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Effect of Microbial Inoculation and EDTA on the Physiology and Phytoremediation Efficiency of *Delonix regia* Plants Growing in Polluted Soil

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Abstract: A pot experiment was conducted to evaluate the growth parameters, photosynthetic pigments, activity of antioxidant enzymes, proline and the concentration of some heavy metals (Pb, Cd and Zn) of *Delonix regia* plants growing in polluted soil, treated with arbuscular mycorrhizal fungus (AMF) and ethylenediamine tetraacetic acid (EDTA). The results showed that the growth parameters values were lower in the polluted soil than those in the unpolluted one. AMF with heavy metals has a superior effect on the growth parameter values either than EDTA or control treatments. Pigment values were lower in polluted soil than those in the unpolluted one. EDTA with heavy metals has a superior effect on pigment content values than those with AMF. The effect of the later in polluted soil exceeded that of EDTA or the control in increasing the proline content due to decreased heavy metals induced oxidative stress and toxicity. The results showed also that the effect of AMF with heavy metals was greater than that of EDTA on antioxidant enzymes activities. In the time of EDTA increased the concentration of Fe, AMF was superiority in increasing the P concentration. Plants grown in polluted soil had higher concentrations of Pb, Cd and Zn than that in the unpolluted soil. Also, in polluted soil, the plants grown with EDTA exceeded these concentrations than with AMF. Roots had the highest concentrations of these nutrients than leaves and stems. The uptake of these nutrients was increased by the plant as a result of increasing the dry matter by different treatments.It was also noticed that using AMF is more efficient than EDTA in remediating Pb polluted soil. As the plants extracted normal amount of Zn and excises amounts of Cd, this means the studied plants are non hyperaccumulator for Cd and Zn. Raising AMF level in soil caused a steady decrease in the extractable values of Pb, Cd and Zn of the treated soil.

Key words: *Delonix regia* · Polluted soil · Arbuscular mycorrhizal fungus (AMF) · Ethylenediamine tetraacetic acid (EDTA) · Phytoremediation

INTRODUCTION

Pollution has been a major issue when taking about the environmental conditions all over the world and in Egypt; especially in urban areas [1]. Cleaning-up soils from pollution is difficult and expensive, often involving a combination of methods to decontaminate an area. Phytoremediation is an emerging cost effective, non-intrusive and aesthetically pleasing technology that used ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues, appear very promising for the removal of pollutants from the environment [2]. Besides the choice of the best plants, chelating agents and microorganisms can also have complementary roles in phytoremediation of the polluted soil [3].

The Royal Poinciana, *Delonix regia* (family Fabaceae), is a tropical or subtropical flowering, deciduous and small to medium-sized tree [4]. It is also known by different names; i.e. Gulmohar, Flamboyant Tree, Peacock Flower, Flame of the Forest and Flame Tree. It grows well in moist soil derived from limestone, where it is common and reproduces well but is also tolerant of well-drained and somewhat droughty conditions [5].

Arbuscular mycorrhizal fungi (AMF) are a direct link between soil and plant roots consequently of great importance in phytoremediation-potentially enhancing heavy metals availability and plant tolerance [6]. The natural role of AMF in maintaining soil fertility is more important than in conventional agriculture, horticulture and forestry where higher use of agrochemicals minimize their significance. AMF recognize their host by signals

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released by host roots, allowing a functional symbiosis. AMF also producing an insoluble glycoprotein, glomalin, which sequester trace elements and it should be considered for biostabilization leading to remediation of contaminated soils [7].

The use of chelating agents constitutes a promising tool for Pb absorption from the soil matrix by forming soluble complexes leading to increasing plant absorption; EDTA is considered the most effective one [8], although main problems of its use are the leaching of Pb-EDTA with subsequent risk of ground water contamination and the major part of Pb-EDTA is taken up in short time (6 hr.) [9].

The objective of this study was to evaluate the effect of AMF inoculation and EDTA as synthetic chelator on the phytoremediation efficiency of *Delonix regia* plants grown on contaminated soil with heavy metals.

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and Studies; the Ornamental Horticulture Department and Soil Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during two seasons 2010 and 2011.

Soil Samples: Two surface soil samples (0-30 cm) were collected; one from Faculty of Agriculture, Cairo University, Giza, Egypt as an unpolluted soil (S1) and the other from Abou Rawash, Giza governorate (S2) which is sandy loam as polluted soil (domestic wastes). The general characteristics and Diethylene triamine penta acetic acid (DTPA) extractable Pb, Cd and Zn are shown in Table 1.

Plant Material: One-year old seedlings of *Delonix regia* (Bojer ex Hook) Raf. were obtained from the nursery and transplanted individually on 1st June, in the two seasons, using 25 cm plastic pots filled with 5 kg of the studied soils. After 30 days from transplanting, in both seasons, the growing media was mixed separately with 100 mmol per kg of soil EDTA-Fe or 500 spores/g of AMF; which obtained from Soils, Water and Environmental Resources, Institute of Agricultural Research Center, Giza, Egypt; and inoculated at a rate of 10g/hole, where the spores dressed around the rhizosphere attached to the secondary roots [10]. The layout of the experiment was factorial (soil treatments × amendment treatments), conducted using a randomized complete blocks design with three replicates. The study included 6 treatments [2 soils × 3 amendment treatments (including the control)], with each block consisting of 12 plants (6 plants/treatment).

Growth Parameters: On 1st August, the experiment was terminated and the growth parameters; fresh and dry weights of plant (g), plant height (cm), number of branches/plant and stem diameter (cm) were recorded.

Chemical Analysis

Photosynthetic pigments and proline: The contents of pigments; chlorophyll a and b and carotenoids (mg/g fresh matter) were determined from fresh leaves samples using the method described by Nornai [11]. The proline content of fresh leaves was determined according to Bates *et al*. [12].

Activities of Antioxidant Enzymes: Preparation the enzymes extraction of leaves tissues was carried out at 40°C at 3:1 buffer: fresh weight (v/v) in a pastel and mortar with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were stored in separate aliquots at 8°C [13]. Antioxidant enzymes were assayed as follows; Catalase (CAT) by measuring the decrease in absorbance due to disappearance of H_2O_2 at 240 nm according to Chance and Maely [14], Peroxidase (POX) by spectrophotochemically according to Amako *et al.* [15] and Superoxide dismutase (SOD) by photochemical method as described by Giannopolitis and Ries [16]. Enzymes activities were expressed as units/min/mg protein.

Table 1: General characteristics and DTPA extractable Pb, Cd and Zn (mg/kg) of the studied soils

	Partical size distribution						Electrical conductivity	Organic		Cation exchange capacity	DTPA extractable		
Soil	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture	pH (1;2.5)	(E.C) (ds/ m)	matter (%)	$CaCo_3$ %	(CEC) (Cmol+/kg)	Pb	Cd	Zn
S1	7	18	40	35	C.L	7.8	0.51	1.1	0.7	33	0.4	0.1	3.8
S2	31	40	11	18	S.L	7.1	0.88	2.3	2.4	14	20.8	1.1	36.0

S1= Soil from Fac. Agric., Giza S2= Soil from Abou-Rawash, Giza

Determination of Elements: Dried leaves samples were digested to extract nutrients and the extract was analyzed to determine; the phosphorus according to Jackson [17] and Fe, Pb, Cd and Zn by Atomic Absorption Spectrophotometer apparatus (Model GBC, 932AA) [18]. The uptake of Pb, Cd and Zn was calculated by multiplying the dry weight of chosen plant parts (leaves, roots and stems) by the concentration of the three heavy metals in the plant tissues. The soils were analyzed at the end of the experiment, for DTPA, extractable Pb, Cd and Zn, as recommended by Lindsay and Norvell [19].

The data were subjected to statistical analysis of variance and the means were compared using the "Least Significant Difference (L.S.D.)" test at 5% level, as described by Little and Hills [20].

RESULTS AND DISCUSSION

Growth Parameters: Growth parameters of *Delonix regia* plants at investigated polluted and unpolluted soils are presented in Table 2. It was generally observed that the growth parameters values of the Poincana plants grown in polluted soil were lower than those grown in the unpolluted one. These results were in agreement with that mentioned by Li *et al.* [21]. They reported that the disturbance in nutrient uptake, water imbalance and alterations in membrane permeability and metabolism as a result of heavy metal might explain this reduction in growth parameters.

Results of this study showed that when comparing the effect of AMF treated by heavy metals on the total fresh and dry weights of Poinciana plants, in polluted soil, to that of control and EDTA plants have the same treatment, in both seasons, it could be stated that the effect of AMF was the superior among the other treatments (Table 2). This effect exceeded that of either the control by 143.9-134.3% for the total fresh weight and by 138.1-145.5% for dry weight, in both seasons and the EDTA by 19.5-27.1% and 29.1-34.9 % in the same order. While the effect of EDTA had overcome that of the control by 103.9-84.3% and 84.4-82.1 % for both characters in the two seasons, respectively. The same trend with some extend was occurred, in both seasons, with the superior effect of AMF treated by heavy metals on the other studied characters; plant height, number of branches/plant and the stem diameter. These observations suggest that AMF play a protective role against excess metals and improve plant growth by assisting root growth and branching, in addition fungi have nutritional value in the acquisition of essential elements that are frequently present at low concentrations compared to nonessential metals in polluted soils [22]. In addition, Sun *et al*. [23] mentioned that the moderate dosage of EDTA could enhance plants growth and it would be helpful for *Delonix regia* to remediate metal-contaminated soils because improvement of plant growth under stressed conditions is critical to the optimum performance of phytoremediation.

Table 2: Growth parameters of *Delonix regia* as affected by AMF and EDTA-Fe under unpolluted and polluted soils during two seasons

Seasons	Treatments (T.)		Total fresh Weight (g/pot)	Total dry Weight (g/pot)	Plant height (cm)	No. of branches/Plant	Stem diameter (cm)
1st	Control	Unpolluted soil (P.)	53.6	24.8	37.7	11.0	0.8
		Polluted soil (P.)	36.7	17.3	25.0	5.7	0.5
		Mean	45.2	21.0	31.3	8.3	0.6
	AMF	Unpolluted soil (P.)	98.2	46.9	56.3	20.0	1.1
		Polluted soil (P.)	89.5	41.2	52.0	15.7	1.0
		Mean	93.9	44.1	54.2	17.8	1.0
	EDTA	Unpolluted soil (P.)	86.2	40.9	41.3	13.7	0.8
		Polluted soil (P.)	74.9	31.9	34.3	9.3	0.7
		Mean	80.5	36.4	37.8	11.5	0.8
2nd	Control	Unpolluted soil (P.)	49.6	24.0	33.0	9.3	0.7
		Polluted soil (P.)	33.8	15.6	24.3	5.0	0.5
		Mean	41.7	19.8	28.7	7.2	0.6
	AMF	Unpolluted soil (P.)	88.0	42.7	53.7	16.0	1.0
		Polluted soil (P.)	79.2	38.3	48.7	13.0	0.9
		Mean	83.6	40.5	51.2	14.5	1.0
	EDTA	Unpolluted soil (P.)	77.3	37.8	37.7	10.7	0.8
		Polluted soil (P.)	62.3	28.4	33.0	9.3	0.6
		Mean	69.8	33.1	35.3	10.0	0.7

		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	L.S.D. (0.05)										
	T.	13.3	8.7	5.7	5.0	3.7	4.6	3.1	2.2	0.1	0.1
	P. × T.	18.8	12.3	8.1	7.1	5.2	6.5	4.4	3.1	0.2	0.1

Arbuscular mycorrhizal fungus (AMF), Ethylenediamine tetraacetic acid (EDTA)

Table 3: Chemical parameters of *Delonix regia* as affected by AMF and EDTA-Fe under unpolluted and polluted soils during two seasons

Seasons		Treatments (T.)	Chlorophyll a content (mg/g f. w.)		Chlorophyll b content (mg/g f. w.)		Carotenoids content (mg/g f. w.)		Proline content (μ moles /g f.w.)	
1st	Control	Unpolluted soil (P.)	0.55		0.31		0.42		15.00	
		Polluted soil (P.)	0.43		0.15		0.29		35.00	
		Mean	0.49		0.23		0.36		25.00	
	AMF	Unpolluted soil (P.)	0.60		0.34		0.54		19.67	
		Polluted soil (P.)	0.52		0.24		0.46		54.33	
		Mean	0.56		0.29		0.50		37.00	
	EDTA	Unpolluted soil (P.)	0.66		0.39		0.57		16.33	
		Polluted soil (P.)	0.57		0.28		0.51		48.00	
		Mean	0.61		0.34		0.54		32.17	
2nd	Control	Unpolluted soil (P.)	0.52		0.30		0.38		11.67	
		Polluted soil (P.)	0.39		0.13		0.27		27.67	
		Mean	0.46		0.22		0.32		19.67	
	AMF	Unpolluted soil (P.)	0.58		0.32		0.52		14.00	
		Polluted soil (P.)	0.51		0.19		0.43		48.33	
		Mean	0.55		0.26		0.48		31.17	
	EDTA	Unpolluted soil (P.)	0.61		0.36		0.55		12.67	
		Polluted soil (P.)	0.55		0.24		0.48		42.33	
		Mean	0.58		0.30		0.52		27.50	
	LSD (0.05)		1st	2nd	1st	2nd	1st	2nd	1st	2nd
	T.		0.01	0.04	0.07	0.06	0.06	0.04	5.54	5.57
	P. × T.		0.02	0.06	0.10	0.08	0.08	0.06	7.83	7.88

Arbuscular mycorrhizal fungus (AMF), Ethylenediamine tetraacetic acid (EDTA)

Chemical Analysis

Photosynthetic Pigments: It was generally observed that the pigments content values of the Poincana plants grown in polluted soil were lower than those grown in the unpolluted one (Table 3). Hou *et al.* [24] confirmed the previous results by mentioning that there are three reasons may be responsible for the inhibitory effect of Chl. a, b and carotenoids in plants grown in heavy metal contaminated soils. Firstly, probably induce production of reactive oxygen species and inhibit the reductive steps in the biosynthesis pathway of these pigments. Secondly, they can directly destroy the structure and function of chloroplast by binding with SH group of enzyme and overall chlorophyll biosynthesis. Lastly, they may activate pigment enzyme and accelerate the decomposition of pigment. Rekha and Mastan [25] added that the reduction in the rate of photosynthesis indicates that the heavy metal impedes the photosynthetic activity by directly interfering in the process of photosynthesis. Heavy metal toxicity is also related to the oxidative damage induced in living systems, which can be promoted both by directly increasing the cellular concentration of reactive oxygen species (ROS) and by reducing the cellular antioxidant capacity [26].

The effect of EDTA in the polluted soil on the contents of the studied pigments (chlorophyll a and b and carotenoids) was the greatest among all other treatments. The chlorophyll (a) content of the plant treated with EDTA, in the polluted soil, exceeded that treated either with AMF by 9.6-7.8% or that of the control by 32.6-41.0% in both seasons (Table, 3). The same trend with some extend was noticed with the contents of the other two pigments (Table 3). Ruley *et al.* [27] referred that the increasing of the pigments in EDTA treatment due to a) increasing Fe concentration in the shoots which is affecting the structure of chlorophyll, b) the formation of heavy metal-EDTA complex which is unable to penetrate the plant membrane and c) EDTA reduces the mobility of heavy metal and then decreases its toxicity.

Meanwhile, Rahmaty and Khara [28] reported that the mycorrhizal fungi can enhance pigment content by increasing P uptake which contributes to pigment biosynthesis in its role as an energy carrier.

Proline Content: Plants have shown proline accumulation under heavy metals stress, besides the control plants maintained a similar level of proline content (Table 3).

Srivastava *et al.* [29] suggested that the accumulation of proline in metal-exposed plants is directly due to metal uptake, rather than to water deficit stress. It is possible that the functional significance of proline accumulation under heavy metal stress might include water balance maintenance, scavenging of hydroxyl radicals or metal chelation.

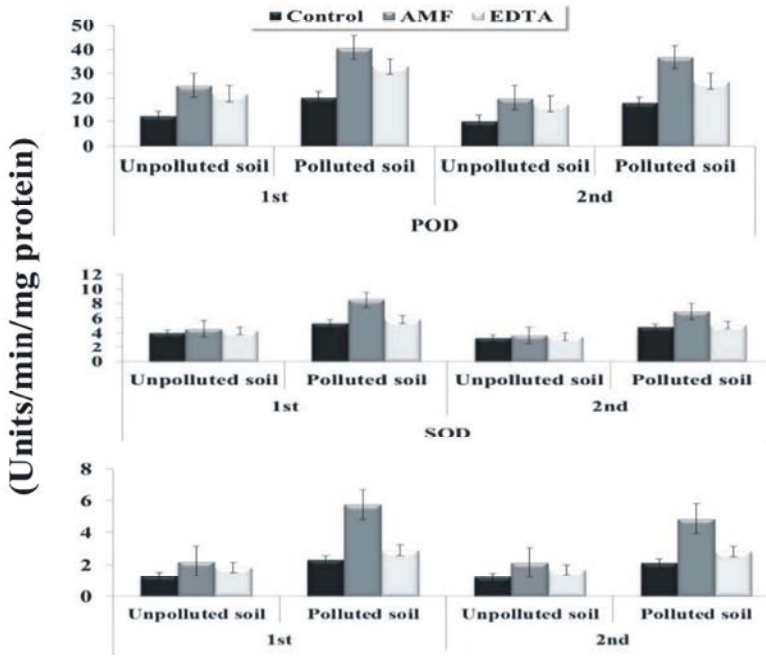

Fig. 1: The activity of SOD, POD and CAT in leaves of *Delonix regia* as affected by AMF and EDTA-Fe under unpolluted and polluted soil during two seasons. Vertical bars represent ±SE.

Adding AMF in polluted soil and comparing to that of EDTA was superior on the proline content. The content of proline of *Delonix regia* plant increased after adding AMF or EDTA in polluted soil due to decreased heavy metals induced oxidative stress and toxicity in the leaves. Xu *et al*. [30] stated that proline serves as a sink for energy to regulate redox potentials, acting as a hydroxyl radical scavenger, as a solute that protects macromolecules against denaturation and as a means of reducing acidity in the cell and it is the most important organic osmotic adjustment. Various environmental stresses such as heavy metals, drought and temperature have been caused to increase the level of proline content.

Activities of Antioxidant Enzymes: The application of chelators has been reported to mitigate Pb-induced oxidative stress by modulating antioxidant enzyme activities and this may also be one of the reasons for better growth of *Delonix regia* plants in the presence of a chelator in contaminated soil. As the heavy metals increase the production of H_2O_2 [31] and induce oxidative stress in plants [32], the antioxidant enzymes (SOD, POD and CAT) in leaves were increased (Fig. 1). So, increasing of SOD, POD and CAT activities may act as an adaptive response of plants exposed to heavy metals and contribute to heavy metals induce phytotoxicity [33].

In addition, Guo *et al*. [34] found that the antioxidant enzymes may work in a cooperative way to minimize the oxidative stress. Optimal protection is only achieved at an appropriate balance between the enzymes.

In addition, the effect of AMF treated by heavy metals was the greater than that of EDTA on SOD, POX and CAT activities in the leaves of Poinciana plants (Fig. 1). The results of this study indicated that the polluted plants inoculated with an AMF or adding EDTA-Fe had maximum SOD, CAT and POD activities compared to the unpolluted plants. This was consistent with previous reports obtained by Hajiboland *et al*. [35]. They implies that AMF colonization could alleviate the damage of ROS protect the plants against the damage occurred by oxidation and improve the extreme heavy metals tolerance of *Delonix regia*. The increased activity of some antioxidant enzymes may be due to nonspecific plant defense responses under heavy metals stress [36].

Concentrations of P and Fe in leaves: Generally from Table 4, plants grown in polluted soil had lower P and Fe concentrations than those of the unpolluted one. The results indicated that the effect of EDTA, comparing to that of AMF or that of the control, was the superior on Fe concentration by 1.5-1.7% or by 98.7-187.0%, respectively, in both seasons. Contrary, the effect of EDTA on the concentration of P came in the second category after that

Table 4: P and Fe (µg/g) in the leaves of *Delonix regia* as affected by AMF and EDTA-Fe under unpolluted and polluted soil during two seasons

	1st season			2nd season		
Treatments (T.)	Unpolluted Soil (P.)	Polluted Soil (P.)	Mean	Unpolluted Soil (P.)	Polluted Soil (P.)	Mean
			P mg/kg			
Control	1100	500	800	800	280	540
AMF	1500	1200	1350	1000	760	880
EDTA	974	700	837	717	440	579
Mean	1191.33	800	---	839	493.33	---
L.S.D. (0.05)	T. = 18.08			T. = 31.37		
	P. × T. = 25.56			P. × T. = 44.36		
			Fe mg/kg			
Control	299	476	388	156	308	232
AMF	422	932	677	674	869	772
EDTA	538	946	742	680	884	782
Mean	419.67	784.67	---	503.33	687	---
L.S.D. (0.05)	T. = 3.07			T. = 3.17		
	P. × T. = 4.35			P. × T. = 4.48		

Arbuscular mycorrhizal fungus (AMF), Ethylenediamine tetraacetic acid (EDTA)

Table 5: Dry weight, concentrations and uptake of lead, cadmium and zinc in the *Delonix regia* plant during two seasons

	1st season						2nd season					
	Unpolluted soil (P.)			Polluted soil (P.)			Unpolluted soil (P.)			Polluted soil (P.)		
Treatments (T.)	D.Mg / pot	Conc.mg / kg	Uptakemg / pot	D.Mg / pot	Conc.mg / kg	Uptakemg / pot	D.Mg / pot	Conc.mg / kg	Uptakemg / pot	D.Mg / pot	Conc.mg / kg	Uptakemg/pot
						Pb						
						Leaves						
Control	2.1	2.6	5.5	1.0	126	126	3.2	2.2	7.0	1.2	112	134
AMF	5.5	2.8	15.4	4.1	198	812	7.3	3.0	22.0	6.0	176	1056
EDTA	2.4	3.1	7.4	1.8	216	389	4.1	3.4	14	2.3	198	455
						Stems						
Control	18.1	9.0	163	12.5	512	6400	16.4	9.2	151	11.5	406	4669
AMF	29.2	10.8	315	25.9	602	15595	25.8	11.0	284	23.9	498	11902
EDTA	26.8	14.0	375	20.5	646	13243	24.9	13.8	344	19.3	586	11310
						Roots						
Control	4.6	26.0	120	3.8	622	2364	4.4	24.2	106	2.8	614	1719
AMF	12.2	32.2	393	11.2	718	8043	9.6	28.4	273	8.5	624	5304
EDTA	11.7	36.8	431	9.7	786	7624	8.9	35.7	318	6.8	688	4665
						Cd						
						Leaves						
Control	2.1	0.10	0.21	1.0	1.2	1.2	3.2	0.10	0.32	1.2	2.2	2.6
AMF	5.5	0.12	0.66	4.1	1.6	6.6	7.3	0.14	1.0	6.0	2.8	16.8
EDTA	2.4	0.22	0.26	1.8	2.4	4.3	4.1	0.18	0.74	2.3	3.6	8.3
						Stems						
Control	18.1	0.20	2.6	12.5	13.8	172.5	16.4	0.22	3.6	11.5	13.8	216.2
AMF	29.2	0.24	7.0	25.9	14.6	387.1	25.8	0.26	6.7	23.9	6.4	392.0
EDTA	26.8	0.30	8.0	20.5	17.3	354.7	24.9	0.32	7.9	19.3	19.4	374.0
						Roots						
Control	4.6	0.30	1.4	3.8	17.0	46.6	4.4	0.30	1.3	2.8	19.0	53.3
AMF	12.2	0.32	3.9	11.2	18.4	206.1	9.6	0.36	3.5	8.5	22.6	192.3
EDTA	11.7	0.38	4.4	9.7	16.2	157.1	8.9	0.42	3.7	6.8	26.8	182.3
						Zn						
						Leaves						
Control	2.1	10.6	22.0	1.0	42.6	43.0	3.2	9.8	31.0	1.2	44.0	53
AMF	5.5	12.8	70.0	4.1	45.4	168.0	7.3	11.2	82.0	6.0	44.8	269
EDTA	2.4	14.2	34.0	1.8	47.0	85.0	4.1	11.4	47.0	2.3	46.2	106
						Stems						
Control	18.1	16.8	304.0	12.5	50.4	630	16.4	14.6	239	11.5	50.4	580
AMF	29.2	17.6	541.0	25.9	52.6	1362	25.8	15.4	397	23.9	52.2	1248
EDTA	26.8	18.2	477.0	20.5	56.2	1152	24.9	16.8	418	19.3	54.6	1054
						Roots						
Control	4.6	15.8	73	3.8	50.0	190	4.4	14.0	62	2.8	51.8	145
AMF	12.2	16.0	195	11.2	54.2	607	9.6	15.2	146	8.5	58.0	447
EDTA	11.7	16.8	197	9.7	54.8	532	8.9	16.2	144	6.8	54.8	373

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of AMF. Since the later exceeded the former, in both seasons, by 71.4-72.7% and the control by 140.0-171.4%, respectively. This may be due to the effect of AMF in increasing the mineralization and nutrient uptake especially the uptake of phosphorus [37]. Also, AMF produce small cysteine-rich proteins known as metallothioneins, which are similar to phytochelatins, to facilitate metal accumulation [38].

In addition, some evidence suggests that the EDTA can desorb heavy metals from the soil matrix to form water-soluble metal complexes and to increase metal uptake by plants. So, EDTA-Fe can be absorbed by plants and translocated to shoots [39, 40].

Dry Weight of Leaves, Stems and Roots, Their Pb, Cd and Zn Concentration and Uptake: The effect of different treatments on dry weight of leaves, stems and roots of plants and the Pb, Cd and Zn concentrations and uptake are presented in Table 5. In both seasons, the dry weights of plants grown in most of the polluted soil were generally lower than those grown in the unpolluted soil. In each soil, raising the AMF level caused a steady increase in the dry weight of plants than raising the EDTA-Fe level where the later exceeded those of untreated plants.

The concentrations of Pb, Cd and Zn in dried plants varied depending on the used soil, parts of plant and treatments. In general, plant grown in polluted soil had higher concentrations of Pb, Cd and Zn than that in the unpolluted soil. The roots had higher Pb, Cd and Zn concentrations than leaves and the later exceeded the stems in these concentrations. Plants grown in polluted soil with EDTA had higher Pb, Cd and Zn concentration than those in polluted soil with AMF. Such results are in agreement with the findings of Chunilall *et al*. [41] on *Amaran*thus *hybrids* and *Amaranusan dubius*.

The Pb concentrations in the dried plants (leaves, stems and roots) grown in polluted soil, in the first season, ranged from 126 to 216, 512 to 646 and 622 to 786 µg/g dry matter, respectively. Meanwhile, in the second season these ranges were from 112 to 198, 406 to 586 and 614 to 688 µg/g dry matter, in the same order. These values are equal, approximately, to the value of 1000 µg/g dry weight reported for Pb hyperaccumulator [42].

The Pb concentration in dried leaves, stems and roots of plant grown in polluted soil were 48 to 70, 46 to 57 and 21 to 24 times, respectively, in the first season and 51 to 58, 42 to 44 and 19 to 25 times, respectively in the second season higher than in the unpolluted soil. On the other hand, the Cd and Zn concentrations in the dried leaves, stems and roots of plants grown in polluted soil were low but still approximately in the normal rang with Zn and little bit higher than the normal range with Cd (excessive range but not hyperaccumulator). These ranges, in the first season, were from 42.6 to 47, 50.4 to 56.2 and 50 to 54.8 µg/g dry matters for Zn and from 1.2 to 2.4, 13.8 to 17.3 and 17 to 18.4 µg/g dry matters for Cd, respectively. In the first season were from 44 to 46.2, 50.4 to 54.6 and 51.8 to 54.8 µg/g dry matters for Zn and from 2.2 to 3.6, 18.8 to 19.4 and 19 to 26.8 µg/g dry matters for Cd, respectively. These values are much lower than the values of 10000 and 100 µg/g dry weights for Zn and Cd, respectively, in hyperaccumulator plant reported by Baker and Brooks [42].

In both seasons, data showed that the Pb, Cd and Zn uptake by the plant was increased as a result of increasing the dry weight of leaves, stems and roots by different treatments compared to the control. Also, raising the AMF and EDTA levels caused a steady increase in the Pb, Cd and Zn uptake in most cases. So, in general, plant treated with AMF showed the highest Pb, Cd and Zn uptake, in both seasons. The result showed also that the plant extracted enormous amounts of Pb. In the first season, the Pb uptake in dried leaves, stems and roots of the plant grown in polluted soil ranged from 126 to 812, 6400 to 15595 and 2364 to 8043 mg/pot, respectively and from 134 to 1056, 4669 to 11902 and 1719 to 5304 mg/pot, in the second season. It means using AMF proved more efficient than using EDTA in remediating Pb polluted soil. Moreover, the results showed that the plants extracted normal amounts of Zn and excises amounts of Cd. This means that the studied plants are non hyperaccumulator for Cd and Zn and therefore, cannot be used to remediate Cd and Zn polluted soils.

DTPA Extractable Pb, Cd and Zn: The effect of the treatments and different soils on extractable Pb, Cd and Zn (µg/g) of the soil used in the experiment before and after treatments are presented in Table 6. In both seasons and in most cases, the extractable Pb, Cd and Zn of each used soils after treatments were markedly reduced as a result of any treatments increased the dry matter of the plants and their Pb, Cd and Zn uptake, compared to the control. Also, raising the AMF level caused a steady decrease in the extractable values of Pb, Cd and Zn of the treated soils with the lowest values has been found in control plants.

In general, soil sample of Abou Rawash gave the highest extractable values of Pb, Cd and Zn after treatments, while, soil of Faculty of Agriculture gave the lowest ones. Generally, in polluted soils, the extractable

Table 6: DTPA extractable Pb, Cd and Zn (µg /g) of the soils used in the experiments before and after treatments during the two seasons

		Lead			Cadmium			Zinc		
			After			After			After	
Soil	Treatments (T.)	Before	1st	2nd	Before	1st	2nd	Before	1st	2nd
Unpolluted soil (P.)	Control	0.40	0.36	0.37	0.1	0.1	0.1	3.8	3.78	3.8
	AMF		0.23	0.24		0.1	0.1		3.76	3.74
	EDTA		0.25	0.27		0.1	0.1		3.77	3.76
Polluted soil (P.)	Control	20.8	13.8	18.6	1.1	1.0	1.0	36.0	35.8	36.0
	AMF		13.2	15.4		0.60	0.60		35.6	35.9
	EDTA		14.8	16.4		0.70	0.70		35.7	35.9

Arbuscular mycorrhizal fungus (AMF), Ethylenediamine tetraacetic acid (EDTA)

Pb after treatment reducing from 20.8 to 13.2 in the first season and from 20.8 to 15.4 µg/g in the second seasons. In contrast, the plants caused only a negligible reduction in the extractable Zn in polluted soils and moderately reduction in the extractable Cd.

In conclusion, the result show that the studied plant can remediate Pb polluted soils, cannot remediate Zn and may be remediating Cd lowest polluted soils.

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