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EFFECT OF *GLOMUS INTRARADICES* ISOLATED FROM PB-CONTAMINATED SOIL ON PB UPTAKE BY *AGROSTIS CAPILLARIS* IS CHANGED BY ITS CULTIVATION IN A METAL-FREE SUBSTRATE

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Abstract: Development and heavy metal tolerance of two cultivation lineages of the indigenous isolate of arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* PH5 were compared in a pot experiment in soil from lead (Pb) smelter waste deposits. One lineage was sub-cultured in original Pb-contaminated soil; the second one was maintained for 13 months in an inert substrate (river sand) without Pb stress. The contribution of these cultivation lineages to the Pb uptake and accumulation by the host plant *Agrostis capillaris* was investigated. The experiment was conducted in a compartmented system where the lateral compartments with *Agrostis* seedlings were separated from the central pot containing 4-week older *Agrostis* plants by a nylon mesh for allowing out-growing of extraradical mycelium (ERM) from the pot.

No differences in mycorrhizal colonization, ERM length and viability were observed between the two lineages of *G. intraradices* PH5 in the soil of the isolate origin. However, the ability to support plant growth and Pb uptake differed between the lineages and also between the plants in the central pots and the lateral compartments. The growth of the plants in the central pots was positively affected by AMF inoculation. The plants inoculated with the lineage maintained in original soil showed larger shoot biomass and higher shoot P content as compared to the other inoculation treatments. The shoot Pb concentration of these plants was lower when compared to the plants inoculated with the lineage sub-cultured in the inert substrate. However the concentration did not differ from non-mycorrhizal control or from the reference isolate *G. intraradices* BEG75 from non-contaminated soil. Also shoot Pb contents were similar for all inoculation treatments. The development of *G. intraradices* BEG75 in the contaminated soil was very poor; this isolate was not able to initiate colonization of seedlings in lateral compartments. In lateral compartments, growth of seedlings in contaminated soil was inhibited by the *G. intraradices* PH5 lineage maintained in the inert substrate. Pb translocation from the seedling roots to shoots was increased for plants inoculated with either lineage as compared to the non-mycorrhizal control; however, the increase for the lineage cultivated in the inert substrate was significantly higher in comparison with that maintained in the original soil. After 13 months of cultivation in a metal free substrate, the *G. intraradices* isolate from Pb contaminated soil did not lose its tolerance to Pb as regards colonization of plant roots and growth of ERM in the soil of its origin. However, its ability to support plant growth and to prevent Pb translocation from the roots to the shoots was decreased.

Keywords: Arbuscular mycorrhizal fungi, Heavy metal tolerance, Lead contamination, Mycorrhiza

Nomenclature: WALKER & TRAPPE (1993)

INTRODUCTION

It has been widely accepted that AMF (arbuscular mycorrhizal fungi) can improve plant fitness under adverse conditions in contaminated soils. Although AMF have been shown to

improve plant tolerance to heavy metal (HM) stress in polluted soils, the role of arbuscular mycorrhiza in the uptake of HM from contaminated soils is not clearly elucidated (LEYVAL et al. 1997). Both an increase (KILHAM & FIRESTONE 1983, WEISSENHORN & LEYVAL 1995) and a decrease (SCHÜEPP et al. 1987, EL-KHERBAWY et al. 1989, HEGGO et al. 1990, WEISSENHORN et al. 1995) of HM content were observed in tissues of mycorrhizal plants as compared to non-mycorrhizal controls. Also decreased translocation of HM from roots to shoots has been described for mycorrhizal plants (LOTH & HÖFNER 1995, TONIN et al. 2001). Among the factors affecting the uptake of HM by mycorrhizal plants, the available concentration of a particular metal (SCHÜEPP et al. 1987, EL-KHERBAWY et al. 1989, HASELWANDTER et al. 1994) and the plant-fungus combination (DÍAZ et al. 1996, SHETTY et al. 1995) are considered as the most important. In a review of literature concerning mycorrhizal symbiosis in metal-contaminated soils, MEHARG & CAIRNEY (2000) concluded that AM associations on highly contaminated sites could be either detrimental, beneficial or neutral with respect to metal assimilation, and local conditions within contaminated soil could regulate the cost-benefit relationship.

Some HM-tolerant AMF have been isolated from polluted soils (GILDON & TINKER 1981, WEISSENHORN et al. 1993, GRIFFIOEN 1994, DÍAZ et al. 1996, HILDEBRANDT et al. 1999). These isolates from contaminated soils might be adapted and more tolerant to elevated levels of particular metals and could differ from non-indigenous reference isolates in their influence on the host plant (DEL VAL et al. 1999). However, the mechanisms involved in metal tolerance of AMF and its stability are still poorly understood. WEISSENHORN et al. (1994) obtained a tolerant isolate from a soil contaminated by Cd nitrate already one year after the contaminant application. With respect to the relatively long generation time of AMF and the large number of nuclei within one spore, they concluded that this fast development of tolerance was based on phenotypic plasticity rather than on the selection of a tolerant genotype. The authors considered the stability of metal tolerance time-limited. During one reproduction cycle (6 months) in non-contaminated substrate, Cd- tolerance of the isolate was lost. On the contrary, LEYVAL et al. (1997) noted that heavy metal tolerance of AMF isolates from contaminated soils can be preserved even during temporary sub-culturing in a non-contaminated substrate.

In the present study a pot experiment was conducted in soil from waste deposits of the lead smelter in order to compare the development and metal tolerance of two cultivation lineages of the indigenous AMF isolate *Glomus intraradices* PH5. One lineage was sub-cultured in original soil, the second one for 13 months in river sand without metal stress. *G. intraradices* BEG75 was used as a reference isolate from non-contaminated soil. The effect of these 3 isolates on Pb uptake and the accumulation by the HM-tolerant host plant *Agrostis capillaris* was investigated.

MATERIALS AND METHODS

The AMF isolate *Glomus intraradices* PH5 originated in a Pb-contaminated waste disposal site of a Pb smelter (Kovohutě Příbram, Czech Republic). Multi-spore cultures of this isolate were maintained either in the original Pb-contaminated soil sterilized by autoclaving (the lineage referred to as *G. intraradices* PH5-OS), or in autoclaved river sand without metal

Table 1. Chemical characteristics of the substrates. Available concentrations are given for macroelements and heavy metals. [1] – extracted by sodium hydrogen--carbonate; [2] – extracted by ammonium acetate at pH 7.0; [3] – extracted by ammonium acetate at pH 4.8.

	Soil	Sand
pH (H_2O)	5.4	6.7
pH (KCl)	4.9	6.0
C (%)	13.2	0.2
N (%)	0.7	0.1
C/N	18.4	2.7
^{1}P (mg kg^{-1})	9.2	0.2
^{2}Mg (mg kg^{-1})	342	28
^{2}Ca (mg kg^{-1})	3090	328
^{2}K (mg kg^{-1})	345	29
^{2}Na (mg kg^{-1})	103	67
SO_4^{2-} (mg kg^{-1})	152	56
^{2}Pb (mg kg^{-1})	2333	0.0
^{3}Fe (mg kg^{-1})	1.7	0.0
^{3}Mn (mg kg^{-1})	10.7	10.0
^{2}Zn (mg kg^{-1})	15.8	0.0
^{2}Cu (mg kg^{-1})	15.8	0.7
^{2}Cd (mg kg^{-1})	15.4	0.0

stress (for 13 months, the lineage referred to as *G. intraradices* PH5-IS). The chemical characteristics of both substrates are given in Table 1. The generation time of *Glomus intraradices* PH5 cultivated in sand is 3 months. The reference isolate of the same species, *Glomus intraradices* BEG75, originates in a non-polluted agricultural area (pH_{H_2O} 8.2) in Switzerland. All AMF were cultivated on maize under the same growth conditions and were fertilized weekly by Vitafeed 102 (Vitax Ltd., UK).

The soil of *G. intraradices* PH5 origin and river sand (the same that was used for AMF sub-culturing) were sieved through a 4-mm sieve and sterilized in an autoclave. Then the soil was put into 1l pots with two 100-ml lateral compartments separated from the central pot by nylon mesh (opening size 42 μm) allowing for the growth of extraradical mycelium (ERM) but not roots into the lateral compartments. One lateral compartment was filled with the soil and the second compartment was filled with sand. This experimental arrangement was used in order to find if ERM can transport Pb from the contaminated soil to the plants growing in sand without HM and to follow AM development after outgrowing of the fungi from the central pot with contaminated soil to a less contaminated environment. Soil in the central pots was either non-inoculated or it was inoculated with one of the following AMF isolates: *G. intraradices* PH5-OS, *G. intraradices* PH5-IS and *G. intraradices* BEG75. Every pot received 5 ml of inoculum suspension, which consisted of the mixture of colonized root fragments, the ERM and spores. There were 6 replicates in each treatment. Microbial populations from original non-sterile soil were reintroduced to the central pots by adding filtrate (Whatman nr. 1 filter) from 100 g of soil shaken for 30 min in 1 litre of deionized water. Seeds of the host plant *Agrostis capillaris* L. were collected from the population growing in the close vicinity of the smelter at the site strongly influenced by the air deposition of Pb. Seeds were surface sterilized in 10% NaOCl for 10 min and sown in each pot. Pots were placed in a greenhouse. Two weeks after seedling emergence, the plants were thinned to 25 per pot. Four weeks later, two plantlets from the seeds pre-germinated in sterilized sand were planted to each lateral compartment. The experiment was harvested after 20 weeks of seedling growth in the lateral compartments.

At harvest, shoot and root dry weights of the plants from the lateral compartments and shoot dry weights of plants from central pots were evaluated after drying at 80 °C. Samples of shoots and roots of plants from the central pots and lateral compartments were ground, digested in HNO_3 and H_2O_2 and analyzed for Pb concentrations by atomic absorption

spectrometry (AAS Spectrometer Unicam 9200X, MOORE & CHAPMAN 1986). In addition, P concentrations in the shoots of plants from the central pots were determined spectrophotometrically (Unicam UV4-100, OLSEN 1982).

Root samples from central pots and lateral compartments were stained with Trypan blue in lactoglycerol (modified from KORMANIK & MCGRAW 1982) and percentage of mycorrhizal colonization was evaluated using the gridline intersect method (GIOVANNETTI & MOSSE 1980) under a stereomicroscope at 40× magnification. For evaluation of ERM length and NADH-diaphorase (NADH-d) activity, 15-ml soil cores were sampled from the central pots or lateral compartments and homogenized by mixing. A sub-sample was put into a household blender with 500 ml of H_2O and blended for 30 s. One ml of supernatant was pipetted onto Whatman membrane filter (24 mm diameter, 0.45 μm pore size) and vacuum filtered. The ERM on the filter was stained with 0.1% solution of Trypan blue in lactoglycerol. The whole filter was scanned and the total length of ERM was evaluated under the microscope at 200× magnification according to BRUNDRETT et al. (1994). The length of the ERM was expressed in meters of total hyphae per 1 gram of air-dried substrate. The remaining supernatant from the blender was poured through a 0.036-mm sieve and clusters of ERM were transferred to an Eppendorf tube with 300 μl of the NADH-d staining solution (SYLVIA 1988). The Eppendorf tubes were incubated for 14 hours in the dark at 28 °C. The ERM length, which contained purple precipitate (NADH-d activity), was estimated on thirty microscope fields of view at 400× magnification.

Data were analyzed by SOLO 4.0/BMDP Statistical Software. All data were logarithmically transformed. Data showing normal distribution were analyzed by ANOVA followed by the Duncan multiple range test, whereas data with non-normal distribution were analyzed by non-parametric the Kruskall-Wallis test followed by the Kruskall-Wallis multiple comparison Z-values test.

RESULTS

Central pots

Inoculation with all AMF significantly increased the shoot biomass of plants in the central pots. *G. intraradices* PH5 cultivated in original soil (OS) caused higher growth stimulation of host plants as compared to the same isolate maintained in the inert substrate (IS) or compared to *G. intraradices* BEG75 (Table 2). The shoot P concentration of plants in the central pots was not affected by inoculation with *G. intraradices* PH5-OS, while inoculation with *G. intraradices* PH5-IS and *G. intraradices* BEG75 resulted in decreased shoot P concentrations compared to non-mycorrhizal plants (Table 2). However, when P concentrations were expressed as total P content per pot, plants inoculated with *G. intraradices* PH5-OS showed significantly higher values as compared to the remaining inoculation treatments. Significant differences in shoot Pb concentrations of host plants were found between the isolates. Only the inoculation with *G. intraradices* PH5-OS significantly decreased the shoot Pb concentration of plants in central pots in comparison with non-mycorrhizal control. However, total shoot Pb contents were not affected by any AMF isolate. Inoculation with AMF did not significantly influence Pb concentrations in roots of the host plants in the central pots (Table 2).

Table 2. Effect of inoculation with different isolates of *Glomus intraradices* (indigenous isolate maintained in original soil – PH5-OS, indigenous isolate maintained in inert substrate – PH5-IS and reference isolate BEG75) on growth, P and Pb uptake and mycorrhizal parameters of *Agrostis capillaris* growing in central pots. Values in columns marked by the same letter are not significantly different at the level $\alpha = 0.05$ according to Duncan multiple range test or Kruskal-Wallis tests.

	Non-inoculated	PH5-OS	PH5-IS	BEG75
Shoot dry weight (g pot^{-1})	2.02 c	3.01 a	2.29 b	2.44 b
Shoot P concentration (mg kg^{-1})	839 a	814 a	674 b	673 b
Shoot P content (mg pot $^{-1}$)	1.7 b	2.5 a	1.5 b	1.6 b
Shoot Pb concentration (mg kg^{-1})	43.8 a	21.5 b	41.7 a	26.8 ab
Shoot Pb content (µg pot $^{-1}$)	88.6 a	61.3 a	95.1 a	65.7 a
Root Pb concentration (mg kg^{-1})	1,484.7 a	1,063.3 a	1,145.3 a	996.0 a
colonization (%)	0	31 a	43 a	12 b
ERM length (m g^{-1} dry soil)	0	6.62 a	10.41 a	0.83 b

Table 3. Effect of inoculation with different lineages of *Glomus intraradices* PH5 (OS – lineage maintained in original soil, IS – lineage maintained in inert substrate) on growth and Pb uptake by *Agrostis capillaris* seedlings growing in lateral compartments. Values marked by the same letter are not significantly different at the level $\alpha = 0.05$ according to Duncan multiple range test or Kruskal-Wallis multiple comparison Z-values test.

	Soil		
	Non-inoculated	PH5-OS	PH5-IS
Shoot dry weight (g compartment $^{-1}$)	0.29 a	0.26 ab	0.20 b
Root dry weight (g compartment $^{-1}$)	0.39 a	0.35 ab	0.24 b
Shoot Pb concentration (mg kg^{-1})	14.8 c	32.2 b	149.3 a
Shoot Pb content (µg compartment^{-1})	4.4 b	8.0 b	30.0 a
Root Pb concentration (mg kg^{-1})	359.3 b	605.8 a	658.8 a
Root Pb content (µg compartment^{-1})	140.0 a	183.3 a	145.4 a
	Sand		
	Non-inoculated	PH5-OS	PH5-IS
Shoot dry weight (g compartment $^{-1}$)	0.04 a	0.02 b	0.03 ab
Root dry weight (g compartment $^{-1}$)	0.11 a	0.04 c	0.07 b
Shoot Pb concentration (mg kg^{-1})	8.7 a	9.4 a	5.8 a
Shoot Pb content (µg compartment^{-1})	0.3 a	0.2 a	0.2 a
Root Pb concentration (mg kg^{-1})	21.7 a	19.0 a	21.7 a
Root Pb content (µg compartment^{-1})	2.4 a	0.6 b	1.5 a

No significant differences in mycorrhiza development in central pots were found between the different lineages of *G. intraradices* PH5. Mycorrhizal colonization and ERM length were significantly higher for this isolate as compared to the reference *G. intraradices* BEG75 (Table 2). Development of the ERM of *G. intraradices* BEG75 was poor and its out-growing from the central pot to the lateral compartments was very limited. The hyphae showed almost no NADH-d activity (data not shown) and colonization of the seedlings in the lateral compartments was not initiated. For this reason data from the lateral compartments with *G. intraradices* BEG75 were not included.

Table 4. Effect of inoculation with different lineages of *Glomus intraradices* PH5 (OS – lineage maintained in original soil, IS – lineage maintained in inert substrate) on mycorrhizal colonization of *Agrostis capillaris* seedlings growing in lateral compartments, the ERM length and NADH-diaphorase activity of ERM. Values marked by the same letter are not significantly different at the level $\alpha = 0.05$ according to Duncan multiple range test or Kruskal-Wallis multiple comparison Z-values test.

	Colonization (%)		ERM length (m g^{-1} dry soil)		NADH-diaphorase activity (%)	
	Soil	Sand	Soil	Sand	Soil	Sand
PH5-OS	17 a	66 a	9.03 a	1.01 b	35 a	79 a
PH5-IS	24 a	35 b	8.30 a	9.53 a	32 a	82 a

Lateral compartments

Unlike in the central pots, inoculation with *G. intraradices* PH5 did not support growth of seedlings in the lateral compartments (Table 3). Plant growth was even decreased in the case of *G. intraradices* PH5-IS in the soil compartments and in the case of both *G. intraradices* PH5 lineages in the sand as compared to the non-mycorrhizal control.

Inoculation with both *G. intraradices* PH5 lineages significantly increased the shoot Pb concentration of seedlings in the soil lateral compartments, however, plants inoculated with the lineage maintained in inert substrate showed a much higher Pb concentration as compared to those inoculated with the lineage maintained in original soil (Table 3). When Pb concentrations were expressed as total Pb content per compartment, differences between inoculated and non-inoculated plants were also more pronounced for *G. intraradices* PH5-IS. Root Pb concentration in seedlings from soil compartments was increased when inoculated with both *G. intraradices* PH5 lineages, although total root Pb content did not differ from the non-mycorrhizal controls. ERM disturbance did not affect Pb uptake by *A. capillaris* seedlings growing in the soil compartments.

In the lateral compartments with sand, inoculation with either *G. intraradices* PH5 lineage did not influence shoot Pb concentration and content in the seedlings (Table 3). No differences in the root Pb concentrations were found between inoculated and non-inoculated treatments. Total Pb content was decreased in the roots of seedlings inoculated with *G. intraradices* PH5-OS.

There were no significant differences in mycorrhizal colonization, ERM length and NADH-d activity of the ERM between both *G. intraradices* PH5 lineages in lateral compartments with soil (Table 4). In compartments with sand, seedlings inoculated with *G. intraradices* PH5-OS showed higher mycorrhizal colonization, while *G. intraradices* PH5-IS developed a significantly higher amount of ERM hyphae. Activity of NADH-d in the ERM in sand did not differ between the lineages.

DISCUSSION

Thirteen months of sub-culturing in inert substrate did not change the behaviour of the indigenous AMF isolate *G. intraradices* PH5 isolated from waste deposits of the Pb smelter as regards to mycorrhiza development in the soil of its origin. No differences between the lineages cultivated either in the original soil or in the inert substrate were found in their ability to colonize the host plant or in the ERM length and viability. At the same time, all mycorrhizal

parameters were significantly lower for the reference non-indigenous isolate *G. intraradices* BEG75. Decreased development of non-indigenous isolates in metal-contaminated substrates was documented already in the first work on this field (GILDON & TINKER 1981). Lower ability of non-tolerant isolates to colonize plant roots and decreased germination of their spores were observed, e.g. by WEISSENHORN et al. (1993, 1994, 1995), WEISSENHORN & LEYVAL (1995). MALCOVÁ et al. (2003a) found that *G. intraradices* PH5 from Pb-contaminated soil showed a higher Pb-tolerance than did the reference isolate *G. intraradices* BEG75 when cultivated in Pb solutions *in vitro*. However, a higher Pb-tolerance of the isolate from polluted soil was not confirmed in a sand-based hydroponics experiment with simulated Pb stress, because root colonization and ERM length did not differ between isolates when exposed to Pb. It is possible that the available Pb concentration in the substrate, which was not determined, was too low to reveal difference in the Pb-tolerance of both isolates. Similarly, in another study MALCOVÁ et al. (2001) did not find any differences in mycorrhizal colonization and ERM length and activity between indigenous and non-indigenous isolate of *G. claroideum* growing with *Calamagrostis epigejos* in original soil containing HM. However, these authors found a negative effect of cultivation in original soil on mycorrhizal parameters of both indigenous and non-indigenous isolate as compared to the sand. This is in agreement with our results, as after outgrowing of both *G. intraradices* PH5 lineages from central pots to less contaminated environment in compartments with sand, higher colonization and ERM viability was found as compared to compartments with soil. However, increased colonization in sand could be at least partly attributed to decreased growth of plants due to the low nutrition status of sand and thus larger proportion of the root system colonized by AMF. MEHARG & CAIRNEY (2000) concluded that a decreased development of AMF with increasing metal stress in the growth matrix is a general phenomenon for both sensitive and resistant strains.

Our results concerning persistent metal tolerance of the *G. intraradices* PH5 lineage cultured in inert substrate are in agreement with LEYVAL et al. (1997) who mentioned that a metal tolerant strain grown without metal stress maintained its metal tolerance. On the contrary, WEISSENHORN et al. (1994) reported that germinating spores from a Cd-tolerant AMF culture lost their metal tolerance already after one reproduction cycle (6 months) in a metal-free substrate. Similarly, MALCOVÁ et al. (2003b) found that the lineage of *Glomus* "cluster-forming" sp. BEG140 from Mn-contaminated soil that was maintained for 27 months in an inert substrate tolerated excessive Mn levels to a lesser extent than the lineage kept in original soil, but it still sustained higher Mn concentration than the reference isolate *G. intraradices* BEG75. This is also in agreement with the findings of ENKHTUYA et al. (2000) who suggested that the ability of AMF isolates from degraded soils to maintain their adaptation and stress tolerance could change during the sub-culturing process depending on the cultivation substrate used.

Although in our study there were no differences in behaviour of the two lineages as to the mycorrhizal parameters tested, there were significant differences in their effects on plant Pb uptake. In the older plants in the central pots, shoot Pb concentration was significantly decreased in plants inoculated with *G. intraradices* PH5-OS, while plants inoculated with *G. intraradices* PH5-IS showed similar values as the non-mycorrhizal controls. This finding

can be ascribed to larger shoot biomass in plants associated with *G. intraradices* PH5-OS as total Pb content was similar in all inoculation treatments (although there was a trend to decreased Pb content in plants inoculated with the lineage maintained in original soil). Dilution of HM in plant tissues due to increased biomass of mycorrhizal plants was reported also by KUCEY & JANZEN (1987) or WEISSENHORN et al. (1995). A different situation was observed for seedlings growing in lateral compartments with soil, where higher shoot and root Pb concentrations were found in plants associated with both *G. intraradices* PH5 lineages when compared to the non-inoculated controls. Also KILLHAM & FIRESTONE (1983) found higher shoot concentrations of Pb and some other metals in mycorrhizal plants than in non-mycorrhizal controls after HM application in simulated acid rain. SHETTY et al. (1995) found that both AMF from a Zn contaminated site and non-contaminated site enhanced Zn uptake by *Andropogon gerardii* and *Festuca arundinacea* compared to the non-mycorrhizal treatment. High Pb concentration in the shoots of plants associated with *G. intraradices* PH5-IS cannot be fully ascribed to the metal accumulation in plants with lower biomass. Similar results were found e.g. by TONIN et al. (2001) for Cd in clover. As similar Pb contents in the roots were found irrespective of the inoculation treatment and the shoot Pb contents were much higher in seedlings inoculated with the *G. intraradices* PH5-IS, the results suggest increased translocation of Pb from the roots to the shoots in plants inoculated with this lineage. On the contrary, for the lineage cultivated in original soil shoot Pb content remained similar to the non-inoculated plants. Similar results were obtained by JONER & LEYVAL (2001) when they compared the effect of indigenous AMF populations (Ind) and one indigenous AMF strain (*G. mosseae* P2 kept in original soil) on the uptake of various HM by maize and clover. They found lower metal concentrations and higher root/shoot concentration ratios in Ind compared to the P2 treatment along with similar colonization levels. They concluded that the former AMF were more efficient in controlling uptake and translocation of metals than the latter. The role of AMF originating either in Pb-contaminated (*G. intraradices* PH5 maintained in original soil) or non-contaminated (*G. intraradices* BEG75) soil in Pb uptake by *Agrostis capillaris* was studied by MALCOVÁ et al. (2003a) in a sand-based hydroponics experiment with simulated Pb stress. They found that Pb concentration in the shoots was not influenced by inoculation regardless of the Pb amount added, while higher root concentration was found for the plants inoculated with *G. intraradices* PH5 at lower applied Pb level. Also TONIN et al. (2001) observed that colonization with AMF from the rhizosphere of metal-tolerant *Viola calaminaria* increased Cd and Zn concentrations in the clover roots without significantly affecting the concentration of metals in the shoots. A higher uptake of HM to roots by mycorrhizal plants, but reduced translocation to the aerial part of the plant was reported also by LOTH & HÖFNER (1995) for Zn and Cu and by DEHN & SCHÜEPP (1989) for Zn and Cd.

In the sand compartments, Pb concentration in the substrate was slightly increased at the end of the experiment (data not shown; average Pb concentration 0.4 mg kg^{-1}) compared to the zero values at the beginning of the experiment probably due to the mass flow and/or passive diffusion of Pb from the central pots. Under these conditions of low Pb availability decreased root Pb content was found in the seedlings inoculated with *G. intraradices* PH5-OS compared to the non-mycorrhizal plants. However, unchanged shoot Pb concentrations and

contents were observed, which does not suggest that ERM plays an important role in Pb transport from the contaminated soil to plants growing in sand.

Different effects of various isolates or cultivation lineages of *G. intraradices* on plant growth and Pb concentrations in plant biomass in central pots and lateral compartments cannot be probably attributed to different root densities or different plant age as proposed by JONER & LEYVAL (2001). These authors observed that HM concentration in plant biomass varied with plant age (3–9 weeks) and with the size of the pots in which host plants were grown. This is not probably the case of our experiment where soil volume per one plant and biomass expressed per "individual plant" in pots (25 plants per pot) and soil lateral compartments (2 plants per compartment) were similar. The four week age difference between grasses in pots and lateral compartments probably might not cause such large differences in Pb uptake by plants 5–6 months old. However, grasses in central pots formed a dense turf during the experiment as compared to the 2 seedlings planted in the lateral compartments.

Inoculation with indigenous or non-indigenous AMF in our experiment did not decrease Pb uptake by the host in comparison with non-mycorrhizal plants grown in contaminated soil. MEHARG & CAIRNEY (2000) concluded that resistant AMF can maintain plant P status at high metal levels benefiting the plant in comparison with sensitive AMF strains and non-colonized controls. According to the authors this seems principally to be a function of the resistant strains maintaining AM colonization at high levels of metal exposure. Our results suggest that this role of tolerant AMF isolates could be negatively affected by their long-term sub-culturing in an environment without metal stress.

It can be concluded that 13 months of sub-culturing in an inert substrate did not affect development of *G. intraradices* PH5 isolated from the waste deposits of a Pb smelter in contaminated soil of its origin. However, the interaction of the fungus with the host plant was changed: the ability of the lineage cultured without HM to support plant growth in Pb-contaminated soil was decreased, while translocation of Pb from plant roots to shoots increased.

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