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Full Length Research Paper

Arbuscular mycorrhiza contribution to the growth performance and heavy metal uptake of *Helianthus annuus* LINN in pot culture

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A greenhouse experiment was conducted to assess the arbuscular mycorrhiza contribution to the growth performance and heavy metals (Cd and Pb) uptake of *Helianthus annuus* L. from polluted soils. Cadmium sulfate ($CdSO_4.8H_2O$) and lead acetate ($(CH_3COO)_2Pb.3H_2O$) were applied at the concentrations: Cd- 0,20,40,60,80 mg kg^{-1} and Pb-0,250,500,750,1000 mg kg^{-1} in 90 plastic pots filled with 5 kg of topsoil each. Propagules of *Glomus mosseae* (GM) and *Glomus intraradices* (GI) at 25 g per pot were applied. Non-inoculated pots served as controls. Each treatment was replicated thrice in completely randomized design. Data were analysed using ANOVA and descriptive statistics. Results showed that arbuscular mycorrhiza (AM) fungi significantly ($p \leq 0.05$) influenced the number of leaves, plant height and stem girth of sunflower plants only at 80 mg Cd kg^{-1} concentration. Highest values of 0.11 mg Cd kg^{-1} and 0.29 mg Pb kg^{-1} were obtained with GI in the dry shoot of *Helianthus annuus* while 0.05 mg Cd kg^{-1} and 0.23 mg Pb kg^{-1} were remediated with GM application. Least values of Cd and Pb were removed when no AM was applied. GM and GI fungi enhanced the growth of *Helianthus annuus* and the uptake of Cd and Pb; but GI performed better. Also, a significant ($p < 0.05$) reduction of AM fungi colonization percentage was obtained with increase in Cd and Pb concentrations.

Key words: *Glomus mosseae*, *Glomus intraradices*, *Helianthus annuus,* phytoremediation, polluted soil.

INTRODUCTION

Soil contamination due to the disposal of industrial and urban wastes generated by human activities has become a major environmental concern. Controlled and uncontrolled disposal of wastes to agricultural soils are responsible for the migration of contaminants into non- contaminated sites (Ghosh and Singh, 2005). Soil contamination by heavy metals may pose a threat to human health, if the metals enter the food chain (Berti and Jacobs, 1996). Soil remediation is therefore needed to eliminate risk to humans from these toxic metals (Lazat, 2002).

Phytoremediation is cost effective (Lombi et al., 2001 and Memon et al., 2001) and a good alternative to the conventional chemical and physical methods of treating contaminated soils (Salt et al., 1998). Much work had been done on phytoremediation technology using sunflower (*Helianthus annuus* LINN) by different scientists. Davies et al. (2002) described sunflower plant as a high biomass plant with high metal accumulation ability. Dushenkov et al. (1995) found out that sunflower plant effectively removed potentially toxic metals such as Cu, Cd, Cr, Ni, Pb and Zn from aqueous solutions. Adewole et al. (2008) also noted that Cu, Cd and Pb were better removed from polluted soils by sunflower plant with organic fertilizer as soil amendment than when inorganic fertilizer was used.

Most fertilizers, especially the inorganic ones have heavy metals like Hg, Cd or Cr as impurities. Therefore, addition of these fertilizers to enhance metal solubility, mobility and bioavailability in phytoremediation (Blaylock et al., 1997), inadvertently is adding to the quantities of these heavy metals in soil. The more the quantities applied for enhancement, the more their accumulation in the soil ecosystem. Good enough, among the rizosphere microorganisms involved in plant interactions with soil are the arbuscular mycorrhiza fungi (Khan et al., 2000).

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Therefore, phytoremediation, enhanced by arbuscular mycorrhiza (AM) fungi offers a natural, efficient and cost effective means of soil remediation. Harrison (1998) observed that AM fungi which are natural constituents of the soil ecosystem have ability to interact with plants' roots thereby enlarging the soil volume for better nutrient uptake. Plants that are capable of forming this association with AM fungi have been shown to accumulate considerable amount of trace metals (Burke et al., 2000). Awotoye (2006) also observed that these fungi help to improve plant growth in soils with low fertility level while at the same time enhancing the uptake of phosphorus.

This study therefore attempts to monitor the growth performance and phytoremediation of contaminated soils of variable concentrations of Cd and Pb using *H. annuus* L. when *Glomus mosseae* and *Glomus intraradices* are used as enhancements under greenhouse conditions.

MATERIALS AND METHODS

Soil sampling, sample and standard solution preparation

The study was conducted at Obafemi Awolowo University, Ile-Ife. Bulk surface soil sample (0 - 15 cm) from an open field within the premises of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife was used. The sampled soil was air-dried; steam sterilized at 121°C for 2 h using the autoclave and sieved using 2 mm mesh. Steam sterilization helped to eliminate native arbuscular mycorrhiza fungi and other micro-organisms.

Heavy metal solutions of Cd and Pb of known concentrations were prepared using soluble compounds of $CdSO_4$. $8H_2O$ and $(CH_3COO)_2Pb$. $3H_2O$ at the levels: Cd-0, 20, 40, 60, 80 mg kg^{-1} and Pb- 0, 250, 500, 750, 1000 mg kg^{-1} respectively, as a follow-up of the soil pollution approach adapted by Adewole et al. (2008). A total of 90 plastic pots having drainage holes at the bottom with 5 kg of soil each were placed on the tables randomly in the greenhouse. Doses of heavy metals and fungi propagules of *G. mosseae* and *G. intraradices* at 25 g per pot were applied. Non-inoculated pots served as control. It was a factorial combination of Cd and Pb of which each treatment was replicated three times in completely randomized design.

Some agronomic practices

The pots were allowed to equilibrate for 4 days and viable seeds of *H. annuus* planted at the rate of 5 seeds per pot. After germination, the seedlings were thinned to two plants per pot at 2 weeks after planting (WAP). The thinned stands were put back to their respective pots. The pots were maintained weed-free and adequate soil moisture was maintained throughout the duration of the experiment. Growth parameters which included plants' number of leaves, heights and stem girths were determined fortnightly for a period of 12 weeks. Plant height was measured with a meter rule from soil level to the terminal bud. The stem girth of the plant was measured with vernier calliper.

At 8 WAP, the plant samples (stem and leaves) were taken from all the levels of the treatments with a clean razor blade and washed using distilled water, oven-dried for 48 h at 70°C, weighed, ground and analyzed for Cd and Pb uptake. At full physiological maturity stage, 16 WAP, total harvesting was done manually. Post-soil test was carried out to evaluate the heavy metal status of the soil in the pots culture.

Soil analyses

The pH of the soil was determined electrometrically using a pH meter in 1:2 soils- 1 M $CaCl_2$ suspension (Mclean, 1982). The particle size analysis was determined using hydrometer method in 5% sodium hexametaphosophate as described by Bouyoucos (1951). Organic matter was determined using Walkley-Black wet oxidation method (Nelson and Sommers, 1982). Total Kjeldahl Nitrogen (TKN) of the soil was determined using the macro Kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus was determined using Bray P_1 method (Olsen and Sommers, 1982).

For heavy metals (Cd and Pb) determination, 5 ml of the acid mixture (conc. HNO_3 and conc. $HClO_4$ in the ratio 2:1 and 5 ml of conc. H_2SO_4) was used to digest 0.5 g of each soil sample for 2 hours at 150°C. The digests were allowed to cool and were made up to 25 ml with distilled water. Concentrations of Cd and Pb in the soil extracts were read on ALPHA 4 Chem. Tech. Analytical model of Atomic Absorption Spectrophotometer (AAS).

Plant analyses

Cd and Pb were determined using 5 ml of the mixture (conc. HNO_3 and conc. $HClO_4$ in the ratio 2:1) with 5 ml of conc. H_2SO_4 to digest 0.5 g of each plant sample for 2 h at 150°C (Odu et al., 1986). The digest was allowed to cool and each was made up to 25 ml with distilled water. The concentrations of Cd and Pb in the plant extracts were read on AAS.

Mycorrhiza colonization

The root infection by the AM fungi was assessed by taking roots samples of sunflower plants and staining the samples for infection according to Brundrett et al. (1984). The grid-intercept method of Giovanetti and Mosse (1980) was used to calculate the percentage root infection.

Statistical analysis

The new Duncan's Multiple Range Test at $P \leq 0.05$ was used to separate the means.

RESULTS AND DISCUSSION

Properties of the soil for the greenhouse experiment were presented in Table 1. The texture of the soil was sandy loam and also slightly alkaline with soil pH of 7.70 in 1:2 soil – 1M $CaCl_2$ ratio. Total N, OC, K and available phosphorus values were: 0.50 mgkg^{-1}, 5.77 g kg^{-1}, 0.58 cmol $kg^{-1}$and 0.05 mg kg^{-1} respectively. The baseline Cd and Pb values were: 0.30 and 0.52 mg kg^{-1} respectively.

Arbuscular mycorrhiza influence on the growth of sunflower

The growth parameters (number of leaves, plants' heights and stem girths) decreased with increase in concentrations of Cd and Pb (Tables 2 and 3). Arbuscular mycorrhiza fungi significantly ($p \leq 0.05$) influenced the number of leaves, plant height and stem girth of sunflo-

Table 1. Results of soil analysis before planting.

Property		Value
pH (1:1 soil – water)		8.00
pH (1:2 soil - 1 M $CaCl_2$)		7.70
Sand (g kg^{-1})		640.00
Clay (g kg^{-1})		170.00
Silt (g kg^{-1})		190.00
Available P (mg kg^{-1})		0.05
Organic carbon (g kg^{-1})		5.77
Total N (g kg^{-1})		0.50
Exchangeable cations (cmol kg^{-1})	Ca	8.10
	Mg	1.10
	Na	0.15
	K	0.58
Textural class		Sandy loam
Cd (mg kg^{-1})		0.30
Pb (mg kg^{-1})		0.52

Table 2. Arbuscular mycorrhiza influence on the growth of sunflower in Cd polluted soils.

Plant growth parameter	Level of soil contamination mg kg^{-1}		Mycorrhiza treatment			F-statistics (df = 2,15)	
			NI	GM	GI	F	Level of significance (p)
Leaf number	0	Mean	13.00	8.00	12.00	0.91	0.42
		SD	4.74	3.24	5.10		
	20	Mean	13.00	12.00	13.00	0.20	0.82
		SD	5.26	3.22	4.66		
	40	Mean	12.00	11.00	10.00	0.34	0.72
		SD	4.37	3.57	3.04		
	60	Mean	13.00	11.00	11.95	0.19	0.83
		SD	4.09	4.03	4.47		
	80	Mean	2.00	14.00	14.00	13.94	0.00*
		SD	2.67	5.33	5.43		
Plant height (cm)	0	Mean	49.76	37.39	40.98	0.62	0.55
		SD	23.38	18.63	16.58		
	20	Mean	44.04	49.46	47.89	0.08	0.92
		SD	22.92	24.39	24.57		
	40	Mean	39.89	42.85	41.38	0.04	0.96
		SD	18.72	19.46	18.03		
	60	Mean	43.18	34.79	47.83	0.56	0.59
		SD	19.07	20.59	25.05		
	80	Mean	4.77	43.17	51.42	11.15	0.00*
		SD	7.70	17.43	25.26		
Stem girth (cm)	0	Mean	1.04	0.90	0.97	2.03	0.17
		SD	0.17	0.12	0.06		
	20	Mean	0.96	0.95	0.96	0.05	0.95
		SD	0.98	0.05	0.05		
	40	Mean	0.99	0.96	0.83	0.51	0.61
		SD	0.10	0.04	0.07		
	60	Mean	1.02	1.04	0.96	8.27	0.00
		SD	0.09	0.22	0.07		
	80	Mean	0.27	1.05	1.10	15.86	0.00*
		SD	0.43	0.18	0.18		

* Significantly different at $p \leq 0.05$.

Legend:

NI – Non-inoculated; GM – Inoculated with *Glomus mosseae;* GI – Inoculated with *Glomus intraradices*.

Table 3. Arbuscular mycorrhiza influence on the growth of sunflower in Pb polluted soils.

Plant growth parameter	Level of soil contamination mg kg^{-1}		Mycorrhiza treatment			F-statistics (df =2,15)	
			NI	GM	GI	F	Level of significance (p)
Plant number of leaves	0	Mean	12.00	10.00	8.00	1.57	0.24
		SD	4.65	4.48	2.73		
	250	Mean	12.00	11.00	11.00	0.13	0.88
		SD	4.51	2.50	5.33		
	500	Mean	13.00	12.00	13.00	0.13	0.88
		SD	5.05	4.12	5.29		
	750	Mean	13.00	11.00	11.95	1.66	0.22
		SD	4.09	4.03	4.47		
	1000	Mean	10.00	12.00	12.00	0.37	0.69
		SD	3.61	5.04	5.68		
Plant height (cm)	0	Mean	42.16	36.10	40.29	0.25	0.79
		SD	15.61	11.28	18.23		
	250	Mean	45.91	34.19	41.80	0.59	0.57
		SD	23.05	10.84	20.97		
	500	Mean	47.90	43.86	43.77	0.07	0.93
		SD	25.24	17.41	19.04		
	750	Mean	50.12	45.32	37.64	0.60	0.56
		SD	22.65	18.49	18.15		
	1000	Mean	44.11	42.39	50.34	0.28	0.76
		SD	16.08	16.26	24.68		
Stem girth (cm)	0	Mean	0.97	0.96	0.94	0.17	0.84
		SD	0.11	0.07	0.12		
	250	Mean	1.02	0.97	0.93	0.95	0.41
		SD	1.92	0.32	0.57		
	500	Mean	1.12	0.97	0.93	3.42	0.06
		SD	0.21	0.07	0.03		
	750	Mean	1.09	1.04	0.15	1.02	0.38
		SD	0.16	0.09	0.16		
	1000	Mean	1.08	0.94	1.07	2.02	0.17
		SD	0.18	0.04	0.02		

Legend:
NI – Non-inoculated
GM – Inoculated with *Glomus mosseae*
GI – Inoculated with *Glomus intraradices*

sunflower only at high concentration (80 mg kg^{-1}) of Cd (Table 2). No significant difference was obtained in the growth parameters of sunflower grown on Pb polluted soils (Table 3).

Arbuscular mycorrhiza colonization percentage

The percentages of arbuscular mycorrhiza colonization for both the inoculated and non-inoculated plants are shown in Table 4. Mycorrhiza inoculated plants had greater colonization than the non-inoculated plants under the same heavy metal pollution regime. Also, pots with GI inoculation had better colonization percentages than GM inoculation, and a significant reduction was observed on both types of mycorrhiza fungi with increase in pollutants' levels.

Arbuscular mycorrhiza influence on the uptake of Cd and Pb by sunflower plant

Cd uptake by sunflower varied (mg kg $^{-1}$) from 0.01 to 0.11 in *Glomus intraradices* (GI), from 0.01to 0.05 in *G. mosseae* (GM) and from 0.02 to 0.03 in non-inoculated pots.

Also, Pb uptake by sunflower varied (mg kg $^{-1}$) from 0.03 to 0.29 in *Glomus intraradices* (GI), from 0.01to 0.23 in *G. mosseae* (GM) and from 0.01 to 0.22 in non-

Table 4. Percentage arbuscular mycorrhiza colonization.

Heavy metal	Concentration level (mg kg^{-1})	Mycorrhiza colonization (%) NI	GM	GI
Cadmium	0	3.2ns	63.2a	86.4a
	20	3.5ns	61.8a	60.8b
	40	3.0ns	42.7b	49.2c
	60	2.8ns	31.3c	47.8c
	80	1.7ns	20.6d	30.4d
Lead	0	3.2ns	63.1a	86.7a
	250	3.3ns	50.7b	71.3b
	500	1.8ns	38.2c	70.8b
	750	2.1ns	37.6c	60.5c
	1000	2.0ns	23.5d	33.2d

Means on the same vertical column followed by different letters are significantly different at $p \leq 0.05$ according to Duncan's multiple range tests.

Legend:
NI – Non-inoculated
GM – Inoculated with *Glomus mosseae*
GI – Inoculated with *Glomus intraradices*
ns – Not significant at $p \leq 0.05$ according to Duncan's multiple range test.

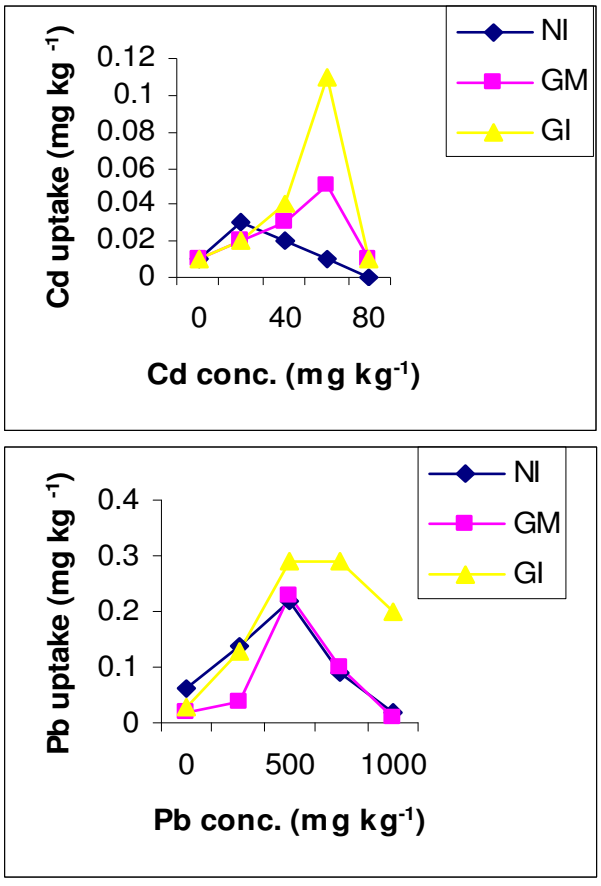

Figure 1. Effects of arbuscular mycorrhiza on the uptake of Cd and Pb by sunflower in greenhouse experiment.

Legend:
NI – Non- inoculated soil
GM – Soil inoculated with *Glomus mosseae*
GI – Soil inoculated with *Glomus intraradices*

inoculated pots.

With increase in the concentrations of Cd and Pb in the soil, there was increased uptake by sunflower plant (Figure 1). When the soils were treated with *G. mosseae*, the uptake of Cd and Pb at threshold levels of 60 mg kg^{-1} and 500 mg kg^{-1} by sunflower were 0.05 mg kg^{-1} and 0.23 mg kg^{-1} respectively. Also, when *G. intraradices* was applied; higher values 0.11 mg Cd kg^{-1} and 0.29 mg Pb kg^{-1} were remediated at 60 mg Cd kg^{-1} and 500 mg Pb kg^{-1} polluted soils respectively. However, at the same levels of soil contamination by Cd and Pb when no AM fungi was applied, least values of 0.01 mg Cd kg^{-1} and 0.22 mg Pb kg^{-1} were taken up by sunflower plant.

The results obtained suggested that arbuscular mycorrhiza (AM) offered protection against heavy metal toxicity. Previous studies of Li and Christie (2001) and Malcova et al. (2003) agreed with the role of AM in alleviating nutritional stress and thus helped to protect sunflower plants against harmful effects of these heavy metals. Khan (2006) had reported the ability of proteins in the cell walls of AM fungi to absorb toxic metals by sequestering them. All the control pots where no AM fungi were applied had low uptake of Cd and Pb by sunflower plants. Hence, AM fungi have the ability to improve plant tolerance to metal stress in polluted soils.

Table 5 showed the mean levels of Cd and Pb that remained in the soil at full physiological maturity growth of sunflower, 16 weeks after planting. These values also increased with increase in the concentrations of the pollutants. Sunflower plants picked up optimally at 60 mg Cd kg^{-1} and 500 mg Pb kg^{-1} with AM application; while it was 20 mg Cd kg^{-1} and 500 mg Pb kg^{-1} without AM fungi. After threshold stage however, the uptake values decreased (Figure 1). The increased concentrations of Cd and Pb resulted to retention of higher values in the soils.

Table 5. Post soil levels of Cd and Pb in the pot culture.

Heavy metal	Level of soil contamination mg kg^{-1}	NI	GM mg kg^{-1}	GI
Cd	0	0.35 ± 0.11	0.30 ± 0.16	0.33 ± 0.17
	20	15.51 ± 0.38	14.05 ± 0.05	13.23 ± 0.45
	40	29.39 ± 0.34	25.60 ± 0.39	22.15 ± 0.25
	60	45.29 ± 0.43	40.18 ± 0.40	24.25 ± 0.39
	80	63.32 ± 0.43	59.39 ± 0.16	40.00 ± 0.12
Pb	0	0.52 ± 0.00	0.51 ± 0.13	0.54 ± 0.16
	250	193.13± 1.19	181.07 ± 0.45	137.25 ± 2.07
	500	279.19 ± 2.19	284.20 ± 0.94	255.55 ± 2.09
	750	665.47 ± 2.81	663.63 ± 2.58	591.45 ± 2.75
	1000	885.83 ± 2.79	815.54 ± 2.70	740.40 ± 2.55

Legend:
NI – Non- inoculated soil±
GM – Soil inoculated with *Glomus mosseae*
GI – Soil inoculated with *Glomus intraradices*

after harvest is evident in pots with zero contamination level of the heavy metals (Table 5). The soil sterilization carried out at the beginning of the experiment is believed to have eliminated native fungi externality contributory factors

Conclusion

The *G. mosseae* and *G. intraradices* fungi enhanced the growth of sunflower. The uptake of Cd and Pb were also enhanced by these arbuscular mycorrhiza fungi. However, *G. intraradices* performed better in the uptake of Cd and Pb. It is therefore recommended that when using sunflower plant in the phytoremediation of soils contaminated by Cd and Pb, propagules of *G. intraradices* should also be applied to enhance the rates of removal, especially in the humid tropics of Africa with fragile soils.

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