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INFLUENCE OF MYCORRHIZAL FUNGI ON PHYTOREMEDIATING POTENTIAL AND YIELD OF SUNFLOWER IN Cd AND Pb POLLUTED SOILS

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Abstract: The influence of mycorrhizal fungi in uptake of heavy metals, pollution response index and yield of sunflower in degraded soils were investigated. It was a greenhouse experiment with 2 arbuscular mycorrhizae (*Glomus mosseae*, *Glomus intraradices*) and a non-inoculation that served as control. The treatments were replicated 3 times in a completely randomized design. Each of the treatment consisted of 30 pots and each pot was filled with 5 kg by weight of dried top soil. Solutions of lead acetate and cadmium sulphate at variable levels of: 0, 250, 500, 750, 1000 mg kg^{-1} and 0, 20, 40, 60, 80 mg kg^{-1} respectively were used to pollute the soils. Increase in pollution-stressed conditions significantly ($P<0.05$) reduced the infection of sunflower roots, and the uptake of Pb and Cd in the dry root of sunflower was also significantly ($P<0.05$) reduced. Also, arbuscular mycorrhizae enhanced the root infection of sunflower, increased the pollution tolerance and consequently increased the yield of sunflower.

Key words: arbuscular mycorrhiza inoculation, heavy metals, phytoremediation, pollution response index, sunflower.

Introduction

Toxicity levels of heavy metals in the environment have been elevated dramatically as a result of human activities. Industrialisation has led to increased release of heavy metals into the environment, particularly the soil. Heavy metals, when present in the environment do not breakdown. In addition, heavy metals pose a threat to human health if the heavy metals enter the food chain (Maquita, 1997). Hence, heavy metal pollution of the environment has been causing serious concern to every stakeholder in Environmental Sciences and related subjects.

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Use of agrochemicals such as synthetic fertilisers and pesticides has resulted into soil and water pollution, and loss of biodiversity. In the humid tropics of Africa, where agrochemicals, especially fertilisers are often wrongly used, arbuscular mycorrhizae (AM), found in natural ecosystem, including agricultural areas do improve soil fertility (Daft and Nicolson, 1969). Many plants are capable of forming association with AM fungi to help boost native soil nutrients (Burke et al., 2000). These fungi are well known to improve plant growth on poor soils.

Arbuscular mycorrhizae have also been observed to play a vital role in metal tolerance (Del Val et al., 1999) and accumulation (Zhu et al., 2001; Jamal et al., 2002). External mycelium of AM fungi provides a wider exploration zone (Khan et al., 2000; Malcova et al., 2003), thus providing access to greater volume of heavy metals present in the rhizosphere.

AM fungi show variation in their susceptibility and tolerance to heavy metals. Del Val et al. (1999) reported six AM fungal ecotypes showing consistent differences with regard to their tolerance to the presence of metals in soil. *Glomus* sp. (isolated from non-polluted soil) was shown to be the most sensitive fungus, while one strain of *Glomus claroideum* (isolated from the most contaminated soil) was the most tolerant. The effectiveness of the different AM fungal isolates in improving plant growth also depends on the level of heavy metals in soil (Awotoye et al., 2009). Furthermore, AM fungi from different soils may differ in their metal susceptibility and both metal-specific and nonspecific tolerance mechanisms may be selected in metal-polluted soil (Weissenhorn et al., 1994).

Sunflower (*Helianthus annuus* L.) has ability to store heavy metals in the roots. Since the natural rate of attenuation of heavy metals in the environment is slow and conventional method of remediation is expensive, labour intensive with negative effect on the soil, inoculation of sunflower with arbuscular mycorrhizal fungi may offer a natural, more efficient, and cost effective means of soil remediation. This study was therefore aimed to determine the influence of two mycorrhizae species (*Glomus mosseae and Glomus intraradices*) on the yield of FUNTUA sunflower, *Helianthus annuus* under heavy metal pollution-stressed conditions.

Materials and Methods

Greenhouse experiment

The study was conducted in a greenhouse at Obafemi Awolowo University, Ile-Ife, Nigeria. Viable seeds of FUNTUA sunflower, *Helianthus annuus* L., were planted into 5-litre pots filled with 5 kg top soil. The physical and chemical characteristics of the soil are shown in Table 1. Prior to the filling of the pots, the top soil was sieved using a 2 mm mesh sieve and then steam sterilized at 121^{o} C for 2 hours using the autoclave. This was to eliminate native arbuscular mycorrhizae fungi propagules as well as other micro organisms.

Table 1. The physical and chemical properties of soil.

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
Textural class	Sandy loam
Available P (mg kg^{-1})	0.05
Organic carbon (g kg^{-1})	5.77
Total N (g kg^{-1})	0.50
Exchangeable acidity (cmol kg^{-1})	0.12
CEC(cmol kg^{-1})	9.93
ECEC (cmol kg^{-1})	10.05
Base saturation (%)	99.00
Cd (mg kg^{-1})	0.30
Pb (mg kg^{-1})	0.52

The experiment was a factorial combination of two heavy metals in a completely randomized design and replicated three times with three arbuscular mycorrhiza levels (*Glomus mosseae*, *Glomus intraradices*, and non inoculated) treatments. Metal solutions of Cd and Pb of known concentrations were prepared using soluble compounds of cadmium II sulphate octahydrate [$CdSO_4.8H_2O$] and lead II acetate trihydrate [$(CH_3COO)_2Pb.3H_2O$] at the levels: $CdSO_4.8H_2O$ – 0, 20, 40, 60, 80 mg kg^{-1} and $(CH_3COO)_2Pb.3H_2O$ – 0, 250, 500, 750, 1000 mg kg^{-1}. These concentrations were used to contaminate 5 kg each by weight of soil. The soils the rate of 25 g per pot. The mycorrhiza treatments consisted of 30 pots each of *Glomus mosseae* (GM), *G. intraradices* (GI) and non inoculated (NI) respectively.

Sunflower seeds were planted at the rate of five seeds per pot. The plants were later thinned to two stands per pot after two weeks of planting and the thinned stands were retained in their pots. Adequate soil moisture was maintained throughout the duration of the experiment with care to avoid leaching of the heavy metals. At full physiological maturity stage (16 weeks after planting, WAP) the seeded heads were cut off from the stems, hand-threshed, weighed and stored. The plant roots were carefully extracted from the soil, rinsed with deionised water, dried at a constant temperature of 70 ^{0}C for 48 hours in a Gallen Kemp oven, weighed and stored. Parts of the roots were collected and stored in 50 % ethanol in McCartney bottles for mycorrhizal infection determination.

Spore extraction

Pre-planting and at harvest mycorrhizal spore extraction of the soils were accessed by taking 100 g each from all the AM treatment pots (including the bulk soil sample used for the experiment after sterilization). After each had been

thoroughly mixed, it was followed by 40 % weight/volume sucrose density centrifugation gradient at 3000 rpm for 4 minutes (Daniels and Skipper, 1982). The spores were thereafter examined and counted under a dissecting microscope.

Mycorrhizal infection determination

Mycorrhiza staining was initiated by heating the root in 10 % KOH for 40 minutes at 80 ^{o}C. Roots were bleached in alkaline H_2O_2 for 10 minutes, after which they were rinsed in water and soaked in 1 % HCl for 10 minutes. The staining solution, Trypan blue used on the roots was prepared by mixing 600 ml of glycerol, 450 ml of water, 50 ml of 1 % HCl and 0.05 % of Trypan blue in water bath, at 90 ^{o}C for 1 hour. Roots were soaked in Trypan blue solution for 2 hours. Stained roots were distained with 50 % glycerol. The degree of mycorrhiza infection was assessed by spreading the root samples evenly on a grid - line intercept plate and observed under the microscope. The total number of spores and infected roots intersecting the grids were counted following the method of Daniels and Skipper (1982).

Laboratory Analyses

The root samples were digested with concentrated H_2SO_4 and 50 % (v/v) H_2O_2 at 90º C. The digest was analyzed for Cd and Pb using a Perkin Elmer Flame Atomic Absorption Spectrophotometer.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) to test for treatment effect. Test of significance for differences in means was done using Least Square differences (LSD) method. All the statistical analyses were done using the SAS package for windows.

Results and Discussion

Mycorrhizal spore count and root infection

The pre-planting mycorrhizal spore count was four per 100 grams of soil using Daniels and Skipper (1982) adapted approach. At harvest, under variable heavy metal pollution levels and arbuscular mycorrhiza inoculation, spore densities and root infections were obtained (Table 2). AM inoculation significantly ($P<0.05$) increased the spore count of sunflower roots. Also, increase in pollution level significantly ($P<0.05$) reduced the infection of sunflower roots. Significant reductions were obtained in sunflower spore counts with increase in soil pollution levels (Table 2). At 500 mg Pb kg^{-1} soil pollution, spore counts per 100 g dry soil in GI, GM and NI pots were 223, 121 and 6 respectively. Also, at 60 mg Cd kg^{-1}

soil pollution, spore counts per 100 g dry soil in GI, GM and NI pots were 151, 99 and 9 respectively, indicating that AM fungi on pollution tolerance appear to be specie specific. At zero pollution level, pots with *Glomus intraradices* (GI) propagules had significantly ($P<0.05$) higher spore count than *Glomus mosseae* (GM) or non-inoculated (NI) pots. High pollution levels might have inhibited sporulation by AM fungi.

Table 2. Relationship between mycorrhizal spore density and root infection under different levels at harvest*.

Heavy metal	Conc. level	AM Treatment	Number of spore / 100 g dry soil	Infection (%)
Pb	0	GI	275a	86.7a
		GM	200b	63.1b
		NI	10e	3.2e
	250	GI	227b	71.3b
		GM	161c	50.7c
		NI	11e	3.3e
	500	GI	223b	70.8b
		GM	121d	38.2d
		NI	6e	1.8e
	750	GI	192c	60.5b
		GM	119d	37.6d
		NI	na	na
	1000	GI	na	na
		GM	na	na
		NI	na	na
Cd	0	GI	274a	86.4a
		GM	200b	63.2b
		NI	10e	3.2e
	20	GI	193b	60.8b
		GM	196b	61.8b
		NI	11e	3.5e
	40	GI	156c	49.2c
		GM	135c	42.7c
		NI	9e	3.0e
	60	GI	151c	47.8c
		GM	99d	31.3d
		NI	na	na
	80	GI	na	na
		GM	na	na
		NI	na	na

*Pre-planting mycorrhizal spore density was 4/100 g dry soil and root infection was also 1.3 %. Mean values at harvest for each fungus and treatment followed by a different letter are significantly different at $P<0.05$ according to Duncan's multiple range tests. Legend: AM=Arbuscular mycorrhizal, GI=Glomus intraradices, GM=Glomus mosseae, NI=Non-inoculated, na=Not available.

Table 3. Extractible Pb and Cd in the root of sunflower at harvest.

Heavy metal (HM)	Rate (mg kg^{-1})	AM Treatment	Conc. in root (mg kg^{-1})	Dry weight of root (g)	HM uptake (mg kg^{-1})
Pb	0	GI	0.18	9.35	0.002f
		GM	0.15	8.67	0.001f
		NI	0.10	2.69	0.0003g
	250	GI	87.35	11.36	0.99c
		GM	49.68	11.97	0.59cd
		NI	28.55	6.00	0.17d
	500	GI	185.00	11.83	2.19a
		GM	136.07	10.99	1.50b
		NI	84.56	9.01	0.76c
	750	GI	171.55	4.89	0.84c
		GM	105.91	4.01	0.42d
		NI	na	na	na
	1000	GI	na	na	na
		GM	na	na	na
		NI	na	na	na
Cd	0	GI	0.15	9.40	0.001f
		GM	0.15	9.00	0.001f
		NI	0.10	3.30	0.0003g
	20	GI	4.83	9.96	0.05e
		GM	3.95	10.03	0.04e
		NI	2.77	4.00	0.01e
	40	GI	9.03	10.56	0.09e
		GM	6.88	9.98	0.07e
		NI	2.05	4.02	0.008f
	60	GI	12.09	11.36	0.14d
		GM	10.00	11.25	0.11d
		NI	na	na	na
	80	GI	na	na	na
		GM	na	na	na
		NI	na	na	na

Mean values at harvest for each fungus and treatment followed by a different letter(s) are significantly different at $P<0.05$ according to Duncan's multiple range tests. Legend: AM=Arbuscular mycorrhizal, GI=Glomus intraradices, GM=Glomus mosseae, NI=Non-inoculated, na=Not available.

Table 3 showed the uptake of Pb and Cd in the dry root of sunflower under different mycorrhizae and soil pollution-stressed conditions. Significantly ($P<0.05$) highest values of 2.19, 1.50 and 0.76 mg Pb kg^{-1} were remediated with GI, GM and NI at 500 mg Pb kg^{-1} soil pollution respectively. However, at 60 mg Cd kg^{-1} polluted soils, 0.14 and 0.11 mg kg^{-1} remediated values with GI and GM were not

significantly ($P<0.05$) different. No remediation could be achieved in pots without fungi inoculation at 60 mg Cd kg^{-1} as the test crop, sunflower could not grow in them. Also, the soil toxicity level from 80 mg Cd kg^{-1} and 1000 mg Pb kg^{-1} with AM inoculation must have been too harsh for the survival of sunflower plant; hence, the sunflower plant could not grow in them. Pollution response index (PRI) has not been previously used to assess mycorrhizal dependency (MD). Many previous works have been based on the contributions of mycorrhizal fungi on plant growth (McGonigle and Miller, 1996); low P and Zn in soil (O'Halloran et al., 1986). This study is able to provide a new dimension to the performance of MD in soil pollution conditions as a direct relationship has been established between them (Figure 1). The PRI was higher in the presence of mycorrhizal fungi than when the pots were not inoculated. Also, the higher the pollution level, the higher was the PRI of sunflower plant until the tolerant level was reached (Table 4).

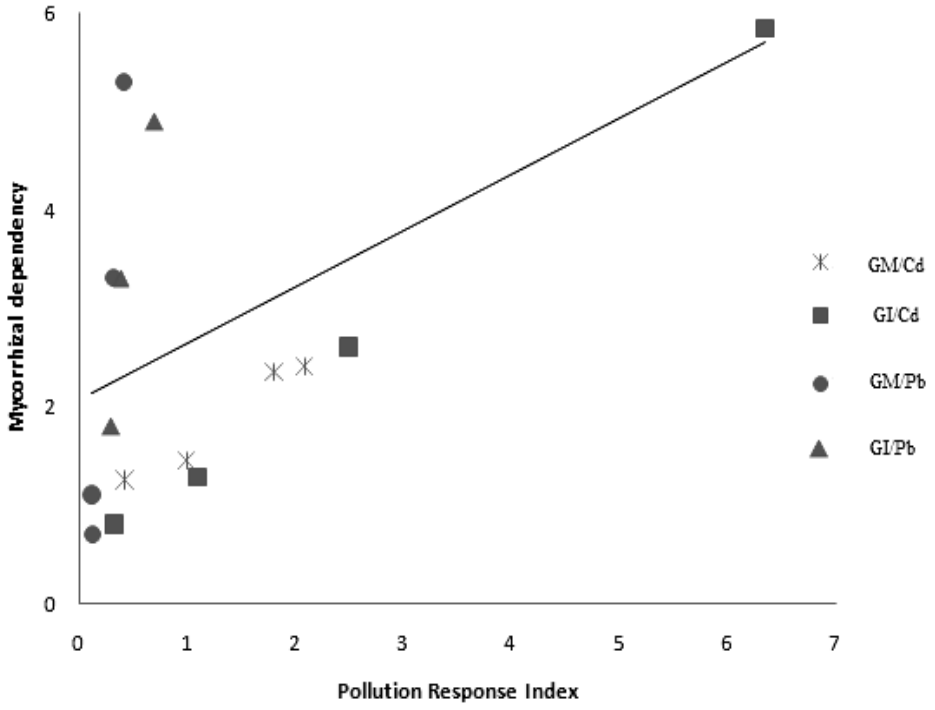

Figure 1. Relationship between mycorrhizal dependency (MD) and pollution response index (PRI). The regression equation for this relationship was: $Y = 0.57X + 2.08$; $r = 0.48$.

Table 4. Pollution response index (PRI) and mycorrhizal dependency (MD) of sunflower plant in Pb and Cd polluted soils.

Heavy metal mg kg^{-1}	Glomus mosseae		Glomus intraradices	
	PRI	MD	PRI	MD
Cd				
20	1.25	0.43	0.80	0.33
40	1.45	1.00	1.28	1.10
60	2.35	1.81	2.84	2.50
80	0.20	0.11	0.32	0.14
Pb				
250	1.10	0.12	1.80	0.30
500	5.30	0.42	3.30	0.39
750	3.30	0.32	6.07	0.70
1000	0.70	0.13	1.36	0.16

Yield of sunflower

Table 5 showed the mean yield of sunflower seed at four months after planting. Singh (2002), working on Nigerian soils gave critical ranges of soil parameters: N< 1.50 g kg^{-1}, P< 8 mg kg^{-1}, K< 0.20 cmol kg^{-1} and OC< 20.00 g kg^{-1} to be low for good sunflower production. Table 1 summarized pre-planting soil properties thus: soil organic carbon (5.77 g kg^{-1}), Total nitrogen (0.50 g kg^{-1}), phosphorus (0.52 mg kg^{-1}) and effective cation exchangeable capacity (10.05 cmol kg^{-1}). Singh (2002) considered these soil properties low for sunflower cultivation. Native Cd and Pb in soil were: 0.30 and 0.52 mg kg^{-1} respectively. Many research works have disagreed on the use of synthetic fertilizers in the phytoremediation of polluted soils as many of these fertilizers have either Hg or Cd as additives. Natural soil enhancers such as *G. mosseae and G. intraradices* for good crop productivity of sunflower under pollution-stressed conditions are good substitutes.

Mycorrhizal associations reduce attack from root pathogen and increase the tolerance of the plant to heavy metal (Awotoye et al., 2009), drought (Osonubi et al., 1991). Plant roots with developed mycorrhizae have greater access to make use of soil nutrients (Habte and Manjunath, 1987).

Also, growth-stimulating effect otherwise referred to as 'mycorrhizal dependency' is influenced by soil fertility and this invariably enhance the yield of crop. The higher the pollution level, the lower was the yield of sunflower (Table 5). This enhancement by AM was however truncated with pollution-stressed conditions. Plenchette et al. (1983) also observed better plant growth under mycorrhizal than non-mycorrhizal inoculation conditions. Karasawa et al. (2002) observed that fallowed land followed by its cultivation to sunflower, maize or soya bean had positive significance on AM colonisation and crop growth. The yield of

sunflower was negatively affected by pollution-stressed condition; though, AM influenced positively. The higher the root infection caused by AM, the higher was the sunflower yield (Tables 2 and 5). Significantly ($P<0.05$) highest yield of 4.05 g/pot at 0 mg Pb kg^{-1} was obtained when compared with 0.17 g/pot at 750 mg Pb kg^{-1} pollution level. The plants from 750 mg Pb kg^{-1} levels without AM did not grow to maturity. The plants died at 6 WAP. A similar trend was obtained in Cd polluted pots from 60 mg kg^{-1} without AM inoculation.

Table 5. Yield of sunflower as influenced by arbuscular mycorrhizae as biofertilisers in polluted soils.

Heavy metal	Rate (mg kg^{-1})	AM	Yield (g/pot)
Pb	0	GI	4.05a
		GM	3.75ab
		NI	3.70ab
	250	GI	3.55b
		GM	2.38c
		NI	2.17c
	500	GI	1.73c
		GM	1.00d
		NI	0.76d
	750	GI	0.29e
		GM	0.17f
		NI	na
	1000	GI	na
		GM	na
		NI	na
Cd	0	GI	3.97a
		GM	3.65ab
		NI	3.10b
	20	GI	1.98c
		GM	1.09d
		NI	0.76e
	40	GI	0.65e
		GM	0.40e
		NI	0.35e
	60	GI	0.08f
		GM	0.04f
		NI	na
	80	GI	na
		GM	na
		NI	na

Mean values at harvest for each fungus and treatment followed by a different letter(s) are significantly different at $P<0.05$ according to Duncan's multiple range tests. Legend: AM=Arbuscular mycorrhizal, GI=Glomus intraradices, GM=Glomus mosseae, NI=Non-inoculated, na=Not available.

Conclusion

AM (GI and GM) enhanced sunflower root infection, mycorrhizal dependency and consequently increased sunflower yield. Cd and Pb removed from the polluted soils were also enhanced with addition of AM. However, better yield of sunflower was obtained under non-polluted soil condition and *Glomus intraradices* mycorrhiza inoculation. Also, better Pb and Cd were removed using sunflower plant when *Glomus intraradices* was used as biofertiliser.

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UTICAJ MIKORIZA NA FITOREMEDIJACIJSKI POTENCIJAL I PRINOS SUNCOKRETA U Cd I Pb ZAGAĐENIM ZEMLJIŠTIMA

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R e z i m e

Ispitivan je uticaj mikoriza u usvajanju teških metala, indeks reakcije na zagađenost i prinos suncokreta u degradiranom zemljištu. Eksperiment je izveden u stakleniku sa 2 arbuskularne mikorize (*Glomus mosseae*, *Glomus intraradices*) i kontrolom bez inokulacije. Tretmani su ponovljeni tri puta po potpuno slučajnom planu. Svaki od tretmana se sastojao od 30 saksija i svaka saksija je bila napunjena sa 5 kg isušenog površinskog zemljišta. Rastvori olovo acetata i kadmijum sulfata pri promenljivim nivoima od: 0, 250, 500, 750, 1000 mg kg^{-1} i 0, 20, 40, 60, 80 mg kg^{-1} su korišćeni kako bi zagadili zemljište. Povećanje zagađenosti značajno ($P<0,05$) je smanjilo zaraženost korenova suncokreta mikorizom, a takođe značajno ($P<0,05$) je smanjeno i usvajanje Pb i Cd kod suvog korena suncokreta. Arbuskularne mikorize su isto tako povećale zaraženost korena suncokreta, toleranciju na zagađenost i kao rezultat toga su povećale prinos suncokreta.

Ključne reči: inokulacija arbuskalarne mikorize, teški metali, fitoremedijacija, indeks reakcije na zagađenost, suncokret.

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