)	2.4 (n.s.)	0.8 (n.s.)
Inoculation (B)		44.6***	35.1***	27.0***	77.9***	0.2 (n.s.)	1.8 (n.s.)	68.1***
A × B		1.8 (n.s.)	1.9 (n.s.)	1.9 (n.s.)	8.0***	2.0 (n.s.)	0.3 (n.s.)	0.4 (n.s.)

The values are given as means (±S.E.) of 12 replicates (P concentrations—6 replicates). Significances according to two-way ANOVA. Means followed by the same letters are not significantly different within each Pb treatment according to Duncan's multiple range test at the level P<0.05. *G. i.*: *G. intraradices*; n.s.: non-significant effect.

* Significant effect at the level $P < 0.001$.

** Significant effect at the level $P < 0.01$.

*** Significant effect at the level $P < 0.001$.

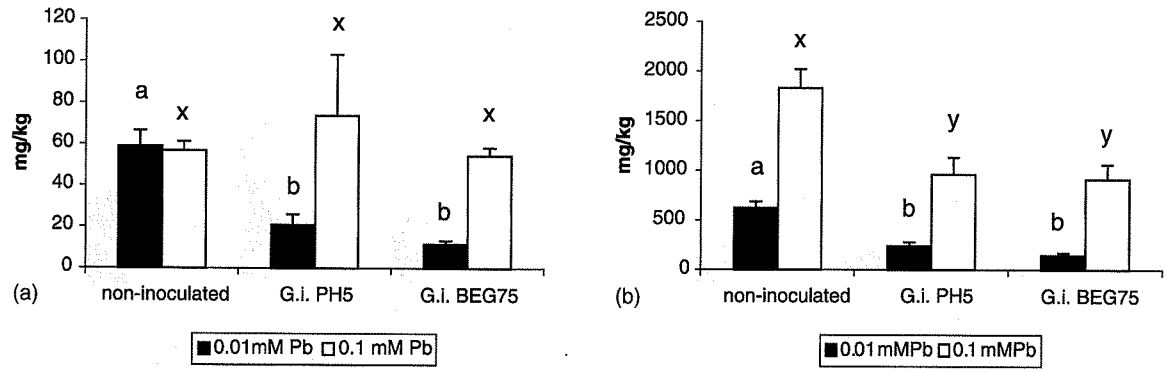

Fig. 1. The effect of inoculation on Pb concentrations in the shoots and roots of *Z. mays* plants: (a) shoots, (b) roots. The columns represent means ±S.E. of six values. The means marked with different letters are significantly different according to Duncan's multiple range test at the level $P < 0.05$.

Table 6
The effects of lead (Pb) and inoculation treatment on Pb contents in the biomass of *Z. mays* and *A. capillaris* plants

Pb (mM)	Inoculation	*Z. mays*		*A. capillaris*	
		Shoot Pb content (μg per plant)	Root Pb content (μg per plant)	Shoot Pb content (μg per plant)	Root Pb content (μg per plant)
0.01	Non-inoculated	328 (± 63) a	1044 (±207) a	43 (±14) a	5202 (±634) a
0.01	*G. i.* PH5	94 (± 34) b	568 (± 249) ab	24 (±7) a	5061 (±1920) a
0.01	*G. i.* BEG75	44 (± 6) b	181 (± 47) b	3 (±1) b	354 (±198) b
0.1	Non-inoculated	161 (±14) x	2810 (± 329) x	108 (±38) x	4319 (±1123) x
0.1	*G. i.* PH5	347 (±139) x	2010 (± 685) xy	85 (±19) x	3962 (±800) x
0.1	*G. i.* BEG75	219 (±23) x	1203 (± 159) y	24 (±8) y	2118 (± 878) x
F-level/significance					
Pb (A)		15.8***	50.2***	33.2***	0.0 (n.s.)
Inoculation (B)		5.3**	12.7***	23.7***	6.8**
A × B		12.1***	1.6 (n.s.)	1.2 (n.s.)	1.1 (n.s.)

The values are given as means (±S.E.) of six replicates. Significances according to two-way ANOVA. Means followed by the same letters are not significantly different within each Pb treatment according to Duncan's multiple range test at the level $P < 0.05$. *G. i.*: *G. intraradices*; n.s.: non-significant effect.

** Significant effect at the level $P < 0.01$.

*** Significant effect at the level $P < 0.001$.

higher Pb concentrations in inoculated *Agrostis* plants was observed. When Pb concentrations were recalculated to total Pb content, *Agrostis* plants inoculated with *G. intraradices* BEG75 contained much less Pb than non-inoculated plants and plants inoculated with the isolate PH5, due to reduced biomass (Table 6). In contrast to maize plants, root-to-shoot Pb ratios were considerably higher at the 0.01 mM Pb treatment than at the 0.1 mM Pb treatment for *Agrostis* plants (data not presented).

4. Discussion

4.1. Tolerance of fungal isolates to Pb

The main question posed in this study was whether AM isolates originating from either Pb polluted or non-polluted soils varied in response to Pb? When cultivated in Pb solutions in vitro, the native isolate *G. intraradices* PH5 showed a higher Pb tolerance than did the reference isolate from non-polluted soil. This

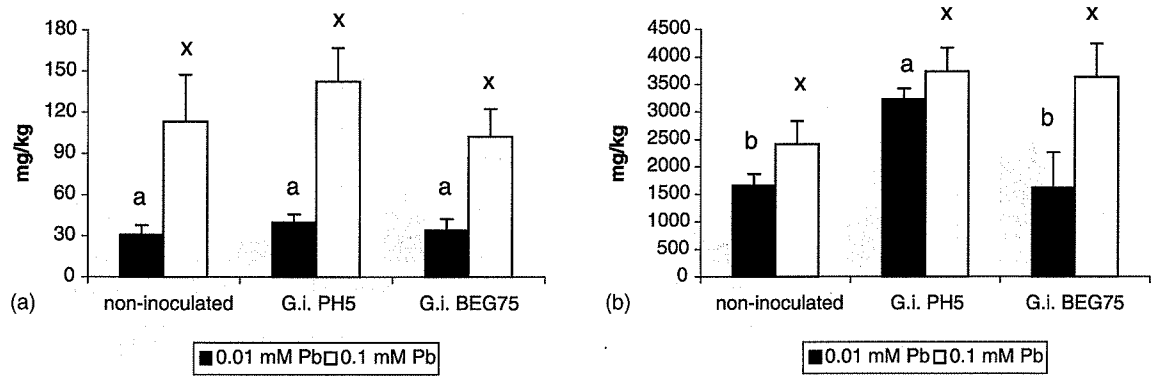

Fig. 2. The effect of inoculation on Pb concentrations in the shoots and roots of *A. capillaris* plants: (a) shoots, (b) roots. The columns represent means ±S.E. of six values. The means marked with different letters are significantly different according to Duncan's multiple range test at the level $P < 0.05$.

finding is in accordance with previous studies showing increased HM tolerance of AM isolates from contaminated soils (Gildon and Tinker, 1983; Weissenhorn et al., 1993, 1994; Weissenhorn and Leyval, 1995; Hildebrandt et al., 1999). Sensitivity of AM symbionts to high concentrations of HM, expressed as a reduction in spore germination, hyphal growth or root colonisation, has been reported previously in a number of studies (e.g. Gildon and Tinker, 1983; McGee, 1987; Leyval et al., 1995; Höflich and Metz, 1997). Using a soil-free proliferation method for testing the response of AM isolates to Pb proved to be viable because factors decreasing HM availability were minimised. In our experiment, intraradical hyphae were able to proliferate even in 0.5 mM Pb media, though their growth was substantially reduced. Such inhibitory concentrations of Pb are comparable to the concentrations reported to reduce mycelial growth of ectomycorrhizal fungi in liquid cultures (Vodnik et al., 1998; Marschner et al., 1999).

However, higher Pb tolerance of fungal isolates from polluted soils was not confirmed in the hydroponic experiment, since root colonisation and ERM length did not differ between isolates when exposed to Pb. None of the Pb concentrations reduced AM development, which indicates that Pb availability in the hydroponic system was considerably lower compared to the in vitro system. This was probably caused by an adsorption of Pb ions on the sand and/or complexation by root exudates or root-derived organic matter. Nevertheless, some of the Pb was available to the plants because high Pb concentrations were detected in the roots and a proportion of the Pb was translocated to the shoots.

4.2. Pb accumulation by plants

Lead was strongly retained in the roots of both species. Most of that Pb is likely to be localised in a precipitated form associated with cell walls or detoxified in vacuoles (Koeppe, 1977; Wierzbicka, 1995). There was a considerable difference between species in Pb accumulation and translocation from the roots to the shoots, regardless of AM inoculation. As shown by the Pb root-to-shoot ratios, *Agrostis* plants accumulated considerably more Pb in the roots than maize plants so that a barrier protecting plant shoots seemed to be more efficient in *Agrostis* than maize, particularly at lower Pb treatment. The observation that maize plants translocated relatively more Pb from the roots to the shoots is in agreement with the findings of Huang and Cunningham (1996), who reported that maize was more efficient in Pb translocation to the shoots compared with other plants tested. The higher Pb accumulation in *Agrostis* roots than in the maize roots might be related to a higher HM tolerance of *Agrostis* originating in contaminated substrate. Higher HM concentrations in the roots of tolerant plants along with more efficient restriction of HM movement from their roots to the shoots compared with non-tolerant plants have been shown repeatedly (Wu et al., 1975; Qureshi et al., 1985; Griffioen and Ernst, 1989).

4.3. Effect of mycorrhiza on plant growth and Pb accumulation

In comparison with maize, negative mycorrhizal growth response was found for *A. capillaris* plants. This contrasts with results given by Griffioen and Ernst (1989), who reported no mycorrhizal growth response for two clones of the same species. Field observations showed that *A. capillaris* frequently forms a symbiosis with AM fungi (Ietswaart et al., 1992; Griffioen, 1994) and the reported levels of root colonisation were similar to our experiment.

The lack of difference in the effect of both native and non-native *G. intraradices* isolates on Pb concentrations in maize corresponds with the results of Weissenhorn et al. (1995), who observed similar Cd uptake in maize plants colonised by either a metal-tolerant or metal-sensitive *G. mosseae* isolate. In contrast, Kaldorf et al. (1999) found lower metal concentrations (including Pb) in maize plants inoculated with the HM tolerant *Glomus* isolate in comparison to the plants inoculated with *G. intraradices* from non-contaminated soil. Likewise, Díaz et al. (1996) observed lower Pb concentrations in the shoots of plants inoculated with an AM isolate from contaminated soil than in the shoots of plants inoculated with an isolate from non-contaminated soil.

The effect of inoculation on Pb accumulation varied between plant species. The inoculation with both *G. intraradices* isolates significantly reduced Pb concentrations in maize plants, while Pb concentrations in *Agrostis* plants were not changed or even increased by the inoculation. Lower Pb concentrations in the shoots and roots of inoculated compared to the non-inoculated maize could not be explained by a dilution effect. Although the inoculation of maize plants decreased the shoot Pb concentration when treated with a lower Pb concentration (0.01 mM), the inoculation did not increased root-to-shoot ratio of Pb concentrations. In contrast, mycorrhiza-induced enhancement of root/shoot barrier for toxic metals was described, e.g. by Dehn and Schüepp (1989) and Joner and Leyval (2001). Reduced Pb concentrations in the shoots (root concentrations were not assessed) of mycorrhizal in comparison to non-mycorrhizal plants were reported repeatedly (Díaz and Honrubia, 1995; Karagiannidis and Hadjisavva-Zinoviadi, 1998; Karagiannidis and Nikolaou, 2000). Kaldorf et al. (1999) observed lower Pb concentrations both in the shoot and roots of maize plants inoculated with *G. intraradices*. In contrast, Weissenhorn et al. (1995) did not observe any difference in Pb shoot and root concentrations between non-mycorrhizal and mycorrhizal maize plants. Killham and Firestone (1983) found higher Pb concentrations in the shoots of mycorrhizal *Ehrharta calycina* plants than in non-mycorrhizal plants when exposed to acidified rain containing HM. Variable effects of inoculation on Pb uptake reported in the literature could be partly attributed to the wide spectrum of host plants tested. Different effects of inoculation on Pb uptake by different plant species have been shown, e.g. by Díaz et al. (1996) and Joner and Leyval (2001). Dependence of HM–AM interaction on plant species was shown also in two studies on AM isolates from the rhizosphere of a metallophyte zinc violet, *Viola calaminaria* (Kaldorf et al., 1999; Tonin et al., 2001). The former found much lower concentrations of HM in maize plants inoculated with *Glomus* Br1 isolate than in non-inoculated control plants. In contrast, the latter reported that colonisation of clover roots with mixed population of AM fungi from the zinc violet rhizosphere significantly increased Cd and Zn concentrations in clover roots without significantly affecting HM concentrations in the shoots. In the present case results, different HM tolerance of the two species could be an important factor influencing the effect of mycorrhizal inoculation on Pb accumulation. Griffioen and Ernst (1989) reported that tolerant and non-tolerant clones of *A. capillaris* showed different HM distribution in response to AM inoculation. Although Cu and Mn shoot-to-root ratios increased as a result of inoculation for both clones, those of the non-tolerant clone increased more. Nevertheless, the relationship between the effect of AM fungi on HM uptake and the HM tolerance of host plants remains to be elucidated.

The fact that inoculated maize plants took up less Pb in the roots and translocated less Pb to the shoots than non-inoculated plants might be due to the binding of metal by ERM, resulting in a lower amount of Pb available to the roots of mycorrhizal plants. As was shown by Joner et al. (2000) for Cd and Zn, ERM has a high HM sorption capacity relative to other microorganisms and the sorption takes place mainly due to passive adsorption on the hyphal surface. Gonzalez-Chavez et al. (2002) reported that ERM

accumulated Cu in the mucilaginous outer hyphal wall zone, in the cell wall, as well as inside the hyphal cytoplasm. In the case of *Agrostis*, much less ERM was produced, which may have resulted in its lower binding capacity for Pb. However, another mechanism, in addition to lower binding capacity resulting from reduced ERM development, was probably employed because even increased Pb concentrations were detected in the roots of inoculated *Agrostis* plants. It might be intracellular sequestration of metal in the fungal structures within cortical cells, as has been suggested by Turnau et al. (1993) and Kaldorf et al. (1999).

In conclusion, the effects of AM inoculation on Pb accumulation differed considerably in maize and common bent plants. This shows that AM fungi have the potential to change the transfer of HM from soil to plants. Our data suggest that the combination of AM fungi and host plants is a determining factor for the result of their interaction under HM stress and consequently for their potential use in phytoremediation of contaminated soils. Future study should thus involve not only comparisons of AM isolates adapted or non-adapted to contamination but also heavy metal tolerant and non-tolerant clones of the same plant species. It is also not clear whether HM tolerance of AM fungi from contaminated soils is a stable characteristic or if it can be lost by subculturing in metal-free substrate; therefore comparisons of the same AM isolate cultured in original soil versus metal-free substrate should be undertaken.

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