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Full Length Research Paper

Contribution of arbuscular mycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal-polluted soil

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The researcher had investigated the role of arbuscular mycorrhizal fungal inoculation in red kidney and wheat in heavy metals tolerance in soil artificially contaminated with high concentrations of zinc, copper, lead and cadmium. Metals accumulated by mycorrhizal wheat plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. Mycorrhizal red kidney plants could accumulate relatively high metal concentrations in their shoots which indicated that internal detoxification metal tolerance mechanisms are also included. From a number of physiological indices measured in this study, mycorrhizal symbiosis significantly increased root and shoot dry weight, chlorophyll content and total lipid in wheat plants. It also significantly increased root and shoot dry weight, protein content and the activity of antioxidant enzymes in red kidney plants, when the two plant species are grown at heavy metals artificially contaminated soils. The beneficial effects of the Am fungus observed in this study aroused an interest in considering the role of arbuscular mycorrhizal fungi in plant-based strategies of remediation of highly heavy metal contaminated soils.

Key words: antioxidant, chlorophyll, dry weight, mycorrhiza, phosphor, phytoremediation, pollution, symbiosis.

INTRODUCTION

Soil ecosystems have been extensively contaminated with heavy metals due to various human activities, possibly including mobilization of these metals up in the food chain, thereby threatening human health. Providing a direct physical link between soil and plant roots, the arbuscular mycorrhiza (Am) fungi are important rhizospheric microorganisms. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that Am fungi can colonize plant roots in metal contaminated soil (Vogel- Mikus et al., 2005), while their effects on metal uptake by plants are conflicting. In slightly metal contaminated soil, most studies show that Am fungi increased shoot uptake of metals (Weissenhorn et al.,1995), while in severely contaminated soil, Am fungi could reduce shoot metal concentration and protect plants against harmful effects of metals (Li and Christie, 2001; Malcova et al., 2003).

Both fungal isolates and plants may vary in their individual or combined tolerances to metals. Optimizing the use of Am fungi to permit growth of plants in soils contaminated with metals may require careful selection of specific fungal and host plant combinations for a given set of soil conditions. It will also require a skillful use of inorganic and organic amendments to maximize plant growth and to capitalize on interactions or competitions between metals and elements such as P and sulfur, whose uptake is generally enhanced in mycorrhizal plants. For example, increased P; through organic or

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Abbreviations: CAT, catalase; NBT, nitroblue tetrazolium; TCA, trichloro acetic acid; SOD, superoxide dismutase; AM, arbuscular mycorrhiza.

inorganic phosphate-amendments; may increase plant biomass and thus perhaps detoxify the potential effects of metals by dilution, precipitation or adsorption of metals onto polyphosphate granules. The non-target ecological effects of plants or fungi which have adsorbed, translocated and sequestered metals also need to be considered in parallel with the efforts to revegetate soils contaminated with high levels of metals. Efforts to phytoremediat, reclaim or restore vegetation to soils contaminated with metals by use of mycorrhizal plant species and inocula is gaining acceptance (Kahn et al., 2000; Entry et al., 2002; Turnau and Mesjasz-Przybylowicz, 2003; Vogel-Mikus et al., 2005.

A number of different types of plants are effective at stimulating the removal of heavy metals in the rhizosphere. Typically, these plants all have extensive and fibrous roots, which form an extended rhizosphere; these plants include many common grasses as well as corn, wheat, soybean, peas and beans (Shen and Shen, 2001; Oudehet et al., 2002; Stolt et al., 2003; Malcova et al., 2003 and Andrad et al., 2004. An experiment was established to (i) determine whether two factors, mycorrhizal infection and rock phosphate amended to the soil, had a marked effect on plant accumulation and tolerance of heavy metals spiked to the soil; and (ii) asses the metal tolerance strategies induced by Am fungal symbiosis in two different plant families; red kidney and wheat; with respect to Zn, Cu, Pb and Cd .

MATERIALS AND METHODS

Soil preparation

An agricultural topsoil contains 78 % sand, 10 % silt and 12 % clay, 7.3 pH. , 4.7 g kg^{-1} Total organic carbon , 3.4 g kg^{-1} total nitrogen, 25 mg kg^{-1} phosphorus. 34 mg kg^{-1} potassium and 1.9 mmoh m^{-1} E.C. It was air-dried, sieved to < 2mm and stored at 20 C before use .The soil was steam sterilized (121 C for 2 h) by autoclaving to eliminate native Am fungus propagules as well as other microorganisms, and left un-spiked or spiked with salts of Zn, Cu, Pb and Cd. Soil microorganisms were reintroduced by adding 10 ml per pot of soil suspension (10 g of the corresponding non-sterilized soil in 200ml water filtered through 20 um filter) and mist-sprayed on the soil during mixing .A water solution of Pb-, Cu, Zn-, and Cd- salts was gradually mixed into the soil over a period of 3 days to ensure a homogeneous distribution of the metals. The total amount of the added metals corresponded to 1500, 500, 500, 20 mg kg-1 of Pb, Cu, Zn, and Cd, respectively. The heavy metal salts were: $Pb(NO_3)_2$, $Cu(NO_3)_2$, ZnN_2O_6- $6H_2O$, and CdN_2O_6-$4H_2O$. De-ionized water was added to the soils to achieve a moisture content of 70% of field capacity. The soils were incubated at room temperature (at about 20^{o}C) for 1 month allowing metals to distribute into various fractions. During the period, soil moisture content was carefully monitored.

Experiments

The experiment examined factorial combinations of 1) two soil types a) non-spiked (non-polluted) soil and b) spiked (polluted) soil, 2) t wo mycorrhizal treatments a) non - inoculated and b) inoculated with Glomus mosseae (Nicol. & Gerd.), 3) two rock phosphate treatments a) non-treated and b) treated. Five replications per each treatment were used to give a total 40 pots for each one of the two plant species used in this investigation.

Rock phosphate (26.4% P_2O_5, Abu Zaabal phosphate fertilizer Co.) at the rate of 0.5% (w/w) of each were mixed with the soil before sowing. Seeds of wheat (Triticum aestivum c.v. Sakha 8) and red kidney (phaseollus vulgaris c.v.prince) plants were made disinfectant by soaking them in 30 % H_2O_2 for 20 min, washing them three times in distilled water and growing them in black plastic pots containing 200 g of the spiked soil. Mycorrhized planted pots received 25 g of a mycorrhizal inoculum's (20 spore's g^{-1} Glomus mosseae was provided from faculty of science, Mansura university Egypt.). Mineral nutrients were added uniformly to the soil at rates of 162 mg N (urea) 126 mg K and 50 mg P (K_2HPO_4) kg^{-1} soil. Pots were sown with 5 pre-germinated seeds of each species and thinned to 3 plants in each pot after 10 days. The pots were arranged in growth chamber at 25 - 20^{o}C day / night, 11 h day, 60-70% relative humidity, and using de-ionized water adjusted regularly to 70% of field water capacity. The plants were harvested 60 days after sowing date.

Analytical methods

The shoots were removed by cutting above the soil surface, and roots were recovered by washing with de-ionized water. Dry weights of roots and shoots were determined after drying at 70^{o}C overnight, before grinding to pass 0.25 mm sieve.

For metal analysis, a 30 mg aliquot of freeze-dried and milled aboveground or underground plant material was mineralized by wet digestion in 3.5 ml of ultra pure mixture of concentrated HNO3 / HClO4 (Merck) = 7/1 (v/v) on an Al block after 24 h incubation period in the same mixture at room temperature. The samples were then diluted with 5 ml of 0.2% HNO_3 and all metal concentrations in extracts were measured (Maff 1986) by atomic absorption spectroscopy (Pye-Unicam SP9).

Photosynthetic pigments (total chlorophyll content) of plant leaves were extracted and determined by the method of (Harbon, 1984) . Protein contents of plant tissue were estimated according to Bradford (1976). Lipid content was extracted from fresh plant samples according to Folch et al. (1957) and total lipids were estimated according to Marsh & Weintein (1966). Phosphorus concentration in plant tissue were measured using the Vanadono molybdophosphorich colometric method (Jackson 1967).

Content of H_2O_2 in plant tissues was determined with Spectrophotomer (Beckmann, Munich, Germany), based on the modified method of Patterson et al. (1984) . Fresh plant samples (150–300 mg) were frozen in liquid nitrogen and ground to powder in a mortar together with frozen 5% TCA (1.5 ml) and with activated charcoal (45 mg). The homogenate was centrifuged at 18000 x g for 10 min at 0°C. The supernatant was filtered through a nylon filter (45 um,) and the filtrate was adjusted to pH 8.4 with NH_4OH. After re-filtration through a nylon filter, a 500-ul aliquot was brought to 1 ml by adding 500 ul of colorimetric reagent. The colorimetric reagent was made daily by mixing 1:1 (v:v) 0.6 mM 4-(2-pyridylazo) resorcinol (disodium salt) (Sigma) and 2% titanium chloride (diluted from 20 % TiCl2 in 1 N HCl and adjusted to pH 8.4) and was maintained in ice until use. The mixture was incubated for 60 min at 45°C and contents of H_2O_2 (E = 3.67 uM^{-1} cm^{-1}) were determined from A508, using H_2O_2 (30 % Sigma) (5-50 uM) as a standard.

All samples of plants were prepared for enzyme analyses by homogenization of the fresh tissue material with a mortar and pestle and a small amount of sand in a solution (5 ml g^{-1} fresh weight) containing 50 mM KH_2PO_4 : K_2HPO_2 (pH 7.0), 10 g l^{-1} polyvinyle-polypyrrolidone (PVP), 0.2 mM EDTA and 10 ml l^{-1} Triton X-100. After the homogenate was centrifuged at 12000 x g for 20 min at 4°C, the supernatant was used for immediate

determination of enzyme activities. All spectrophotometer analysis were conducted on a spectrophotometer (Beckmann, Munich, Germany). Activity of CAT was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 2 ml 29.8 mM H_2O_2 in KPO_4 buffer (pH 7.0) and 1 ml extract [Beers and Sizer,1952]. Activity of SOD was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the modified method of Becana et al. (1986). The reaction medium comprised 0.25 ml 50 mM Na–phosphate buffer (pH 7.8) with 0.1 mM Na2 EDTA, 2.73 ml O_2--generating solution and 20.45 ml extract. The O_2--generating solution contained 2.2 uM riboflavin, 14.3 mM methionine, and 82.5 uM NBT. Glass cells containing the mixture were placed in a cylindrical bath lined with aluminum foil at 25°C and fitted with a 22 W fluorescent lamp. The reaction was initiated by turning the light on and the reduction of NBT was followed by reading the A560 for 10 min. Blanks were run the same way but without illumination. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under the assay conditions. Activity of peroxidase (POX) was measured by monitoring the H_2O_2 -dependent oxidation of reduced guaiacol at 470 nm (Chance & Maehly 1955) . One unit was defined as the enzymic amount which oxidizes 1 uM guaiacol min-1 (e =26.6 mM^{-1} cm^{-1}).Total activities (U) of enzymes and contents and (nmol) of H_2O_2 were expressed on afresh weight basis.

Immediately after harvest part, of the root system was washed carefully in $4^{O}C$ water to remove the adhering soil particles and cut to 0.5 – 1.5 cm segments. The roots were cleared and stained by using the methods by Philips and Hayman (1970) and percentage of mycorrhizal colonization (F %) was estimated by the methods of Trouvelot and Gianinazzi (1986).

The translocation factor (TF) for metals within a plant was expressed by the ratio of (Metal) Shoot / (Metal) Root to show metal translocation properties from roots to shoots (Stoltz and Greger, 2002).

Tolerance indices (Ti) of Am and non-Am plants to heavy metals in the soil were determined as:

$$Ti = \frac{\text{Dry weight of plant at polluted soil x 100}}{\text{Dry weight of plant at non-polluted soil of the same treatments}}$$

Statistical analysis

A statistical comparison of means was examined with ANOVA and Pearson correlation coefficient available in the SPSS statistical package. Significance was set at *P <0.05 and ** P < 0.01.

RESULTS

Shoot and root heavy metal accumulation

Observations during the experiment and at the harvest revealed that some symptoms of toxicity or deficiency were recorded on non-mycorrhized plants grown in polluted soil, irrespective of the presence and absence of rock phosphate relating to either of the heavy metal (Zn , Cu, Pb, Cd) treatments (data not shown). Initially, plants grown in the non-polluted (np) soil grew markedly better than those in the polluted (p) soil.

The highest Zn concentration was observed in root and shoot for wheat and red kidney respectively in the presence of Am fungi in polluted soil amended with rock phosphate (Figure1a) . At polluted soil Am and non-Am red kidney as well as non- Am wheat plants accumulated significantly higher Zn in its shoots than in its roots. Whereas Am wheat plants showed higher Zn concentration in its root than that in its shoot especially in the polluted soil amended with rock phosphate.

The results of Figure 1b also showed that in red kidney plants Cu concentrations were higher in roots than in shoots in the absence of Am fungi, while it exhibited higher concentrations in shoots than that in roots in the presence of Am fungi . On the contrary , non-Am wheat plants showed higher Cu concentrations in shoots than in roots, whereas Am wheat plants accumulated more Cu concentrations in roots than in shoots . The results of the Table 1 revealed that total Cu accumulations in tissues of red kidney and wheat plants positively correlated to mycorrhizal symbiosis in the soil.

The results of Table 1 also showed that there are significant effects and positive correlations between Am symbiosis and rock phosphate with total Pb and Cd accumulations in the two plant species however, these effects were clearly different in the two plants (Figure1c & d). In red kidney plants, Pb and Cd root concentrations were higher than that in shoots in the absence of Am fungi in polluted soil. However, the presence of Am fungi leads to more accumulations of Pb and Cd in shoots than that of roots especially in the presence of rock phosphate. Meanwhile, an Am wheat plant significantly accumulates Pb and Cd in roots much higher than in shoots; however this accumulation was increased in the presence of rock phosphate.

The translocation factors (TF), the ratio of the shoot to root metals, indicate internal metal transportation. The data presented in Table 2 showed that the rate and extent of translocation within plants depend on Am symbiosis and the plant species concerned. Zn accumulated by the plant studied was largely translocated to shoot as shown by general TF values > 1. Exceptions occurred on Am wheat plants (TF < 1). However, Cu metal was accumulated in shoots of Am red kidney and non-Am wheat plants (TF > 1) meanwhile, it was retained in roots of other treatments (TF < 1). On the other hand, Pb and Cd metals accumulated by Am wheat plant in roots as shown by general TF values < 1 (Table 2). On the contrary, the presence of Am fungi infected red kidney plants might increase the translocation of these heavy metals from roots to shoots; therefore Pb and Cd metals would become more concentrated in Am red kidney shoots than roots as indicated by TF > 1.These results indicated that the effect of Am fungi on metal uptake and translocation within plant would depend on the type of mechanisms by means of which Am fungi would protect host plant from heavy metal accumulation.

The development of Am symbiosis varied between host plants (Table 2) .While the average value of mycorrhizal colonization in wheat roots reached

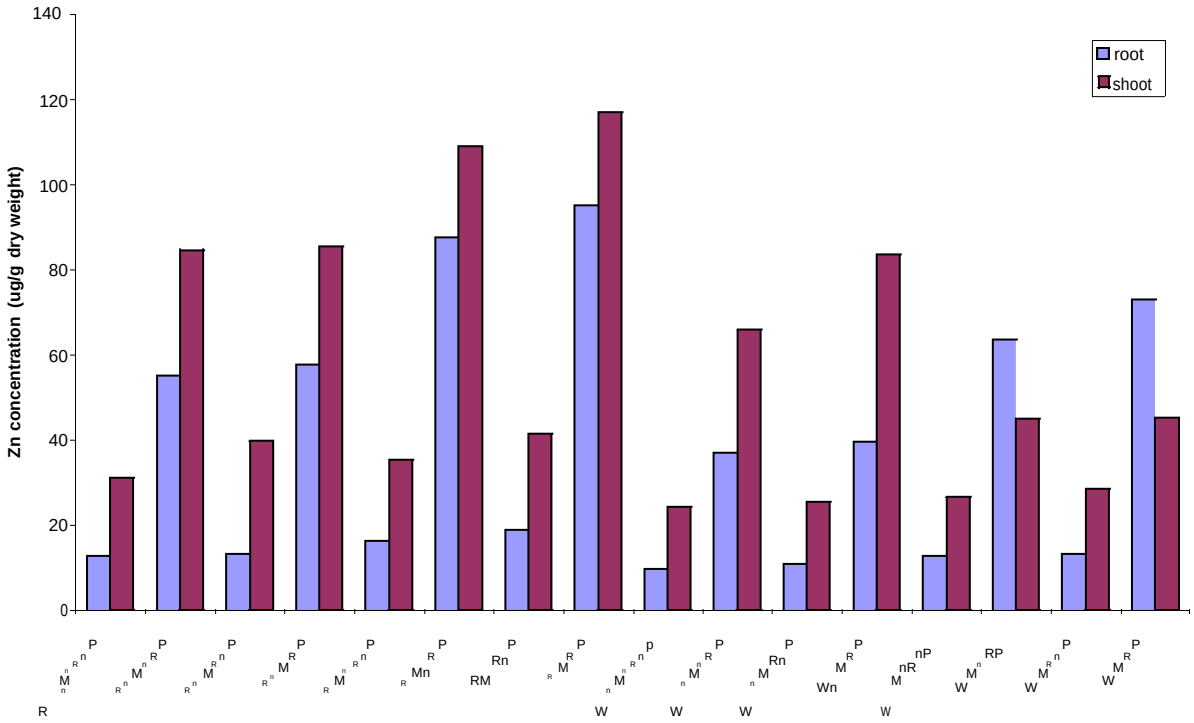

Figure 1a. Zinc concentrations in Am and non-Am plants in polluted and non-polluted soil. R=red kidney, W=wheat, nM=non-mycorrhized, M=mycorrhized, nR=no rock phosphate, R=rock phosphate, np=non-polluted, P= polluted soil.

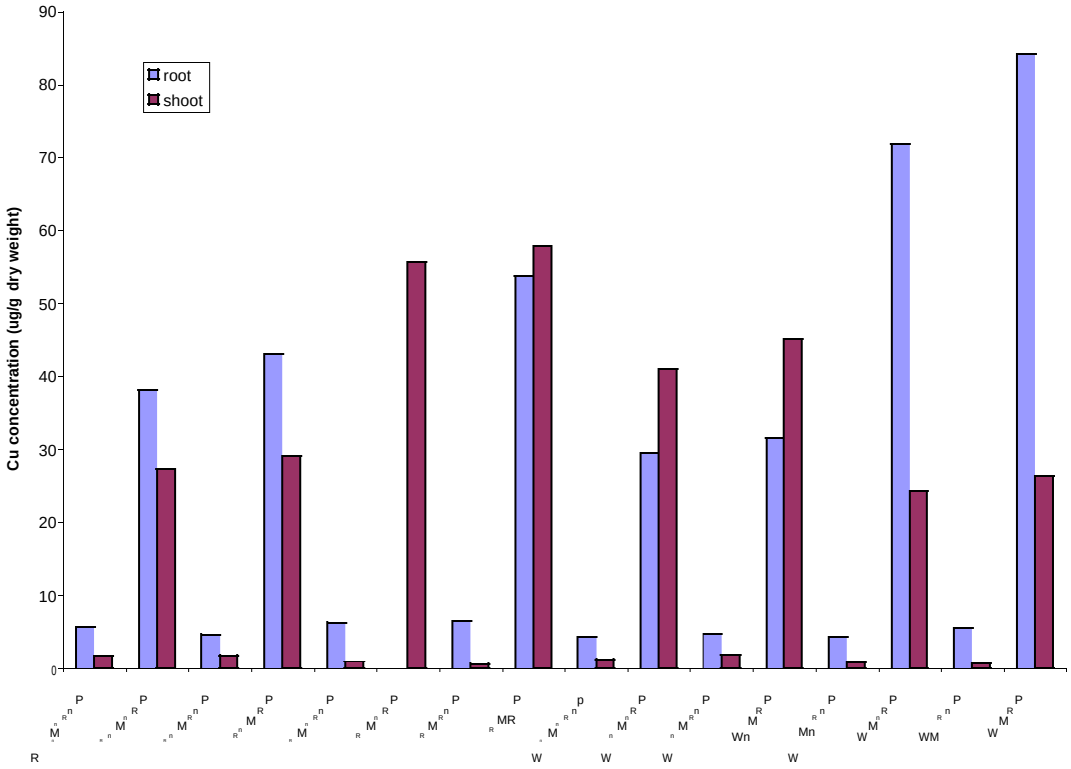

Figure 1b. Copper concentrations in Am and non-Am plants in polluted and non-polluted soil. R=red kidney, W=wheat, nM=non-mycorrhized, M=mycorrhized, nR=no rock phosphate, R=rock phosphate, np=non-polluted, P= polluted soil.

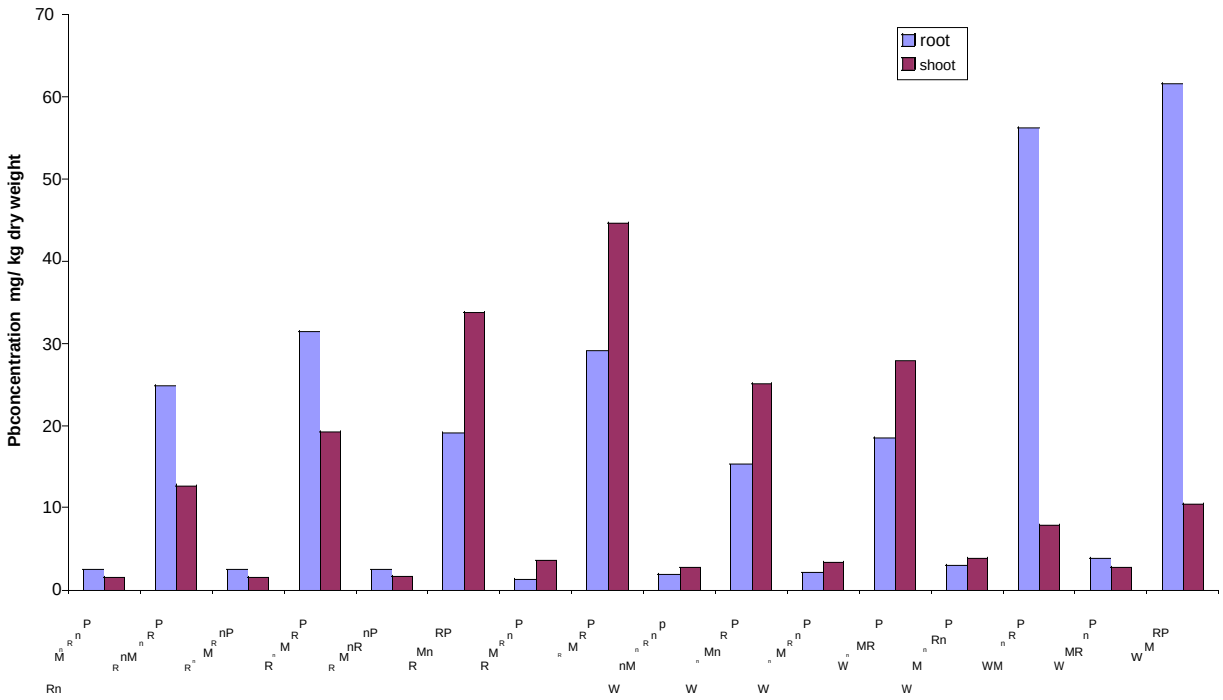

Figure 1c. Lead concentrations In Am and non- Am plants in polluted and non-polluted soil (R= red kidney), W= Wheat, nM= non- Mycorrhized, M = mycorrhized, nR= no rock phosphate, R= rock phosphate, np= non-polluted, P=polluted soil.

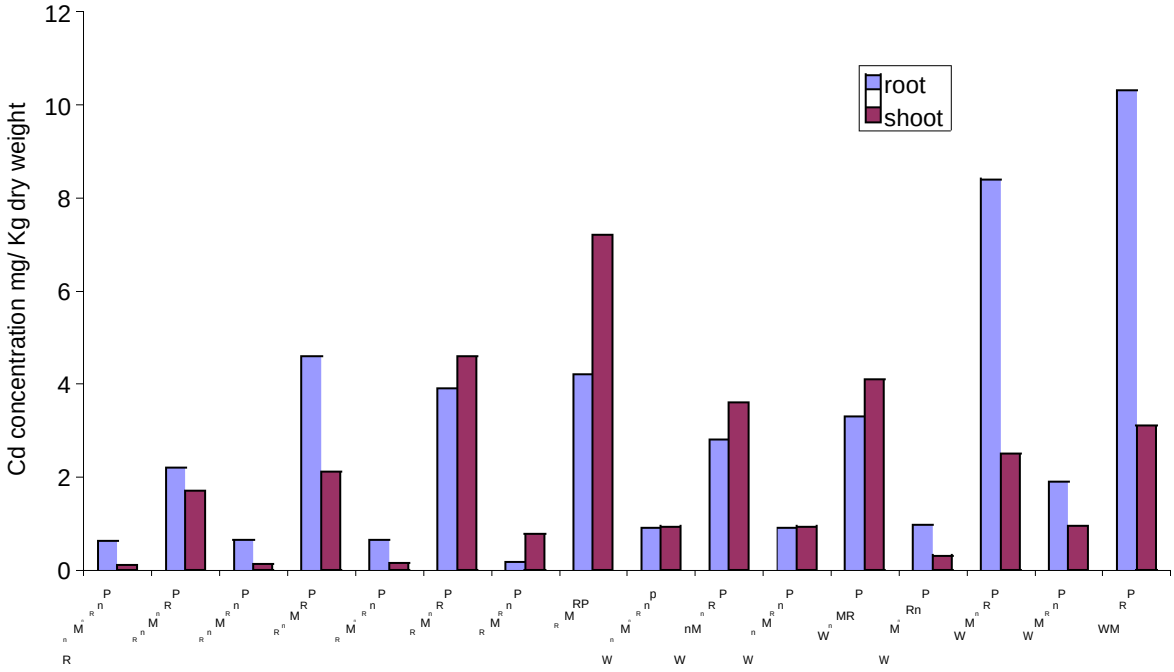

Figure 1d. Cadmium concentrations in Am and non-Am plants in polluted and non-polluted soil (R=red kidney), W=wheat, nM=non-mycorrhized, M=mycorrhized, nR=no rock phosphate, R=rock phosphate, np=non-polluted, P= polluted soil.

Table 1. ANOVA (significant F) and correlation coefficient (r) for metal concentrations of Am and non Am plants grown in soil with or without heavy metal pollutants and rock phosphate.

Variables	Plant	Statistics	Zn	Cu	Pb	Cd
Heavy metal polluted soil	Red kidney	Sig F	0.025*	0.015*	0.016*	0.013*
		Sig r	0.549*	0.528*	0.811**	0.719*
	Wheat	Sig F	0.002**	0.027*	0.043*	0.032*
		Sig r	0.881**	0.582*	0.565*	0.675*
Mycorrhizal symbionts	Red kidney	Sig F	0.065	0.009**	0.05*	0.029*
		Sig r	0.431	0.681*	0.532*	0.531*
	Wheat	Sig F	0.079	0.041*	0.006**	0.017*
		Sig r	0.432	0.526*	0.851**	0.641*
Rock phosphate amendment	Red kidney	Sig F	0.263	0.159	0.348	0.38
		Sig r	0.369	0.374	0.183	0.071
	Wheat	Sig F	0.089	0.238	0.05*	0.041*
		Sig r	0.193	0.351	0.513*	0.591*

* = significant difference (P< 0.05).
** = highly significant difference (P< 0.01).

Table 2a. Translocation factors (TF = $\{Metal\}_{shoot}$ / $\{Metal\}_{root}$) and tolerance index in Am and non Am plants grown in soil with or without heavy metal pollutants and rock phosphate.

Plant	AM state	Rock phosph.	Soil state	Zn	Cu	Pb	Cd	AM infection F %	Tolerance index
Red kidney	-ve	-ve	np	2.4	0.3	0.0	0.0	0.0	42
			p	1.5	0.7	0.4	0.2	0.0	
		+ve	np	3.0	0.4	0.0	0.0	0.0	55
			p	1.5	0.7	0.5	0.2	0.0	
	+ve	-ve	np	2.2	0.1	0.0	0.0	58	87
			p	1.2	1.2	1.2	1.1	51	
		+ve	np	2.2	0.1	0.0	0.0	75	91
			p	1.2	1.1	1.3	1.2	64	
Wheat	-ve	-ve	np	2.5	0.3	0.0	0.0	0.0	47
			p	1.8	1.4	1.16	1.1	0.0	
		+ve	np	2.4	0.4	0.0	0.0	0.0	59
			p	2.1	1.4	1.2	1.14	0.0	
	+ve	-ve	np	2.1	0.2	0.0	0.0	78	78
			p	0.7	0.3	0.04	0.06	66	
		+ve	np	2.2	0.1	0.0	0.0	79	83
			p	0.6	0.3	0.04	0.05	69	

+ve Am = plant infected with *G. mosseae*
-ve Am = plant non-infected with *G. mosseae*

Table 2b. ANOVA (significant F) and correlation coefficient percentage of mycorrhizal infection of Am plants grown in soil with or without heavy metal pollutants and rock phosphate.

Variables	plants	Statistics	Heavy metal polluted soil	Rock phosphate amendment
Mycorrhizal infection F %	Red kidney	Sig F	0.039*	0.008**
		Sig r	-0.681*	0.721*
	Wheat	Sig F	0.026*	0.003**
		Sig r	-0.578*	-0.659*

approximately 64% , it was about 73% in the roots of red kidney plants . Root colonization of the two plant species was significantly reduced and negatively correlated by the presence of heavy metals in soil (Table 2), it was decreased from 79% to 69% for red kidney and from75% to 64% for wheat in non-polluted and polluted soil respectively. On the other hand, rock phosphate amendment to the soil showed highly significant influence and positive significant correlation with mycorrhizal root colonization in red kidney and wheat plants in the presence or absence of heavy metal in the soil.

The effect of heavy metal pollutants on the plant studied as indicated by tolerance index was recorded in Table 2. The results showed that the tolerance index of wheat plants was higher than that of red kidney in absence of Am fungi. On the contrary, in the presence of Am fungi, red kidney plants showed higher tolerance index than that of wheat plants . On the other hand, the results also showed that the tolerance index of red kidney and wheat plants markedly increased by the presence of Am fungi especially in the presence of rock phosphate. Therefore, it was proposed that Am infection has a potentiality of increasing heavy metal tolerance for plants.

Physiological indices

Growth responses

The results presented in Table 3 and Figures 2, 3& 4 reveal the growth responses of red kidney and wheat plants grown in heavy metal polluted soil in the presence and absence of Am fungi with and without rock phosphate .The dry weight of red kidney and wheat plants, either roots or shoots, were significantly affected by soil pollution and negatively correlated with the presence of heavy metal in the soil (Table 3). However, Am fungi inoculations had a significant influence on root and shoot dry weight of both red kidney and wheat plants, where the presence of Am fungi caused a decrease in the inhibiting effects of heavy metal pollutants on dry weight of roots and shoots in the studied plants (Figure 2). Although the results of Table 3 showed that rock phosphate amendment to the soil had insignificant effect on dry weight of the two plant species, the dry weight was higher in soil amended with rock phosphate in comparison with non-amended soil (Figure 2).

The data given in Figure 2 show that the chlorophyll content of Am and non-Am plants were generally reduced by the presence of heavy metal pollutants in the soil. The value obtained for the Am plants remained greater than those given by non-Am plants, where the % reduction in chlorophyll content was lowered from 48% to 19% in red kidney and from 63% to 8% in wheat in the presence of Am fungi. In addition, the plants grown in soil amended with rock phosphate still had higher values than that grown in non-amended soil.

As shown in Table 3 heavy metals in polluted soil significantly diminished protein content in red kidney while it had an insignificant effect on protein content in wheat plants. In the presence of Am fungi, the protein content significantly increased for the two plant species studied (Figure 3) . However, the protein content of Am red kidney plants at polluted soil increased by 27% of that at non-polluted soil in soil amended with rock phosphate, meanwhile the protein content of Am wheat plants were closely similar in polluted and non-polluted soil.

The results of Table 3 showed that heavy metals in polluted soil had an insignificant effect on total lipid of red kidney while it had significant reduction in total lipid content of wheat. Mycorrhizal symbiosis showed a significant effect and positive correlation with the lipid content of red kidney and wheat plants grown in polluted and non-polluted soil. The inoculation of Am fungi to wheat plants minimized the negative effect of heavy metals on total lipids (Figure 3). The percentage reduction in lipid content of wheat plants decreased from 56% in absence of Am fungi to 16% in presence of Am fungi in soil amended with rock phosphate. The results of Table 3 also revealed that rock phosphate amended to the soil showed a significant effect on the lipid content of the two plant species, where the lipid content of the plants grown in soil with rock phosphate was still higher than that without rock phosphate especially in the presence of Am fungi (3).

The data in the present investigation show that the phosphorus content of Am and non- Am plants were generally decreased by the presence of heavy metal pollutants in the soil. Am inoculations had a significant effect on phosphorus content (Table 3) where the value of this element obtained in the Am plants remained greater than those given by non-Am plants (Figure 4).

H_2O_2 production and antioxidant enzymes

The results in the Table 4 showed that the presence of heavy metal pollutants in the soil were correlated positively and significantly with H_2O_2 production in the studied plants , and also had significant and highly significant influence on H_2 O_2 content of wheat and red kidney respectively. In this connection, H_2O_2 contents in the red kidney and wheat plants grown in polluted soil were much higher than that in non-polluted one (Figure 5). Am fungi infected to the plants studied had an insignificant effect and correlation with H_2O_2 contents in the red kidney and wheat plants (Table 4). Nevertheless, in the presence of heavy metals, Am plants showed lower H_2O_2 levels compared with non-Am plants while, in absence of heavy metals, there is no significant differences in H_2O_2 levels between mycorrhized and non-mycorrhized plants (Figure 5).

Table 3. ANOVA (significant F) and correlation coefficient for root and shoot dry weight, chlorophyll, protein, lipids and phosphorus contents of Am and non-Am plants grown in soil with or without heavy metal pollutants and rock phosphate.

Variables	Plants	Statistics	Root dw	Shoot dw	Chlorophyll	Protein	Lipids	Phoshorus
Heavy metal polluted soil	Red kidney	Sig F	0.035 *	0.045 *	0.073	0.041 *	0.143	0.083
		Sig r	-0.649 *	-0.572 *	-0.311	-0.489	-0.371	-0.449
	Wheat	Sig F	0.007 **	0.427	0.156	0.082	0.026 *	0.076
		Sig r	-0.681 *	-0.354	-0.265	-0.275	-0.635 *	-0.485
Mycorrhizal symbionts	Red kidney	Sig F	0.005 **	0.007 **	0.092	0.049 ^	0.016 ^	0.045 ^
		Sig r	0.831 **	0.581 '	0.479	0.511 '	0.559*	0.684 '
	Wheat	Sig F	0.017 *	0.041 *	0.046 *	0.079	0.014 *	0.032 *
		Sig r	0.932 **	0.593 *	0.551 *	0.401	0.612 *	0.728 *
Rock phosphate amendment	Red kidney	Sig F	0.063	0.159	0.348	0.38	0.05 ^	0.157
		Sig r	0.419	0.374	0.183	0.071	0.463	0.239
	Wheat	Sig F	0.074	0.148	0.185	0.081	0.034 *	0.356
		Sig r	0.293	0.191	0.213	0.491	0.573 *	0.296

* = significant difference (P< 0.05)
** = highly significant difference (P< 0.01)
dw = dry weight

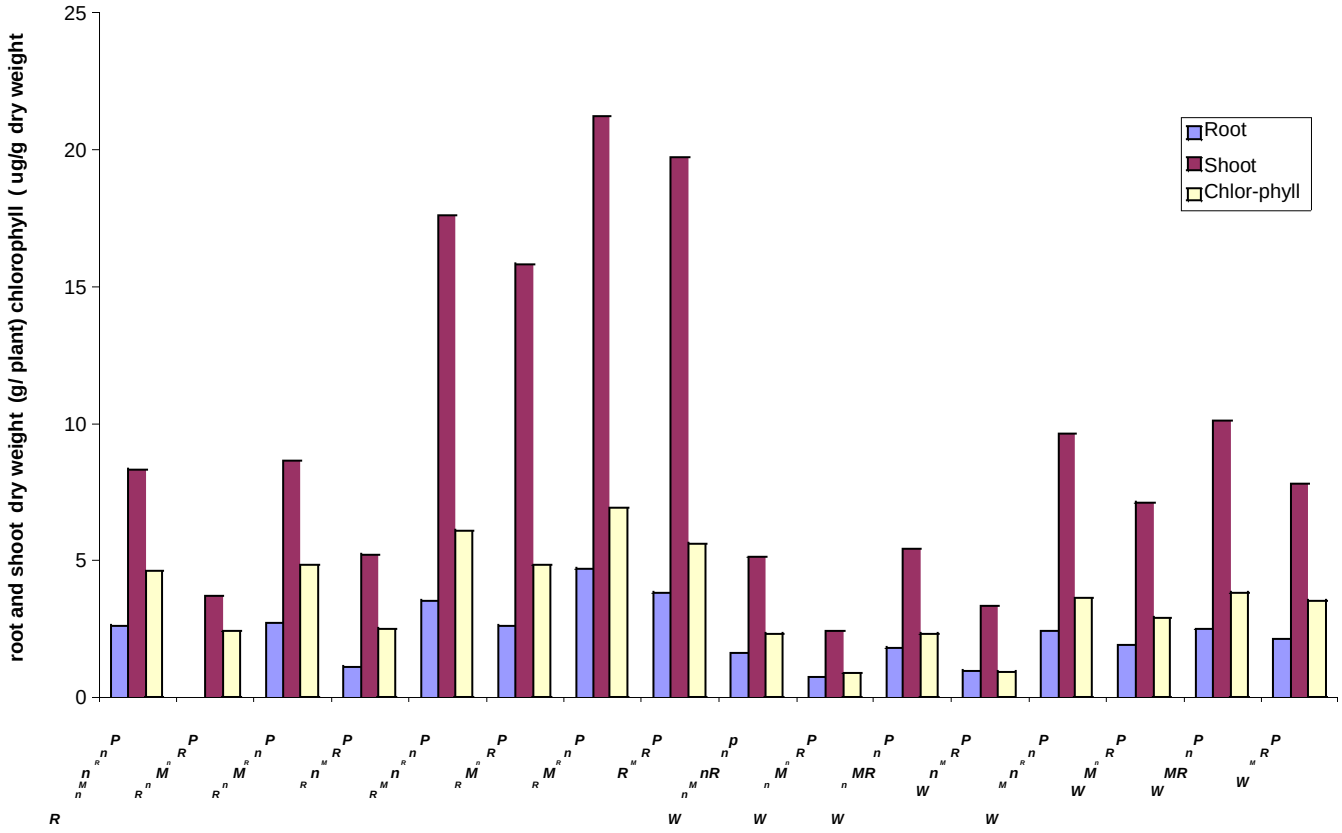

Figure 2. Root,shoot and chlorophyllof Am and non-Am plants grown in soil with and without heavy metal pollutants (R=red kidney, W=wheat, nM=no mycorrhiza, M=mycorrhiza, nR=no rock phosphat, R=rock phosphate, nP=non-polluted, P=polluted soil).

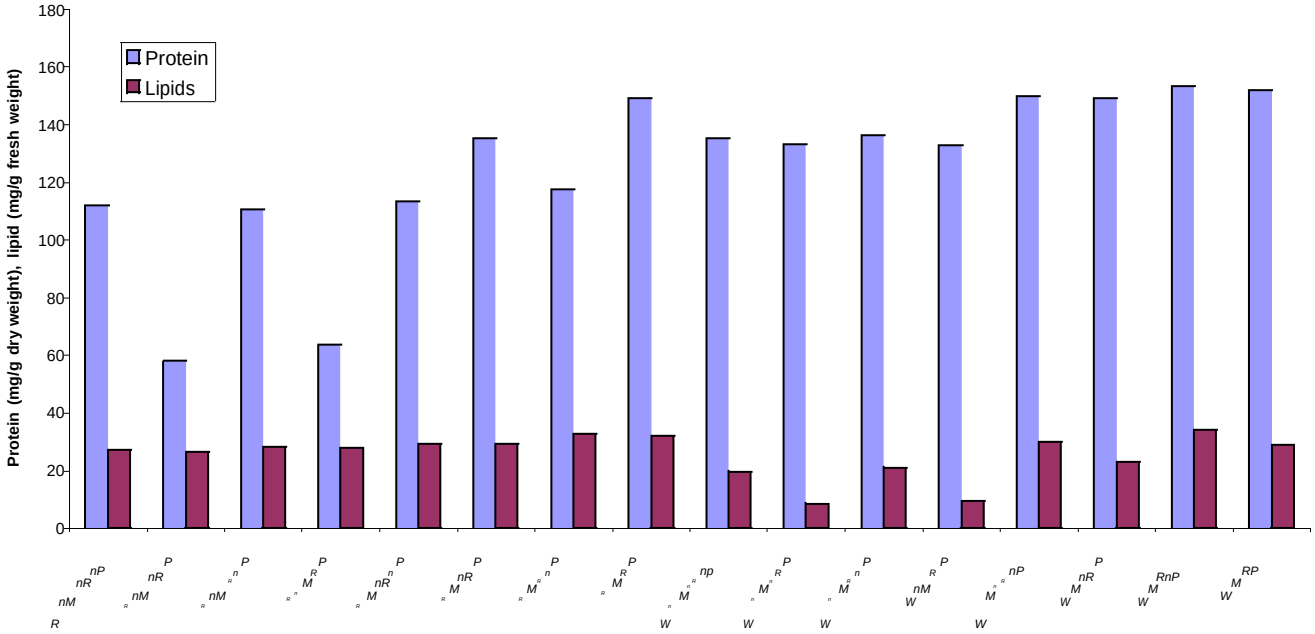

Figure 3. Protein and lipid contents of Am and non-Am plants grown in soil with and without heavy metal polluted soil (R=red kidney, W=wheat, nM=no mycorrhiza, M=mycorrhiza, nR=no rock phosphate, R=rock phosphate, nP=no polluted, P=polluted soil).

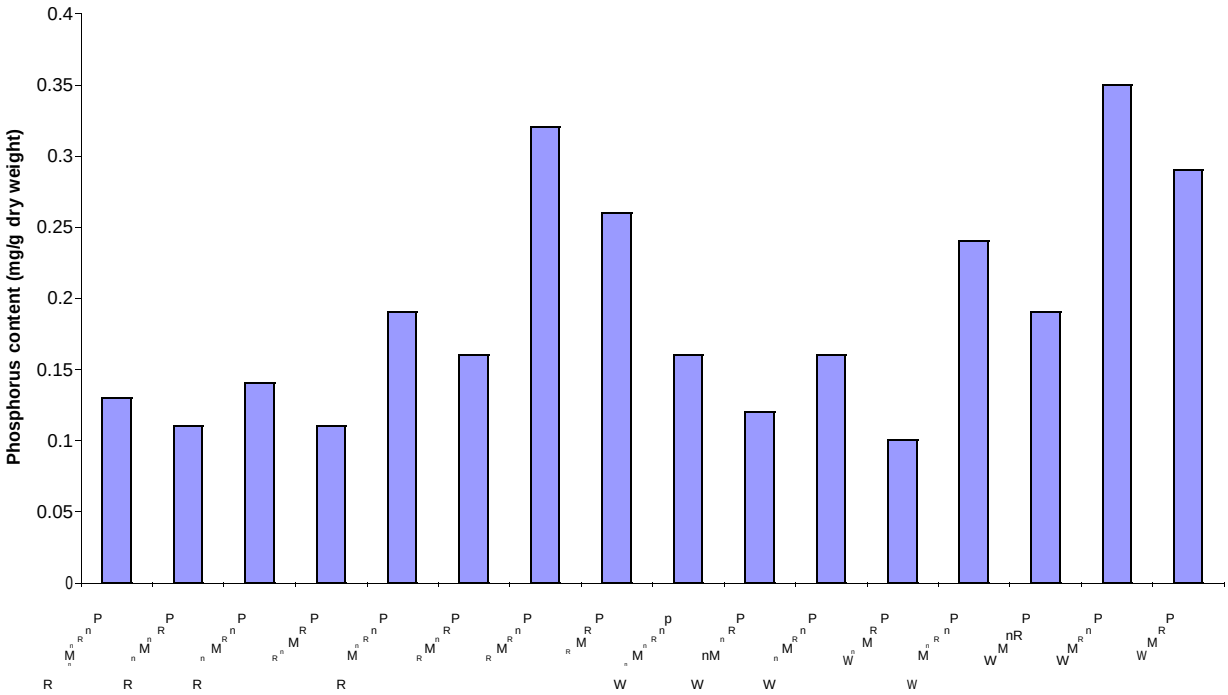

Figure 4. Phosphorus content of Am and non-Am plants grown in soil with and without heavy metal pollutants (R=red kidney, W=wheat, nM=no mycorrhiza, M=mycorrhiza, nR=rock phosphate, R=rock phosphate, nP=no polluted, P=polluted soil).

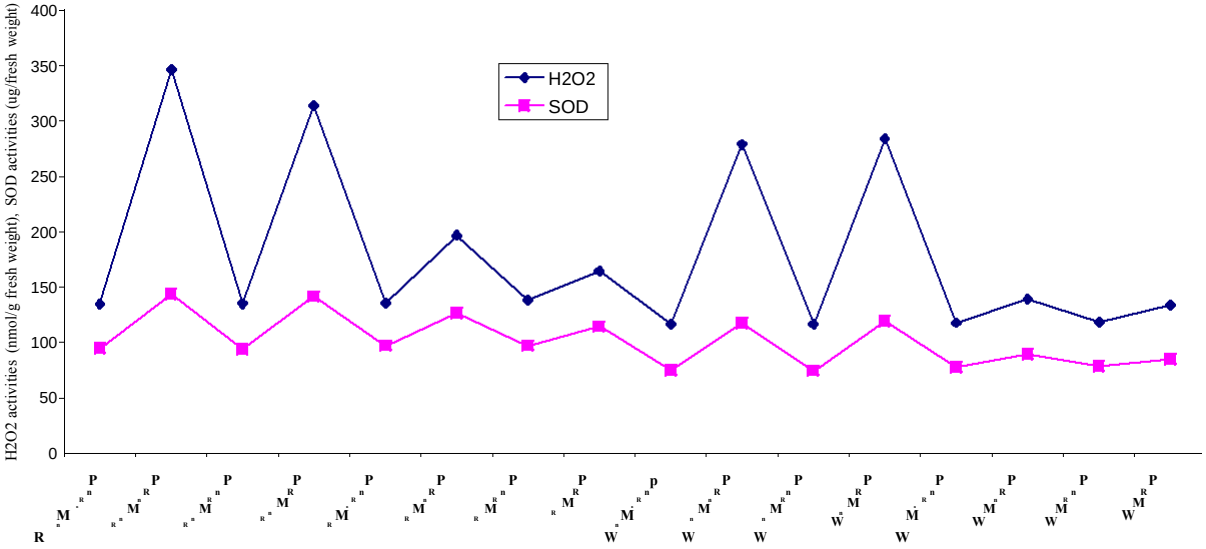

Figure 5. Activities of H_2 O_2 and antioxidant enzymes in Am and non-Am plants grown in soil with and withought heavy metal pollutants. (R=red kidney, W=wheat, nM=no mycorrhiza, M=mycorrhiza, nR=no rock phosphate, nP=no polluted, P=polluted soil).

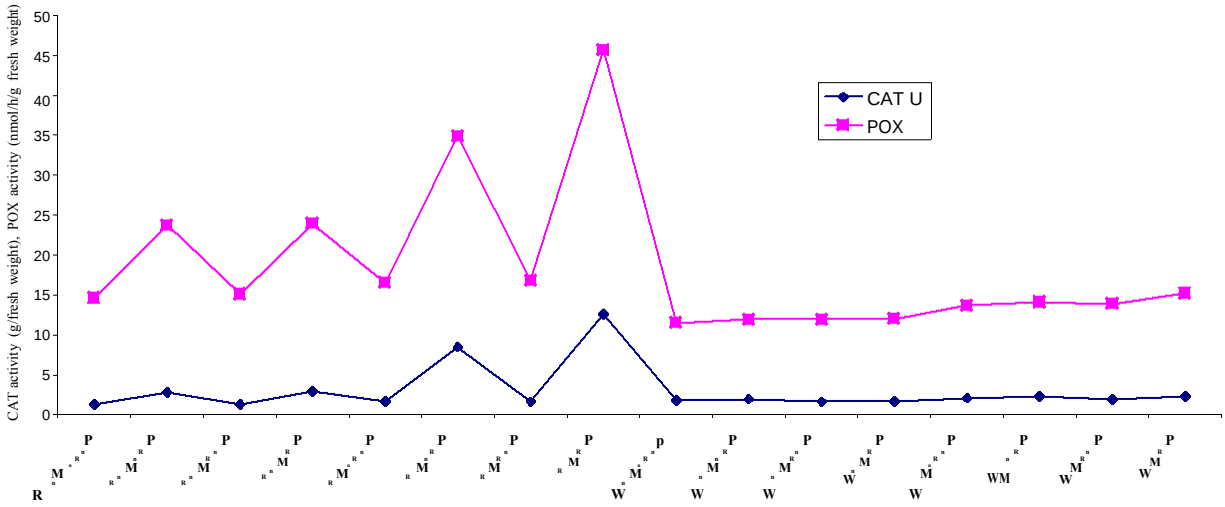

Figure 6. Activities of CAT and POX enzymes in Am and non-Am plants grown in soil with and without heavy metal pollutants (R=red kidney, W=wheat, nM=no mycorrhiza, M=mycorrhiza, nR=no rock phosphate, R=rock phosphate, nP=non-polluted, P=pollu

Compared to non-polluted soil, The activity of SOD markedly increased significantly in red kidney and wheat plants grown in heavy metal polluted soil (Figure 5). On the other hand in the presence of Am fungi, the SOD activities in red kidney significantly increased in polluted soil than in the absence of Am fungi (Figure 5). On the contrary at heavy metal polluted soil, Am wheat plants showed SOD activities closely similar to that in non- Am wheat plants. Careful examination of Figure 5 proved that the increased SOD activities paralleled the levels of H_2O_2 formed in red kidney and wheat plants in this study.

Examination of two enzymes, which decompose the H_2O_2 generated by SOD, indicated that the activities of CAT and POX also significantly and insignificantly increased in red kidney and wheat plants respectively in response to heavy metal exposure (Figure 6). CAT and POX activities in red kidney plants showed a highly significant effect and positive correlation with the

presence of Am fungi infected plants grown in heavy metal polluted soil (Table, 4). The Am red kidney plants had a marked increase of CAT and POX activities in polluted soil than that of non-Am plants specially in soil with rock phosphate (Figure 6). The results also indicated that insignificant slight increases were observed for CAT and POX activities in Am wheat plants in compared to non-Am plants grown in heavy metal polluted soil (Figure 6). Moreover, there is no significant marked differences between CAT and POX activities in wheat plants that were grown in either polluted and non-polluted soil, where the activities of CAT and POX enzymes were closely similar in heavy metal polluted soil and non-polluted one.

DISCUSSION

Both red kidney and wheat plants species accumulate higher amount of Zn , Cu , Pb and Cd in their tissues in polluted soil than that in non-polluted one (Figure 1d). Metal accumulation by plants in response to increased inputs is not consistent. Studies by Pichtel et al.(2000) have found no increase . Other work was not affected by soil metal concentrations (Oudeh et al., 2002). In contrast, increases in metal resulted in higher plant concentrations even at low soil concentrations(Khan, 2001; Romkens, et al., 2002 ; Rufyikiri et al., 2004).

In the present study, mycorrhizal acquisition may account for a high proportion of Zn, Cu, Pb and Cd in red kidney and wheat plant tissues that grew at heavy metal contaminated soil. The higher heavy metal concentration in Am plants could be explained by the fact that Am infection increased plant uptake of metals by mechanisms such as enlargement of the absorbing area , volume of accessible soil, and efficient hyphal translocation (Yu et al., 2004). In addition, although heavy metal concentrations in Am plants were much higher than that in non-Am plants, some metal toxicity (data not shown) was observed only on non-Am plant . This result suggests that Am infection offers some protection against metal toxicity. Most reports note a positive effect of Am inoculation on the growth of plants in metal-contaminated soils. This protective benefit may be related to the adsorptive or binding capability for metals of the relatively large fungal biomass associated with the host plant roots, which may physically minimize or exclude the entry of metals into host plant (Cairney and Meharg, 2000). Protective responses of Am fungi to metal toxicity among Am plants have been variable, but generally existent, depending on host plant and fungal isolate sources (Andrad et al., 2004) . In this connection, Zn, Cu, Pb and Cd concentrations in roots and shoots of Am red kidney and wheat plants tissues varied among the two species at the same conditions.

Accumulation and exclusion are two basic strategies by which plants respond to elevated concentrations of heavy metals. In metal accumulator species shoot/root ratios greater than 1 are common, whereas in metal excluder species the factors are typically lower than 1 (Stoltz and Greger, 2002). Accumulation of Zn, Cu, Pb and Cd is suggested as a tolerance strategy by Am red kidney on the basis of translocation factor as shown in Table 2, which is in line with results of Malcova et al. (2003), who found that Am maize plant was more efficient in Pb translocation to the shoots compared with other plant tested. Dang et al. (2004) reported that species able to accumulate relatively high metal concentrations in aboveground tissues could be good candidates for phytoextraction. Therefore, the present results with certain limits (because the aboveground parts are used by human) suggested that Am red kidney would be a good choice for extracting Zn, Cu , Pb and Cd from heavy metal contaminated soil .However, according to Stoltz and Greger (2002), exclusion of heavy metals was suggested as a tolerance strategy by Am wheat plants on the basis of translocation factor as shown in Table 2. Shoot/root ratios of less than 1 were common for Zn, Cu, Pb and Cd, which is typical of excluders (Dahmani-Muller et al., 2000). These results suggested that the Am fungi acts in wheat plants as an heavy metal filter to maintain low heavy metal concentrations in aboveground plant tissues. If so Am wheat plants could be good candidates for the revegetation and phytostabilization of heavy metal polluted soil. In this connection, Dang et al. (2004) reported that plant species which have strong ability to reduce metal translocation from roots to shoots are suitable as phytostabilizers for revegetation of metal contaminated lands.

As shown in Table 2 the percentage of Am infection was slightly reduced in the presence of heavy metals in soil. Nevertheless the results of mycorrhiza-plant interactions (Figures 2-6) proved overwhelmingly that Am fungi still function in heavy metal polluted soil. These results indicated that the tested concentrations of heavy metals in the soil were not harmful to Am fungus (Glomus mosseae). This finding is in line with results of Leyval et al. (1995) ; Hildebrandt et al. (1999) ; Vogel-Mikus et al. (2005) who reported that sensitivity of Am symbionts to heavy metal contaminated soil expressed as a reduction in spore germination, hyphal growth or root colonization, had been proved previously in number of studies. Another interesting result in Table 2 was that the presence of Am fungi inoculations would increase the metal tolerance index of red kidney and wheat plants compared with non-Am plants that grew in heavy metal polluted soil. This result emphasizes that Am fungi could be potentially effective in protecting plants exposed to high levels of heavy metal. The Am fungi ability to alleviate heavy metal stress of plants grown in heavy metal contaminated soil was previously proved by Rufyikiri et al. (2000) ; Hildebrandt et al. (2002) ; Burleigh et al. (2003).

The data in Figures 2,3 &4 showed that physiological indices accounted for Am plants were significantly higher than that for non-Am plants grown in heavy metal contaminated soil in this investigation. This finding supported results from previous studies reporting that Am fungi has the ability to alleviate many anthropogenic stresses including effects of metals , polychlorinated aliphatic and phenolic compound and polycyclic aromatic hydrocarbons compounds (Entry et al., 2002 ; Yu et al., 2004 ; Rabie, 2004& 2005).These results indicated that Am fungi effects on physiological growth of infected plants probably improved plant development and indirectly minimized the stress caused by excess heavy metals in the soil.

Heavy metal contaminated soil cause oxidative-stress; as shown by the increased H_2O_2 formation associated with an increased activity of SOD for reactive oxygen species (O_2^-) conversion (Jacob et al. 2001 ; Schutzendubl et al. 2001). In addition, Ott et al. (2002) who found that if the induction in SOD activity reflects increased O_2^- production rates, an enhanced production of H_2O_2 may be expected . From this perspective, enzymes like POX and CATs involved in detoxification of H_2O_2 were induced to maintain the cellular redox balance. In this connection the present study showed that H_2O_2 content SOD activity were decreased in Am red kidney and wheat plants compared with non-Am plants grown at heavy metal polluted soil. Meanwhile, CAT and POX activities were significantly increased in the presence of Am fungi but these activities are still higher in red kidney than that in wheat plants. Based on these data, it is conceivable to conclude that the Am fungi plays a prime role for induction and activation of the antioxidative system in the two plant species grown in heavy metal polluted soil and the results also suggested that Am-plant symbiosis can prevent such unfavourable conditions that might result in plants exposed to heavy metal stresses.

The data of the present study clearly showed that in the presence of rock phosphate amended soil, the heavy metal concentration, tolerance index, mycorrhizal infection, physiological indices , CAT and POX enzyme activities in Am red kidney and wheat plants increase more than that in the absence of rock phosphate in heavy metal polluted soil. These results indicated that the known beneficial effects of plant mycorrhization appeared to be mainly due to the improvement of P uptake by the mycorrhizal fungus. Numerous studies reported similar results on the enhancement of P uptake and this beneficial effect of plant mycorrhization was attributed to an active uptake of P from the soil and its translocation to plants by the extraradical mycelium of Am fungi (Ryan and Angus, 2003 ; Rufkyikri et al., 2004; Vogel-Mikus et al., 2005). In this study the results suggest that increased P may increase plant biomass as well as growth parameters and thus perhaps detoxify the potential effects of metals by dilution, precipitation or adsorption of metals onto polyphosphate granules and thus limiting its delivery to root cells. In this connection, Andrad et al. (2004) suggested that the symbioses provided the host with P, thereby remaining functional in relation to P supplies. P may be also involved in plant Zn detoxification by means of molecules of phytates that can neutralize excess metals, or P can provide metabolic energy indirectly as ATP for possible compartmentalization within the cell vacuoles.

One of the objectives of this study was the evaluation of the Am fungi behavior for increasing the heavy metal tolerance in the two plant species, representing two different families, grown in heavy metal polluted soil. As previously reported from the data of Table 2 Am red kidney plants accumulate more heavy metals in shoots than that in their roots. Moreover, extrapolation of data in Figures 2-6 indicates that protein content and antioxidant enzyme (SOD, CAT and POX) activities of Am red kidney plants showed significant higher values in polluted soil than in non-polluted one. Based on these results, the mechanisms related to physiological interactions between the Am fungus and red kidney plants involve increased protein synthesis as well as induction of antioxidant enzymes to avoid heavy metal-mediated oxidative stress. If so, the author attributed the reduced heavy metal toxicity effects in Am red kidney plants to antioxidative protection through detoxification of heavy metals, chelation through metal-binding proteins (peptides) and dilution through increased plant growth induced by Am fungi. This finding supports results from some dissipated studies reporting that the role of protein, antioxidative enzyme system as well as improved growth as possible mechanisms for plant protection against high accumulated toxic heavy metals in the shoots (Ott et al., 2002 ; Burleigh et al., 2003 ; Tong et al., 2004). Contrary to red kidney plants, Am wheat plants accumulated higher concentrations of heavy metals in roots than that in shoots meanwhile; heavy metals in shoots were greatly reduced in Am plants compared with non-Am in polluted soil. This suggests that the Am fungus Glomus mosseae can protect wheat plants, by depositing metals within the fungal mycelium and cortical cells of Am roots and thereby preventing metal translocation from roots to shoots . The increase of metal retention in the roots to reduce metal concentration in shoots was hypothesized as the main mechanism involved, and the intraradical hyphae markedly contribute to this retention in the host roots. Besides, Am fungi can protect wheat plants against heavy metal accumulation thereby increasing lipid production in Am plants which compensates lipid destruction by lipid peroxidation enhanced by heavy metals exposure. This finding supports results from numerous studies reporting that Am fungi often protect plants against high accumulation of toxic elements in the shoots, as it was reported for Al (Rufyikiri et al., 2000), Cd (Yu et al., 2004), Zn (Burleigh et al., 2003), Pb (Malcova et al., 2003), U (Rufyikiri et al., 2004), Cu (Gonzalez-Chavez et al., 2002) and As (Fitz and Wenzel,

2002).

These results overwhelmingly indicate that the behavior of Am fungi in the protection of plants from heavy metal toxicity might differ according to host plant which can not generalized. Therefore additional researches are needed to explore the behavior of Am fungi in various plant species and families for plant protection in heavy metal polluted soil. Nevertheless, the beneficial effects of the Am fungus, observed in this study, arouse an interest in considering the role of Am fungi in plant-based strategies of remediation of highly heavy metal contaminated soils.

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