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INTERACTION EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND HEAVY METALS ON MAIZE PLANTS GROWN IN CADMIUM, LEAD AND ARSENIC CONTAMINATED SOIL

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ABSTRACT

Pot experiments were carried out to evaluate the effects of different concentrations of arsenic, cadmium and lead on the growth response, phosphorus content and levels of mycorrhizal colonization of maize plants. Furthermore, the effects of mycorrhizal fungi on the growth and metals contents in maize plants grown in soil contaminated with heavy metals was also investigated. Heavy metals stress reduced, in most cases, shoot fresh and dry weights of maize plant, phosphorus content and levels of mycorrhizal colonization as compared to control untreated plants. However, no significant differences in these levels were obtained between different concentrations of heavy metals. Mycorrhizal colonization increased growth response, in general, of maize plants grown in soil contaminated with heavy metals as compared to equivalent non-mycorrhizal plants. In general, arbuscular mycorrhizal (AM) inoculation reduced heavy metals concentration in roots and shoots. This study shows that growing maize with AM inoculum can minimize heavy metals toxicity and increase growth and P uptake. In this regard, the AM fungus have a protective role, to some extent, for the host plant and therefore are of value in phytoremediation of heavy metals in soil contamination.

Keywords: Arbuscular mycorrhizal fungi, Heavy metals, Growth responses, Maize, Phosphorus uptake, Phytoremediation

INTRODUCTION

Metal contamination of soils and the subsequent uptake by plants constitute a major environmental problem in some areas of Saudi Arabia. Soil contamination by heavy metals is derived from various sources, such as atmospheric deposition, the use of sewage sludge in agricultural land and exploitation of mineral resources. Plant establishment and growth in metal-contaminated sites are inhibited by adverse soil factors such as poor physical structure, toxic metals and nutrient deficiencies (Chen *et al.,* 2007; Lin *et al.,* 2007; Repetto *et al.,* 2007). In order to promote plant establishment, it is essential to improve the physical and chemical properties of the soil. Arbuscular mycorrhizal (AM) fungi can form potentially beneficial associations with the roots of more than 80% of terrestrial plant species (Smith and Smith, 1979; Abdel-Fattah *et al.,* 2009) and there is increasing evidence that AM fungi are important components of soil phytoremediation in terms of increasing plant growth and nutrient uptake (Ahmed *et al.,* 2006; Shen *et al.,* 2006; Cavagnaro, 2008; Wang *et al.,* 2008).

The principal role of AM fungi in assisting plant growth in metal contaminated soils is their capability to supply the host plants with mineral nutrients, particularly phosphate and trace elements (Bagayoko *et al.,* 2000; Jakobsen *et al.,* 2002; Shen *et al.,* 2006). AM fungi can also improve soil structure through the actions of external hyphae and glomalin excreted by external hyphae (Gonzalez-Chavez *et al.*, 2004; Repetto *et al.,* 2007; Soares and Siqueira, 2008), and maintain plant biodiversity and ecosystem stability (Koide and Dickie, 2002). In addition, AM fungi exude enzymes that participate in the immobilization process of soil contamination in which case accumulation in plant is reduced (Weissenhorn *et al.,* 1993; Audet and Charest, 2006).

The effects of heavy metals on AM fungi colonization in their hosts are varied. Some studies suggest that high concentrations of heavy metals in soil may significantly decrease root colonization by AM fungi (Gildon and Tinker, 1983) or inhibit spore germination (Weissenhorn *et al.,* 1993). However, other studies have reported high levels of mycorrhizal in agricultural soils contaminated with metals of different origins (Weissenhorn *et al.,* 1995; Turnau *et al.,* 1996). Metal polluted soils seldom have elevated concentrations of one trace metal. They are usually contaminated by mixtures of metals and metals may interact in the soil-plant system. Schuepp *et al.* (1987) reported that mycorrhizal colonization reduced the shoot concentrations of cadmium and zinc in field grown maize and lettuce when the soil had high available concentrations of both metals.

AM fungi are associated with improved growth of many plant species due to increased nutrients uptake, production of growth promoting substances, tolerance to drought, salinity and transplant shock, bio-agents against plant pathogens and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilizer. Little is known about the beneficial role of AM fungi to overcome the toxicity of heavy metals. In this study, pot experiments were carried out to evaluate the effects of different concentrations of arsenic, cadmium and lead on the growth response, phosphorus content and levels of mycorrhizal colonization of maize plants. Furthermore, the effects of mycorrhizal fungi on the growth and metals contents in maize plants grown in soil contaminated with heavy metals was also investigated.

MATERIALS AND METHODS

Experimental materials

Maize (*Zea mays* L.) seeds were surface sterilized with 10% v/v hydrogen peroxide for 10 min, washed with sterile water and germinated in the dark on moist sterilized filter paper at room temperature. Seedlings were selected for uniformity of size prior to planting.

The mixture of AM fungi inocula used in these experiments were *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *Glomus etunicatum* (Becker & Gerdmann) and *Glomus intraradices* (Schenck & Smith). These fungi were propagated in pot culture for 10 weeks on maize plants grown in soil collected from Durab farm, Experimental Station, Collage of Food and Agricultural Sciences. Spores of the mycorrhizal fungi were collected from stock maize soil by a procedure of decanting and wet sieving (Gerdemann and Nicolson, 1963) through a series of 250, 150 and 5o µm. The mycorrhizal inoculum consisted of growth medium comprised of the sandy soil containing a mixture of spores (approximately 450 spores per 100 g) together with infected maize root fragments. The inoculum was placed 3 cm below the surface of the soil before planting to produce mycorrhizal plants. Non-mycorrhizal plants received a soil inoculum free of arbuscular mycorrhizal propagules to equilibrate soil microbiota between mycorrhizal and non-mycorrhizal treatments.

The soil used in this experiment was collected from Durab farm had the following properties: pH (water soil) 8.0, organic matter (0.52%) and $NaHCO_3$-extractable P (12.3 mg kg^{-1}). The soil was sieved (1 mm), and steam sterilized (121 ºC for 30 min three successive times) to serve as non-mycorrhizal treatments.

Experimental design

The experiment was a 2 x 4 x 3 factorial design with mycorrhizal colonization (inoculated or non-inoculated) with four levels (0, 15, 30 and 6o mg kg^{-1} soil) of three heavy metals (cadmium as $CdCl_2$, lead as $PbCl_2$ and arsenic as As_2O_3 in aqueous solution) with 5 replicates, giving 120 tubes in total. The soils were equilibrated for 2 weeks after the metal solutions were thoroughly mixed in. The tubes were arranged in a fully randomized design.

Growth medium

The experiment was conducted in a growth chamber (Plate 1) at the Plant Production Department, College of food and Agricultural Sciences. Plants were grown in sterilized plastic tubes (one plant for each tube) containing soil collected from Durab farm. Half of the tubes were inoculated with mixture of mycorrhizal spores (approximately 450 spores per 100 g) together with infected maize root fragments to produce mycorrhizal treatments. The other tubes received equal amounts of sterilized inoculum plus 1 ml of mycorrhizal fungal-free filtrate from the inoculum suspension were added to produce the non-mycorrhizal treatments. All treatments received 10% Hoagland solution minus phosphorus (Hoagland and Arnon, 1950). The plants were left to grow in a controlled growth chamber with 16 h 30 ºC day and 8 h 20 ºC night. De-ionized water was required to maintain moisture content near 60% of water holding capacity.

Harvest and chemical analysis

Plants were grown for 8 weeks and shoots and roots were harvested separately. Root samples were carefully washed with tap water and then deionised water to remove adhering soil particles. Sub-samples of fresh roots were collected for the determination of AM colonization levels. Shoot height and root volume were estimated. Dry weights of shoots were determined after oven drying at 80 ºC for 48 h. Oven-dried shoots and roots were milled and digested by 5 ml concentrated HNO3 at 160 ºC using microwave accelerated reduction system (Mars 5, CEM Co. Ltd, USA). The dissolved samples were analyzed for P, Cd and Pb concentrations and measured by inductively coupled plasma-optical emission spectroscopy using a Perkin Elmer Optima

2000 Dv (Pearson and Jakobsen, 1993). Arsenic (As) concentrations were determined using an atomic fluorescence spectrometer (Modle AF 610A, Bezorbtech Analytical Instrument Co.).

The percentage of root length colonized by the mycorrhizal fungi was estimated randomly from selected root segments. Sub-samples of fresh roots were cut into 1 – 0.5 cm long, cleared in 10% KOH at 90 ºC for 45 min. in a water bath, rinsed three times with tap water and stained with 0.05 % (w / v) Trypan blue in lactophenol at 90 ºC for 15 min. according to the procedure of Phillips and Hayman (1970). Percentage of root colonization levels in stained segments were determined by the method of Trouvelot *et al.* (1986).

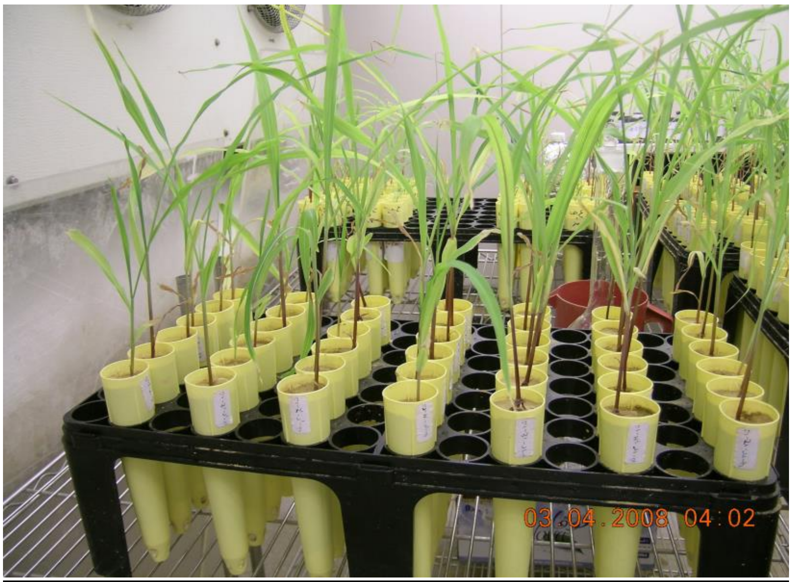

Plate 1. Growth of maize (*Zea mays* L.) in tubes (1.5 cm x 30 cm) containing heavy metals contaminated soil in growth chamber.

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA). Pairs of treatment means were compared using Duncan's multiple range test at the 5% level. The SAS version 8.02 software package was used. Data presented in the Tables and Figures are mean values ± SE (*n*=4).

RESULTS

I- Effect of heavy metals on mycorrhizal maize plants

Plant growth

Shoot fresh and dry weights, root volume and shoot height of mycorrhizal maize plants were affected by the addition of heavy metals to the soil. Application of heavy metals, in general, significantly reduced growth responses of mycorrhizal maize plants (Table 1). Shoot fresh mass and shoot height of maize plants were highly decreased with increasing heavy metals levels in soil. However, no significant differences were found in shoot dry weight and root volume of mycorrhizal maize plants grown in different concentrations of arsenic and lead metals. Growth responses of mycorrhizal maize plants at 60 mg Cd Kg^{-1} were significantly lower compared to 0 and 30 mg Cd Kg^{-1}soil.

Table (1): Effect of different concentrations of heavy metals (arsenic, cadmium and lead) on growth response of mycorrhizal maize plants (mean ± SE, *n* = 4).

Heavy metals addition rate (mg kg^{-1} soil)	Shoot fresh weight (g / plant)	Shoot dry weight (g / plant)	Shoot height (cm / plant)	Root volume (ml / plant)
Arsenic				
0	1.057 ± 0.11 a*	0.145 ± 0.013 a	33.75 ± 1.86 b	1.88 ± 0.17 a
15	1.068 ± 0.20 a	0.157 ± 0.157 a	38.92 ± 5.35 a	1.93 ± 0.39 a
30	0.730 ± 0.12 b	0.117 ± 0.020 a	32.25 ± 3.71 ab	1.47 ± 0.30 a
60	0.720 ± 0.19 b	0.113 ± 0.027 a	31.43 ± 5.63 ab	1.50 ± 0.33 a
Cadmium				
0	1.275 ± 0.09 a	0.182 ± 0.018 a	39.37 ± 3.81 ab	2.59 ± 0.28 a
15	0.934 ± 0.21 ab	0.162 ± 0.045 ab	31.75 ± 4.27 b	1.91 ± 0.71 ab
30	1.234 ± 0.20 a	0.180 ± 0.029 a	42.50 ± 6.52 a	2.47 ± 0.46 a
60	0.814 ± 0.22 b	0.109 ± 0.033 b	31.75 ± 1.83 b	1.57 ± 0.51 b
Lead				
0	1.031 ± 0.36 a	0.139 ± 0.054 a	32.50 ± 5.73 ab	2.00 ± 0.56 a
15	1.021 ± 0.26 a	0.131 ± 0.036 a	37.81 ± 5.89 a	2.47 ± 0.59 a
30	0.902 ± 0.19 ab	0.125 ± 0.034 a	33.19 ± 6.01 ab	2.23 ± 0.51 a
60	0.860 ± 0.19 b	0.119 ± 0.033 a	30.06 ± 4.62 b	1.98 ± 0.50 a

***Values in each column for each heavy metal followed by the same letter are not significantly different by LSD multi-comparison at the 5% level**.

Phosphorus content

Data concerning P content of shoots of mycorrhizal maize plants treated or not with different concentrations of heavy metals are illustrated in Figure (1). Neither type of heavy metal application nor concentration level showed any significant effect on shoot phosphorus content of maize plants grown in contaminated soil. However, total phosphorus content of mycorrhizal maize plants were significantly decreased by application of cadmium when compared to lead and arsenic treatments regardless of heavy metal concentration (except at 30 mg Cd kg^{-1} concentration).

Heavy metals (As. Cd and Pb) uptake

Table (2) indicated that arsenic, cadmium and lead concentrations in shoots of mycorrhizal maize plants increased dramatically with increasing the corresponding heavy metal addition level to the soil, but the increase was more distinct in mycorrhizal plants grown in soil contaminated with cadmium treatments. However, there was no constant trend in the metals uptake by mycorrhizal fungi of maize plants treated by different concentrations of heavy metals. In this connection, arsenic treatments had no significant effect on cadmium (except at 60 mg As kg^{-1}) and lead concentrations of shoots of mycorrhizal plants. In contrast, total As and Pd concentrations in shoots of mycorrhizal plants were significantly increased as Cd level in the soil increased.

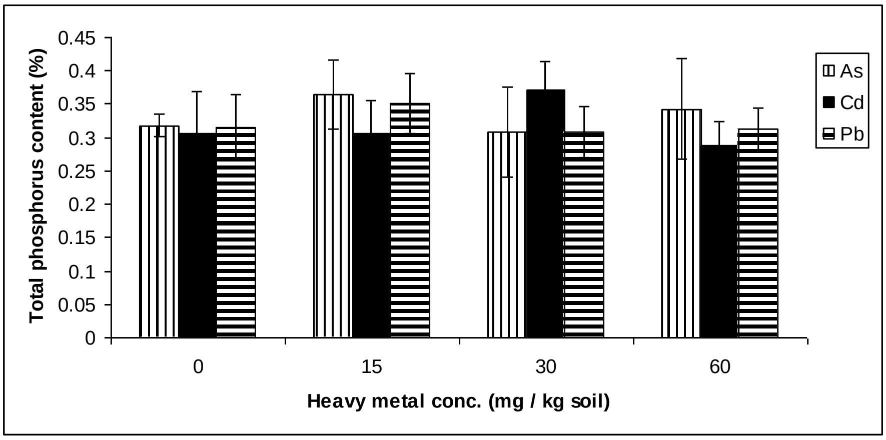

Fig. 1: Effect of different concentrations of heavy metals (arsenic, cadmium and lead) on total phosphorus content of mycorrhizal maize shoot plants (mean ± SE, *n* = 4).

Table (2): Arsenic, cadmium and lead concentrations (PPM) in shoots of mycorrhizal maize plants grown under increasing soil heavy metals (mean ± SE, *n* = 4).

Heavy metals addition rate (mg kg^{-1} soil)	As	Cd	Pb
Arsenic			
0	23.57 ± 09.1 b*	1.08 ± 0.48 a	3.58 ± 1.31 a
15	20.84 ± 07.8 b	0.92 ± 0.61 a	2.98 ± 1.19 a
30	43.09 ± 21.4 a	0.99 ± 0.32 a	3.44 ± 1.25 a
60	33.32 ± 16.7 ab	0.80 ± 0.53 ab	3.73 ± 1.15 a
Cadmium			
0	19.2 ± 06.3 ab	00.43 ± 0. 18 b	1.15 ± 0.45 b
15	13.1 ± 05.3 b	10.55 ± 0.28 ab	1.17 ± 0.55 b
30	25.4 ± 11.3 a	23.19 ± 3.70 a	7.43 ± 1.30 a
60	28.3 ± 12.5 a	26,12 ± 4.84 a	7.71 ± 1.83 a
Lead			
0	03.81 ± 0.52 a	3.23 ± 0.52 a	17.4 ± 08.2 b
15	04.85 ± 1.90 a	2.70 ± 0.33 ab	19.4 ± 07.3 ab
30	04.40 ± 3.60 a	0.75 ± 0.49 b	29.5 ± 13.6 a
60	4.52 ± 4.62 a	0.64 ± 0.29 b	31.1 ± 17.9 a

***Values in each column for each heavy metal followed by the same letter are not significantly different by LSD multi-comparison at the 5% level**.

Mycorrhizal root colonization

Frequency of mycorrhizal infection (F%), intensity of infection (M%) and frequency of arbuscules (A%) in infected roots of maize plants were not highly affected by each type and concentration of heavy metals addition to the soil. However, in most cases, Cd application significantly reduced the levels of mycorrhizal infection in roots of maize plants when compared to As and Pb application, regardless the concentration of heavy metals (Figure 2).

As compared with all treatments, high concentration of Pb (60 mg kg^{-1}) dramatically increased the levels of mycorrhizal infection. On the other hand, frequency of arbuscules in infected roots of maize plant grown in Cd contaminated soil decreased with increasing Cd level addition to the soil.

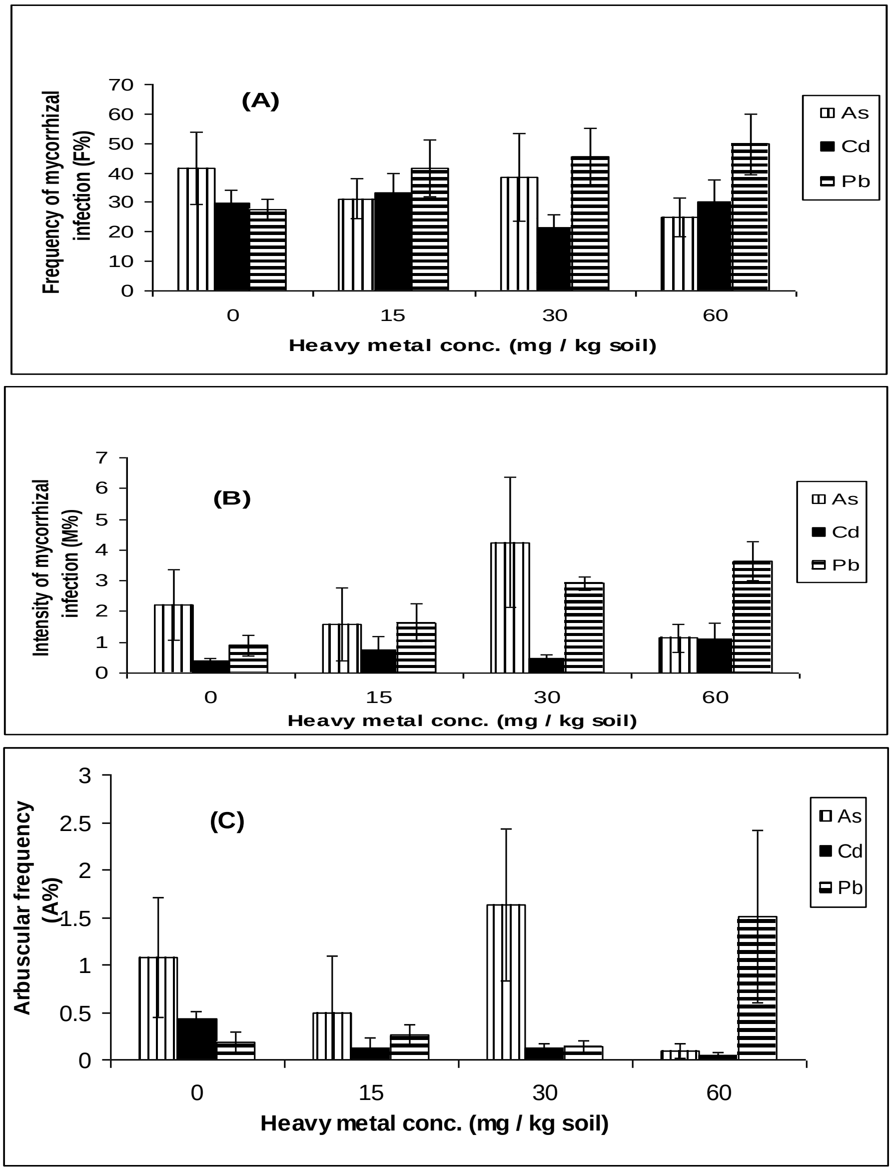

Fig. 2: Frequency of mycorrhizal infection (A), intensity of mycorrhizal infection (B) and arbuscular frequency (C) in roots of maize plants growing under effect of different concentrations of soil heavy metals (mean ± SE, *n* = 4).

II- Effect of arbuscular mycorrhizal colonization on maize plants grown in contaminated soil

Plant biomass

Shoot and root dry weights of maize plants were highly affected by both heavy metals application and mycorrhizal colonization (Table 3). In most cases, plant biomass of maize plants decreased with increasing heavy metals level in the soil, but the decrease was more obvious in mycorrhizal and non-mycorrhizal plants grown in arsenic contaminated soil. In control and high concentration of arsenic (0 and 60 mg As kg^{-1} soil respectively), mycorrhizal colonization significantly increased shoot and root dry weights of maize than those non-mycorrhizal plants. However, no significant differences were observed in the dry biomass between mycorrhizal and non-mycorrhizal plants grown in soil contaminated with different concentration of lead (except at 15 mg Pb kg^{-1} soil where shoot dry weight in mycorrhizal plants was significantly lower than non-mycorrhizal plants).

Shoot and root dry weights were not significantly affected by Cd levels and also mycorrhizal colonization. However, plant biomass at 30 mg Cd Kg^{-1} soil was significantly lower in mycorrhizal plants as compared to non-mycorrhizal ones.

Table (3): Effect of arbuscular mycorrhizal fungi (AMF) on the growth of maize plants grown in soil contaminated with different concentrations of heavy metals (mean ± SE, *n* = 4).

Heavy metal addition rate (mg kg^{-1} soil)	Shoot dry weight (g / plant)		Root dry weight (g / plant)	
	- AMF	+ AMF	- AMF	+ AMF
Arsenic				
0	0.337 ± 0.021 b	0.422 ± 0.042 a	0.233 ± 0.081 b	0.278 ± 0.050 a
15	0.412 ± 0.034 a	0.445 ± 0.056 a	0.198 ± 0.022 b	0.341 ± 0.061 a
30	0.445 ± 0.036 a	0.214 ± 0.036 b	0.193 ± 0.026 a	0.172 ± 0.041 a
60	0.108 ± 0.034 b	0.198 ± 0.057 a	0.118 ± 0.017 b	0.169 ± 0.017 a
Cadmium				
0	0.424 ± 0.051 a	0.478 ± 0.054 a	0.202 ± 0.033 a	0.262 ± 0.052 a
15	0.487 ± 0.082 a	0.367 ± 0.077 a	0.271 ± 0.046 a	0.223 ± 0.091 a
30	0.475 ± 0.021 a	0.338 ± 0.049 b	0.247 ± 0.051 a	0.188 ± 0.016 b
60	0.402 ± 0.052 a	0.365± 0.044 a	0.239 ± 0.032 a	0.258 ± 0.020 a
Lead				
0	0.419 ± 0.018 a	0.310 ± 0.053 a	0.244 ± 0.041 a	0.337 ± 0.026 a
15	0.427 ± 0.045 a	0.241 ± 0.079 b	0.282 ± 0.053 a	0.208 ± 0.043 a
30	0.465 ± 0.039 a	0.316 ± 0.039 a	0.194 ± 0.024 a	0.219 ± 0.033 a
60	0.355 ± 0.052 a	0.371 ± 0.091 a	0.206 ± 0.039 a	0.256 ± 0.061 a

-AMF and +AMF represent uninoculated treatment and inoculation with mycorrhizal fungi, respectively. Different letters in each parameter indicate significant differences between inoculation and uninoculated treatment by LSD multi-comparison at the 5% level.

Plant arsenic (As) concentrations

Shoot and root As concentrations of maize plants were highly affected by mycorrhizal colonization and level of arsenic metal in the soil (Figure 3). Arsenic concentration in plant tissues increased with increasing As addition rate. Shoot and root As uptake were significantly higher in mycorrhizal plants than in equivalent non-inoculated plants grown at 60 mg As Kg^{-1} soil. However, shoot As concentrations were higher in mycorrhizal plants than in non-mycorrhizal at 30 mg As kg^{-1} soil . Root As concentration was lower in mycorrhizal plants than in non-inoculated plants and the decline was very pronounced at 60 mg As kg^{-1} soil .

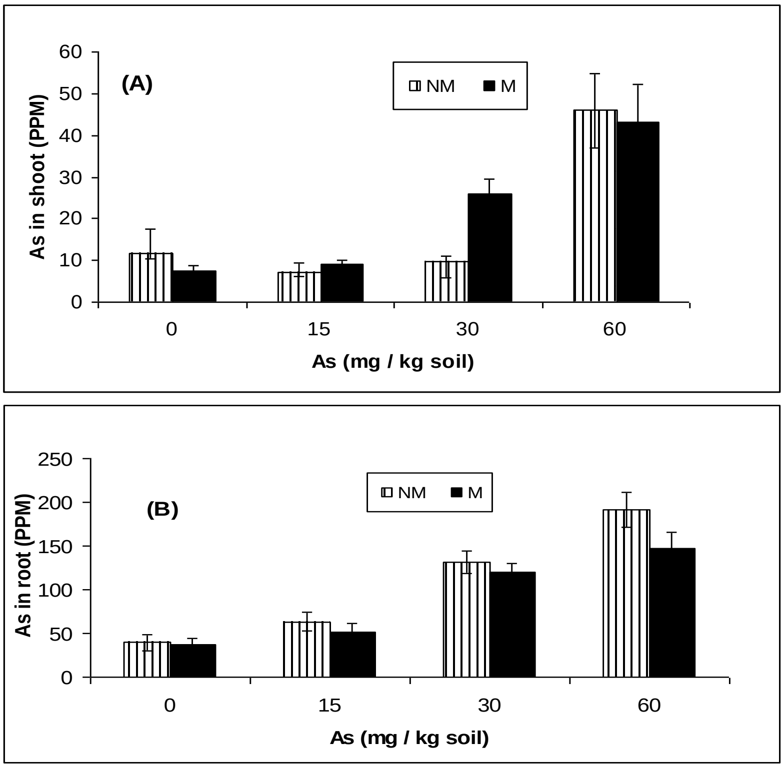

Fig. 3 Effect of arbuscular mycorrhizal fungi (AMF) on concentrations of arsenate in shoots (A) and Roots (B) of maize plants grown in soil contaminated with different concentrations of arsenic (mean ± SE, *n* = 4).

Plant cadmium (Cd) concentrations

Shoot and root cadmium concentrations increased with increasing Cd level to the soil. Mycorrhizal colonization had no constant trend on Cd concentrations in shoot and root of maize plants grown in Cd contaminated soil. However, root Cd concentration of mycorrhizal plants was significantly lower than non-mycorrhizal plants at 15 and 30 mg Cd kg^{-1} soil (Figure 4). Similar trend in shoot Cd concentration of maize plants at 60 mg kg^{-1}. No significant differences were found in root Cd concentrations between mycorrhizal and non-mycorrhizal plants grown at the higher Cd level (60 mg kg^{-1} soil).

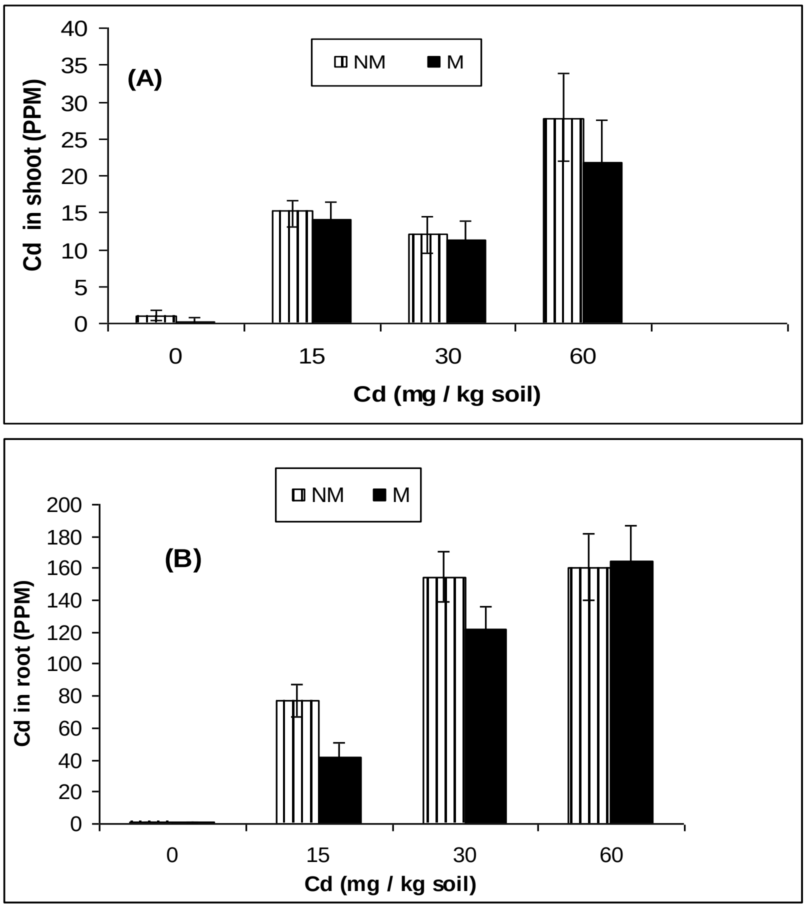

Fig. 4 Effect of arbuscular mycorrhizal fungi (AMF) on concentrations of cadmium in shoots (A) and Roots (B) of maize plants grown in soil contaminated with different concentrations of cadmium (mean ± SE, *n* = 4).

Plant lead concentrations

Shoot and root lead concentrations of mycorrhizal and non-mycorrhizal maize plants were increased by increasing Pb in the soil (Fig. 5). Mycorrhizal colonization significantly reduced shoot and root Pb concentration when compared to non-mycorrhizal maize plants grown at the higher Pb concentration (60 mg kg^{-1} soil). However, no significant differences in these concentrations were found between mycorrhizal and non-mycorrhizal control plants.

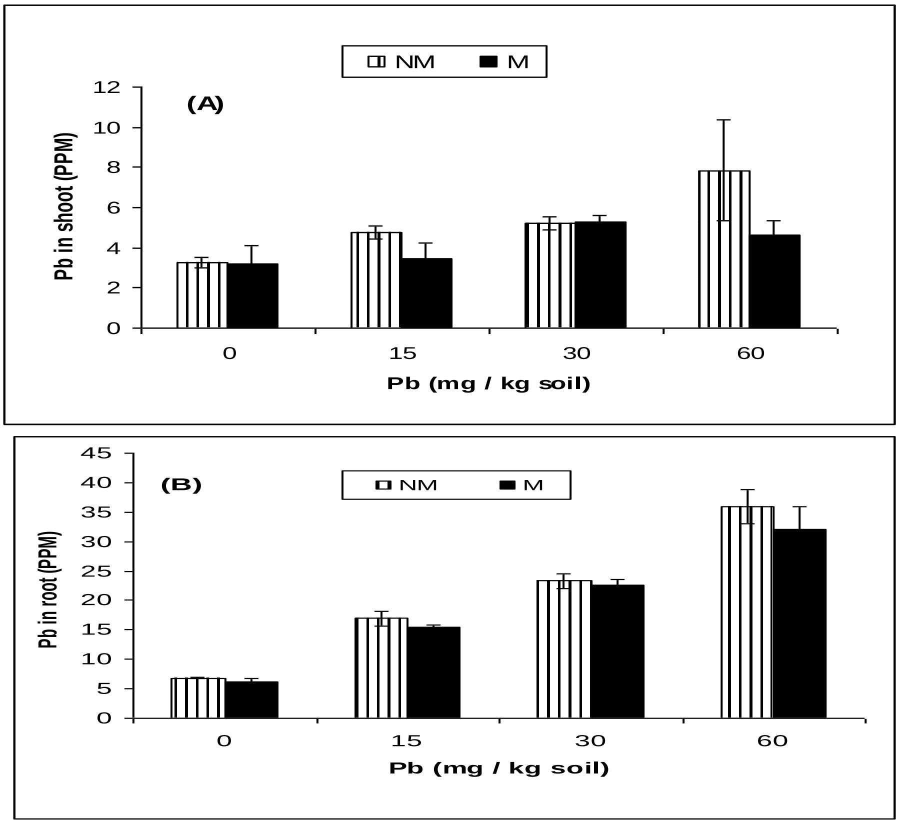

Fig. 5 Effect of arbuscular mycorrhizal fungi (AMF) on concentrations of lead in shoots (A) and Roots (B) of maize plants grown in soil contaminated with different concentrations of lead (mean ± SE, *n* = 4).

DISCUSSION

Phytoremediation is defined as the use of plant system to clean up contaminated environment. The effects of arbuscular mycorrhizal (AM) fungi (in the context of phytoremeditation) on plant growth and uptake of heavy metals are varied (Heggo *et al.,* 1990; Khan *et al.*, 2000; Ahmed *et al*., 2006; Shen *et al*., 2006., Gohre and Paszkowski, 2006; Repetto *et al*., 2007; Soares and Sliqueira, 2008; Wang *et al*., 2008). In our experiment, mycorrhizal colonization significantly increased shoot and root dry weights of maize than those on non-mycorrhizal plants. However, no significant differences were observed in the dry biomass between mycorrhizal and non-mycorrhizal plants grown in soil contaminated with different concentrations of lead (except at 15 mg Pb kg^{-1} soil where shoot dry weight in mycorrhizal plants was significantly lower than non-mycorrhizal plants). These results are in agreement with those reported by Shen *et al*. (2006) who reported that mycorrhizal inoculation increased growth of maize plants with enchancement of P nutrition, perhaps increasing plant tolerance to Cd and Zn or by lowering the concentrations of soluble heavy metals in the soil dilution and / or by adsorption onto the extrametrical mycelium of mycorrhizal fungi.

Arsenic had more pronounced effect on plant biomass than did cadmium and lead at the higher rate of application (60 mg kg-1). Mycorrhizal plants had higher shoot and root yields than non-mycorrhizal plants. Plant growth responses to mycorrhizal colonization are difficult to interpret. because, they are influenced by P nutrition in addition to added Zn and Cd (Audet and Charest, 2006; Gohre and Paszkowski, 2006). In contaminated field sites, excessive heavy metals are always associated with a shortage of available mineral nutrition, especially P (Shetty *et al.*, 1994; Shen *et al.,* 2006). Under metal contamination P deficiency may increase the severity of metal phytotoxicity (Cavagnaro, 2008). Mycorrhizal fungi may alleviate P deficiency, thus avoiding the need to supply P fertilizers (Kaldorf *et a*l., 1999).

In spite of the effect of heavy metals on the levels of mycorrhizal colonization of maize roots were varied, however the percentage of arbuscular frequency in maize root of inoculated plants decreased with increasing arsenic and cadmium levels to the soil. In contrast, the arbuscular frequency of mycorrhizal root colonization significantly increased with increasing lead concentrations in the soil. The fluctuation of the results obtained here are in agreement with the study of Gildon and Tinker (1983) who reported that high concentrations of heavy metals in soil may significantly decrease root colonization by AM fungi, or inhibit spore germination (Weissenhorn *et al.,* 1993; Shen *et al.,* 2006). In some extreme conditions, AM fungal inoculation can be entirely inhibited due to heavy metal toxicity (Weissenhorn *et al.,* 1994). However, other studies have reported high levels of mycorrhizal colonization in agricultural soils contaminated with metals of different origins (Audet and Charest, 2006; Wang *et al.,* 2008). The inconsistence of the results may be probably due to the origin of mycorrhizal fungus, plant species and the dose of heavy metal used (Bradely *et al.,* 1982).

Of particular interest in this study, the concentrations of arsenic and lead in shoots and roots of mycorrhizal maize plants were significantly lower than that in non-mycorrhizal plants grown at higher rates of both heavy metals to the soil. These results corroborate those by Soares and Siqueira (2008) who reported that mycorrhizal colonization reduced the shoot concentrations of Cd and Zn in field growing maize and grass when the soil had high available concentrations of both metals. A possible reason for such reduction may be that AM plants yielded higher biomass, which contributed to dilute metals in the shoot tissue (Cavagnaro, 2008) or that the AM mycelium retained the absorbed metals (Chen *et al.,* 2007; Repetto *et al.,* 2007). Metal immobilization in fungal tissues can occur as metal sequestration in fungal wall components such as the glycoproteins-glomalins, which have high affinity to metals (Gonzalez-Chavez *et al.,* 2004). Accordingly, the mycorrhizal fungi may have immobilized soil contaminants and prevented these from being taken up by the host plant, especially under increasingly toxic soil- Zn concentrations (Weissenhorn *et al.,* 1995). Cadmium, arsenic and lead contents in the root tissues of maize plant were generally higher than in the shoots in all treatments.

Shoot arsenic concentrations were higher in mycorrhizal plants than in non-mycorrhizal plants grown in soil contaminated with 30 mg As Kg^{-1}. These results are in agreement with the study of Liu *et al.* (2007) who reported that inoculation by mycorrhizal increased heavy metals uptake in three leguminous plants. In contrast, Ahmed *et al*. (2006) reported that mycorrhizal inoculation reduced As concentration in roots and shoot of lentil irrigated with arsenic contaminated water. The outcome of mycorrhizal colonization on clean-up of contaminated soils depends on the plant-fungus-heavy metal combination and is also influenced by soil conditions (Gohre and Paszkowski, 2006).

Conclusion

In spite of the fact that arbuscular mycorrhizal (AM) inoculation have little protective effects on maize in heavy metals (Cd, As and Pb) contaminated soil, they exhibit reduced metal translocation and enhanced shoot growth, maintaining metal concentration at tolerable levels below toxicity-critical content. Probably, AM fungi may have facilitated plant metal tolerance in a number of ways: 1- The mycorrhiza increased plant biomass diluting the metals in the plant tissues. 2- Reduced the concentration of soluble metals in the soil possibly by adsorption of metals on the extraradical hypha and 3- Enhancing P nutrition with a possible further contribution to enhanced biomass. However, further investigations on the effects of heavy metals on AM fungi in interaction with host plant species will be of interest to a better understanding of the mycorrhizospheric network.

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تأثير تفاعل فطريات الجذور التكافلية الشجيرية والعناصر الثقيلة على نبات الذرة النامي في تربة ملوثة بعناصر الكادميوم والزرنيخ والرصاص

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أجريت هذه الدراسة لمعرفة تأثير الدور المتبادل لكل من العناصر الثقيلة وفطريات الجذور التكافلية الشجيرية على نمو نباتات الذرة النامية فى تربة ملوثة بتركيزات مختلفة من الكادميوم والزرنيخ والرصاص. وكذلك دراسة الدور الحيوي لفطريات الجذور الشجيرية في حماية نباتات الذرة من سمية هذه العناصر الثقيلة وتم التوصل إلى النتائج التالية:-

١- أدت زيادة تركيزات العناصر الثقيلة في التربة إلى نقص في الوزن الطازج والجاف للجذور والساق والمحتوى الفسفوري وكذلك مستويات الإصابة بفطريات الجذور التكافلية لنبات الذرة إذا ما قورنت بالنباتات الضابطة (الكنترول).

٢- اتضح إن معاملة النباتات النامية تحت تأثير سمية العناصر الثقيلة بفطريات الجذور التكافلية أدى إلى حد ما إلى زيادة معدلات النمو إذا ما قورنت بالنباتات الغير مصاحبة لهذه الفطريات بالرغم من عدم وجود اتجاه ثابت في هذا الاتجاه مع زيادة تركيزات العناصر الثقيلة المستخدمة المضافة إلى التربة.

٣- أوضحت النتائج ان تركيزات المعادن الثقيلة المستخدمة في المجموع الخضري للنبات تتأثر بدرجة كبيرة بتركيز العنصر المستخدم. وفى هذا الإطار كان تركيز عنصر الزرنيخ في سيقان نبات الذرة أكثر بدرجة واضحة بالمقارنة بالعناصر الأخرى.

٤- تبين من خلال النتائج التي تم الحصول عليها إن لفطريات الجذور التكافلية دورا ايجابيا في حماية نبات الذرة من خطورة العناصر الثقيلة من خلال تقليل تركيز هذه العناصر فى السيقان عن مثيلاتها فى الجذور بالمقارنة بالنباتات الغير مصاحبة لهذه الفطريات مما يؤكد إن لهذه الفطريات دورا حيويا هاما فى تخفيف أو تقليل انتقال هذه العناصر من الجذر إلى الساق والاحتفاظ بها داخل هيفاتة الخارجية وتحويلها إلى مواد متبلورة تتحد مع الكايتين الموجود في جدار هيفات الفطر. ويوصي الباحثين بعمل حصر كامل للفطريات الجذرية التكافلية المصاحبة للنباتات فى الأماكن المعرضة للتلوث بالمعادن الثقيلة ومحاولة التعرف عليها على مستوى الجنس والنوع.