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Effect of inoculation with mycorrhizal fungi on lead uptake by corn((*Zea mays*) in a calcareous soil

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Abstract

Pot experiment was conducted to study the influence of two arbuscular mycorrhizal fungi (AMF) species as a bioremediation agent for soil contaminated with lead. maize (*Zea mays*) was grown in a calcareous soil and supplemented with four pb addition levels of 0,01,0.05,0.1, and 0.5 mM soil in the form of $Pb(NO_3)_2$. two AM fungal inocula namely, *Glomus macrocarpium*, and *Glomus fasciculatum*, the first one was isolated from contaminated soil and were applied to the soil. The plants were harvested after 60 days of growth. Mycorrhizal colonization rate, plant dry weight (DW), pb concentrations and pb uptake were determined and uptake efficiency, translocation efficiency and phytoextraction efficiency were calculated. *G. macrocarpium*-treated plants had higher mycorrhizal colonization rates than other inoculation-treated plants. Two mycorrhizal species increased shoot and root DW, and *G. macrocarpium* was more effective than the other. Mycorrhizal plants accumulated more pb in roots but large reductions in shoots. The uses of AM fungal for phytoremediation of the contaminated soil lead to more absorption of pb in plant. The comparisons of two AM fungal species indicate that the AM fungal represented by *G. macrocarpium* can benefit against potentially toxic pb .

Key words: Fungi, Lead uptake, Corn

Introduction

Soil pollution by heavy metals disseminated from human activities is a major world concern because metals are very toxic and remain persistent in soils. Metals are not biodegradable and tend to accumulate in soils and organisms where anthropogenic impact is intense (Redon et al; 2009). AM fungi isolated from contaminated sites have been reported to possess higher HM tolerance in comparison with those collected from non-contaminated substrates (Weissenhorn and Leyval, 1995).

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 (Meharg and Cairney, 2000).

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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. The most important variables which control metal availability are: pH, redox

potential, texture, organic matter, mineral composition, temperature and water regime (Kabata-Pendias 2004). Besides these abiotic factors, the biological components of soils (plant, microorganisms) play also a major role on metal fate. In particular, soil microorganisms affect root morphology, plant physiology and metal fractionation (Tao et al. 2005). AM fungi increase nutrient acquisition by exploring a vast soil volume (Harley and Smith 1983) and can be beneficial to host plant growing in unfavorable soil conditions as in nutrient-deficient soils or in polluted areas. In heavy-metal contaminated soils, AM fungi can also improve the growth of host plant by reducing metal stress (Rivera-Becerril et al. 2002).Dıaz 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.

In the present study, we hypothesized that AM colonization increases the uptake and tolerance to pb in *Zea mays* plant. To test this hypothesis, we study the ability of two AM species (isolated either from Pb-contaminated or non-contaminated soil) to colonize *Zea mays* grown in a soil and uptake of pb by plants across a gradient of pb concentrations from uncontaminated to potentially toxic levels and to confirm whether AM fungi can be applied as an aid in amelioration toxicity produced by pb contamination under calcareous soil conditions.

Material and Methods

Soil

Surface calcareous soil sample (0-15 cm) was collected from El- Nubaria at km 59 Alexandria – Cairo desert road. The sample was air- dried, ground to pass into 2mm sieve and thoroughly mixed. It has the following general properties: pH,8.00; organic matter, 2.34 g kg^{-1}; total $CaCO_3$, 14.6%; EC ,1.7 dSm^{-1}; clay content,24.21%; silt content, 22.32%; sand content,53.47 % ; available P, 3.5mgkg^{-1} soil; , available lead 0.001 mg/kg-1; total nitrogen 0.09%.The methods used for soil analysis were those described by (Page et al., 1982). The soil was enriched with pb at the rate of 0,01,0.05,0.1, and 0.5 mM soil in the form of $Pb(NO_3)_2$. The different soil treatments were well mixed, and exposed to repeated drying rewetting cycles for two weeks, then stored in plastic pots for soil pb extraction and pot experiment.

Mycorrhizal inocula

Two arbuscular mycorrhizal fungi (AMF) species belonging to the genus *Glomus* were used in this study. The used species were *Glomus macrocarpium* and *Glomus fasciculatum*, the first one was isolated from contaminated soil and the second one was isolated from non-contaminated soil then were applied to the soil.

Pot experiment

Pot experiment was carried out at the green house of the Agriculture faculty (Saba bacha**),** Alexandria University. Plastic pots, 120 cm deep and 50cm in diameter with holes in their bottom, were filled with 6 kg of the enriched soil with pb leaving the upper 5cm without soil. Seeds of *Zea mays* were treated by 0.05% NaOCl solution and planted in each pot. About fifty grams of inoculum *Glomus macrocarpium* and *Glomus fasciculatum* (colonized root segments) were placed 2 cm below the seeds. four weeks after planting, plants were thinned to one plant per pot. The pb and AMF treatments were distributed in completely randomized design with three replicates. all pots were irrigated with tap water every three days to keep the soil at 70% of its field capacity by regular weighing of pots. At harvesting (60 days after planting), samples of plants were dried, ground and dry weight of plants (shoots and roots) were recorded and analyzed to determine pb concentrations and the uptake of pb . A 0.5 g of dried plant materials was digested in H_2SO_4-H_2O_2 mixture according to Lowther (1980). In the digested solution pb was determined by atomic absorption spectrophotometer(A Analyst 400). Also, samples of the soil were collected at harvest from each pot and analyzed to estimate available pb as described by Lindsay and Norvell(1978).The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observations. The gridlines intersect method of Giovannetti and Mosse (1980)used to estimate the mycorrhizae infection percentages using the equation:

Also, the mycorrhizal dependency (MD) of plant growth was calculated according to the following formula (Plenchette et al., 1983) Data were subjected to analysis of variance or regression analysis (Snedecor and Cochran, 1972). and L.S.D test at 0.05 level of probability was used to compare between means.

Results and Discussion

Soil extractable lead

The effect of pb application to the soil on DTPA-extractable pb after cropping without AMF inoculation and after cropping with the inoculation with the tested two AMF species are found in Table 1. The data summarized in Table 1, also indicates that after cropping with two AMF inoculation, concentrations of extractable soil pb tended to be higher with increasing pb addition level than those of cropping without AMF inoculation. It is clear also, Soil pb concentration in two mycorrhizal treatments were generally higher than corresponding values of the uninoculated treatments, but the species *Glomus*

Pb rate mM/kg	Available pb mg/kg soil			
	without inoculation	***G.macrocarpu***	***G.fasciculatum***	**Mean of pb rate**
0	0.11	0.21	0.31	**0.21e**
0.01	1.15	1.47	1.27	**1.30d**
0.05	1.33	2.23	1.28	**1.61c**
0.1	1.64	2.**84**	1.57	**2.02b**
0.5	2.02	3.18	2.24	**2.48a**
Mean AMF inoculation	**1.25 c**	**1.99 a**	**1.33b**	
$L.S.D_{0.05}$	0.05			
pb rates $L.S.D_{0.05}$	0.08			
Interaction AMF×pb	0.14			

Table 1 : Available pb in soil after cropping of *Zea mays* as affected by two arbuscular mycorrhizal fungi (AMF) .

macrocarpium was more effective mostly than the second one in increasing the DTPA-pb. In the same time, there were significant differences between the mean values of DTPA-pb over all pb application rates for the soils after cropping with two mycorrhizal inoculation species. also our results showed that lead immobilized in the soil by forming low soluble, stable compounds and have a greater adsorption in the exchange complex. These result are agreement with Moral et al. (2004) who found that the elevated sorption of metals in carbonated, alkaline soils is regulated by three different mechanisms: surface adsorption of colloids, complexation with surface functional groups, and the precipitation with hydroxides, phosphates and carbonates.

The process is mainly regulated by strong covalent bonds (McBride, 1994). As well as the possibility to form salts (Bradl, 2004). This type of low soluble, stable salt has been proposed in the bibliography as retaining pb in these media (phosphates and carbonates) and suggests that the carbonated minerals fraction (calcite, dolomite) in the soil have been largely responsible for their retention.

Root colonization rate

Table 2 showed that High levels of colonization rates (52-36% of root length) by two mycorrhizae inoculation were observed at zero application rate of pb to soil. Also, the results showed notably, that *Glomus fasciculatum* was very sensitive to the presence of height metal of lead added in soil, and its propagules practically very low or even zero colonization and disappeared at 0.5 mM/kg pb added to soil, while under low or moderate pb application levels the *Glomus macrocarpium* showed more extensive mycorrhizal colonization in the roots of *Zea mays* plant and the infection decreased significantly with increasing pb rates up to 0.1 mM/kg soil.

This is in line with (Audet P, Charest C (2007)) who found that high levels of heavy metal concentration in soil decrease the AM fungi colonization of Sunflower roots. Metal toxicity can affect AM fungi at different life stages, from spore germination to hyphal elongation and root colonisation;tolerance can thus occur at different levels and is difficult to define (Redon et al 2009) Therefore, based on the present results, G. *macrocarpium* fungi colonizing *Zea mays* roots and more resistance to metals such as pb . These findings are agreement with (Weissenhorn et al. 1995) who found that AM fungi collected from contaminated soils seemed to have developed a strategy adapted to metal stress and more tolerance to heavy metals .Statistically there were significant difference between two inoculation .

Plant growth

The effects of AM fungi and soil pb treatments and their interaction were significant on dry mass which was higher in AMF than non-AMF plants at all pb rates added to soil . The results in Table 2 revealed that shoot and root dry weights of *Zea mays* plant significantly decreased with increasing pb application rate. Compared to the non-*G.macrocarpum* but the values significantly decreased with *G.fasciculatum* . Opposite trend was noticed for the dry weights of pb-treated *Zea mays* roots which did not differ significantly as affected by *G.fasciculatum* and *G.macrocarpum*, The inhibition of plant growth of mycorrhizal plants with 0.5 mM/kg pb added was more pronounced with inoculation by *G.fasciculatum* .The results of the

AMF. type	Pb rate mM/kg soil	AMF infection%	*Zea mays* plant growth (g/plant).			MD%
			Roots Dry weigth	Shoots Dry weigth	Whole plant	
Non-AMF	0	4.10	2.10	5.27	7.37	----------
	0.01	0	0.89	1.21	2.1	----------
	0.05	0	0	0	0	----------
	0.1	0	0	0	0	----------
	0.5	0	0	0	0	----------
Mean		**0.82 c**	**0.60 c**	**1.30** c	**1.90 c**	----------
G.macrocarpum	0	52.12	2.68	5.23	7.91	7
	0.01	29.21	2.33	4.27	6.60	68
	0.05	19.11	1.52	3.12	4.64	100
	0.1	12.13	0.90	1.29	2.19	100
	0.5	10.02	1.02	1.13	2.15	100
Mean		**24.52 a**	**1.69 a**	**3.01 a**	**4.70 a**	----------
G.fasciculatum	0	36.20	2.16	4.20	6.36	0
	0.01	12.43	1.24	3.21	4.45	53
	0.05	10.40	1.25	1.78	3.03	100
	0.1	6.61	0.76	1.43	2.19	100
	0.5	0	0	0	0	100
Mean		**13.13 b**	**1.10 b**	**2.12 b**	**3.21 b**	----------
L.S. D $_{0.05}$		1.08	0.19	0.27	0.21	
Mean effect of pb rate	0	30.81 a	2.31 a	4.90 a	7.21 a	
	0.01	13.88 b	1.49 b	2.90 b	4.38 b	
	0.05	9.84 c	0. 92 c	1.63 c	2.56 c	
	0.1	6.25 d	0.55 d	0.91 d	1.46 d	
	0.5	3.34 e	0.34 e	0.38 e	072 e	
L.S.D $_{0.05}$		1.08	0.19	0.27	0.21	
Interaction (AMFxpb rate) L.S.D $_{0.05}$		*	*	*	*	

Table 2: The effect of pb rates and inoculation with two mycorrhizal fungi species on mycorrhizal infection and *Zea mays* growth

mycorrhizal treatment, two mycorrhizal inoculation species increased significantly both of shoot and root dry weights at each rate of applied pb.

Shoot dry weights (average values over pb rates) did not differ significantly as affected by current experiments show that mycorrhizal colonization improves the ability of *Zea mays* plant to resist pb toxicity under the higher pb rates added to soil, mycorrhizal colonization decreased pb concentrations in the shoots than in roots, with a concomitant increase in root and shoot biomass. This may aid in plant tolerance as most of the pb absorbed could be sequestered in roots thereby minimizing the

disruption of biochemical processes in shoots. Our results agree with others which showed that AM fungi participate to soil remediation by sequestering toxic metals in fungal tissues, then decreasing their bioavailability (Audet and Charest 2007).

Table 3: pb content of *Zea mays* plants as affected by arbuscular mycorrhizal fungi (AMF) species and pb application rate.

* significant at 0.05 probability level.

AMF. type	Pb rate mM/kg soil	Pb content mg/kg	
		Roots	Shoots
Non-AMF	0	0.10	0.03
	0.01	12.04	0.06
	0.05	0	0
	0.1	0	0
	0.5	0	0
Mean		2.43 c	0.02c
G.macrocarpum	0	0.14	0.06
	0.01	14.81	1.20
	0.05	20.27	2.05
	0.1	24.33	2.60
	0.5	32.00	2.74
Mean		18.31 a	1.73 a
G.fasciculatum	0	0.11	0.05
	0.01	10.00	1.19
	0.05	12.00	1.54
	0.1	17.00	2.17
	0.5	0	0
Mean		7.82 b	0.99 b
L.S.D $_{0.05}$		1.40	0.47
Mean effect of pb rate	0	0.12 e	0.05d
	0.01	12.28 d	0.82 c
	0.05	10.76 c	1.20 b
	0.1	13.78 a	1.60 a
	0.5	10.67 b	0.91 c
L.S.D $_{0.05}$		0.99	0.16
Interaction (AMF x pb rate)		*	*
L.S.D $_{0.05}$		2.7	0.68

As mentioned previously, the obtained results indicated that the two tested species had significant effects in enhancing plant growth (Table 2). Shoot and root dry weights increased by 132 % and 182 % in *G.macrocarpum* treatment,63 % and 83 % in *G.fasciculatum* relative to non-inoculated plants, respectively.

Lead content and uptake by *Zea mays* Plants

Table 3 showed, compared with non-inoculated plants that, mycorrhizae inoculation species increased significantly lead concentration in *Zea mays* shoots and roots. Also significant differences were observed in roots pb concentration as affected by two inoculation treatments. Lead concentrations reaching 32 mg/kg were observed in roots of *Zea mays*, but reached to 2.74 mg/kg in shoot. However concentrations of pb was much higher in the roots than in the shoots, the idea that mycorrhizae may act by reducing metal uptake/translocation into the host plant (Marques et al., 2006). A possible reason for such a reduction may result on the fact that AMF plant yielded higher biomass, which contributed to dilute metals in the shoot tissue (Lambert et al. 1979; Eivazi and Weir 1989) or that the AM mycelium retained the absorbed metals (Kaldorf et al. 1999; Chen et al. 2003). The strong metal binding capacity of arbuscular mycorrhiza may have played an important role in alleviation of pb phytotoxicity. Furthermore, It is likely that the AM association contributed to an enhanced mineral acquisition in order to alleviate pb toxicity. (Ma, Y., 2003)concluded that the high root pb content in mycorrhizal birch seedlings, correlated with increased P content, suggests a detoxifying mechanism through polyphosphate-pb binding. This may be the case in our study since P concentrations were higher in AMF than non-AMF plants (data not shown). The results in Table 3 indicated that data elucidate the ability of root tissues to accumulate heavy metals at higher application of lead. These results are in line with (Weissenhorn et al., 1995) who found that AM fungal inoculation decreases lead concentrations in plants especially in shoots.

The data in Table (4) also revealed that pb uptake in *Zea mays*, shoots and roots significantly increased with more pb added for both non-mycorrhizal and mycorrhizal treatments.

Mycorrhizal colonization had clearly facilitated pb transport from the roots to the aerial parts, while for non-mycorrhizal plants, pb uptake values generally were low with maximum values at 0.01 mM/kg pb addition level in soil with and became zero at 0.05, 0.1 and 0.5 mM/kg because of the plant growth inhibition (dying the plants).

AMF type	pb rate (mM/kg soil)	lead uptake µg /plant		Total pb uptake µg/plant
		Roots	Shoots	
Non-AMF	0	0.21	0.16	0.37
	0.01	10.72	0.07	10.79
	0.05	0	0	0
	0.1	0	0	0
	0.5	0	0	0
Mean		**2.19 e**	**0.05 d**	**2.23 e**
G.macrocarpum	0	0.38	0.31	0.70
	0.01	34.50	5.12	39.62
	0.05	30.81	6.40	37.21
	0.1	21.90	3.35	25.25
	0.5	32.64	3.10	35.74
Mean		**24.05 a**	**3.66 a**	**27.70 a**
G.fasciculatum	0	0.24	0.21	0.45
	0.01	12.40	3.82	16.22
	0.05	15	2.74	17.74
	0.1	12.92	3.10	16.02
	0.5	0	0	0
Mean		**8.11 d**	**1.97 c**	**10.09 d**
L.S.D $_{0.05}$		0.94	0.52	0.50
Mean effect of pb rate	0	**0.27 d**	**0.23 e**	**0.51 e**
	0.01	**19.21 a**	**3.00 a**	**22.21 a**
	0.05	**15.27 a**	**3.05 b**	**18.32 b**
	0.1	**11.61 b**	**2.15 c**	**13.76 c**
	0.5	**10.88 c**	**1.03 d**	**11.91 d**
L.S.D $_{0.05}$		0.94	0.52	0.50
Interaction (AMF x pb rate) L.S.D $_{0.05}$		*	*	*
		2.10	1.17	1.11

Table 4: **Means of lead uptake as affected by arbuscular mycorrhizal fungi (AMF) species and pb application rate**
* significant at 0.05 probability level.

In the present study, when pb was added shoot pb uptake was increased up to 0.01 mM/kg soil and root pb accumulation increased up to 0.01 mM/kg pb soil rate. When pb was added mycorrhizal colonization resulted in higher pb uptake by the roots but had no marked effect on shoot pb uptake. Therefore, enhanced binding of pb by mycorrhiza may have played an important role in metal tolerance of the host plants under pb contamination. These results are in line with (Weissenhorn et al., 1995) as mentioned previously.

Mycorrhizal colonization showed different effects on pb uptake by the host plants under different pb addition levels. There may have been a critical plant tissue or soil available pb concentration below which pb uptake by plants and partitioning to shoots could be increased by mycorrhizal colonization, and above which the protective effects of the mycorrhiza would appear. Clearly, this value of a critical plant tissue or soil available pb concentration is likely to differ according to soil type, plant species.

The present results showed that P contents decreased according to the increase in soil pb concentrations, which could be attributed to the possible P precipitation with added metal (Ma et al. 1997). When comparing the mycorrhizal inoculation *G.macrocarpum* stimulated P uptake by two times compared with *G.fasciculatum*. The highest P uptakes were observed in the plant treated with *G.macrocarpum*. On the other hand significantly higher P uptake were found in the shoots than in roots. (data not shown)

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