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EFFECTS OF LEAD, CADMIUM, VA-MYCORRHIZAL FUNGI AND THEIR INTERACTIONS ON THE GROWTH OF RADISH PLANT (*Raphanus sativus*, L.).

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ABSTRACT

Lead and cadmium at 100 and 200 ppm decreased length of either root or shoot system and their fresh and dry weights as well as leaf area per plant in both seasons. Photosynthetic pigment concentrations in the leaves; carbohydrate and endogenous phytohormones (IAA, GA_3 and cytokinins) concentrations in the shoot system were also decreased, while ABA and accumulation of both lead and cadmium in the root and shoot systems were increased. Cadmium was more concentrated in the shoot, whereas lead in the plant root. Cadmium application led to more deleterious effect compared with lead. On the other hand, inoculation with VA-mycorrhizae increased all the above mentioned parameters, while suppressed not only the accumulation of ABA in the shoot system, but also accumulation of the two applied heavy metals in both root and shoot systems. Anatomically, treatments with lead and cadmium decreased root diameter, cortex thickness, diameter of vascular cylinder and metaxylem vessel diameter. Leaf blade thickness and mesophyll tissue thickness were also decreased due to reducing both palisade and spongy tissue thickness. The size of the midvein vascular bundle was decreased as indicated by its width and length. Inoculation with VA-mycorrhizae induced all the above mentioned anatomical parameters. It is also interesting to mention that inoculation with VA-mycorrhizal fungi alleviated the adverse effects of lead at all concentrations used and partially alleviated effects of cadmium, leading to improving plant performance under the heavy metals stress.

INTRODUCTION

Pollution of the cultivated soils with heavy metals, particularly lead (Pb) and cadmium (Cd) has become a major environmental concern during the last decades. Toxic levels of both lead and cadmium in some agricultural soils may occur from industrial pollution, motor vehicles, road dust composition; fertilizers, smelting, pesticides application and addition of sewage sludge to the soils. These metals tend to accumulate in the plough layers of soils and are easily absorbed by roots and translocated to different parts, thereby, leading to inhibition of metabolic processes and reducing plant growth and yield (Khan and Frankland, 1983; Sheoran *et al.*, 1990 and El-Saied, 2001).

Toxic effects of lead and cadmium on plant growth and development are often accompanied with morphological and anatomical responses (Suzan A. Sayed, 1999 and Fouda and Arafa, 2002). Most of these responses tended to have an inhibitory effect on some physiological processes such as photosynthetic activity (Krupa and Baszynski, 1995) and photosynthetic apparatus (Sheoran *et al.*, 1990). They added that photosynthesis has been found to be the most sensitive process affecting by cadmium toxicity.

Moreover, both lead and cadmium alter carbohydrate status, endogenous hormones of the shoot system in various plant species (Godbold and Kettner, 1991b and Hassan and Wahdan, 1991).

Mycorrhizae (VAM) play an important role not only in stimulation of plant growth (Mankarious *et al.*, 1995), but also in protection of the host plants against metal toxicity and improve plant performance under heavy metals stress (Weissenhorn *et al.*, 1995 and Fouda and Arafa, 2002). The enhanced effects of mycorrhizae on plant growth directly may be due to increased nutrient uptake (Fouda and Arafa, 2002) and indirectly to increasing other endogenous plant hormones produced by the host plants (Duke *et al.*, 1986 and Torelli *et al.*, 2000).

The present study aimed to investigate effects of lead and cadmium on growth, certain physiological aspects and the possible changes in the anatomical structure of root and leaves of radish plant. On the other hand, VAM was applied to alleviate the adverse effects of either lead or cadmium on plant growth.

MATERIALS AND METHODS

Two pot experiments were carried out during the two growing seasons of 2000/2001 and 2001/2002 in the greenhouse and Lab. of Agric. Bot. Dept., Fac. of Agric., Mansoura University, Egypt. Each pot (30 cm diameter) was filled with 3 Kg caly/sand soil (1:1 w/w).

1. Heavy metals treatments:

The pots were divided into two sets: first set was contaminated with lead chloride and the second with cadmium chloride at 0, 100 and 200 ppm. Heavy metal treatments were achieved by adding 500 ml aqueous solutions of different concentrations prepared in distilled water to each pot. After carefully mixing the heavy metal solution with the soil, it was allowed to stabilize for a period of 5 days before sowing. All treatments was applied with and without mycorrhizal inoculum.

2. Mycorrhizal inoculum:

The used mycorrhizal inoculum were obtained from Fac. of Sci., Mansoura University. The mycorrhizal inoculum consisting of soil with spores, hyphae and onion root fragments infected with *Glomus mosseae*.

The inoculum was placed 3 cm below the soil surface at the rate of 10 g/pot at the moment of sowing.

Uniform seeds of radish (*Raphanus sativus*, L. var. sativus) were sown on the 10th of May in both seasons and irrigated with tap water when ever required.

The pots were arranged in a complete randomized design with three replications to form the following treatments (Each treatment consisted of 6 pots):

1. Control.
2. Inoculation with VA-mycorrhizae (VAM).
3. Pb 100 ppm.
4. Pb 200 ppm.
5. Pb 100 ppm + VAM.
6. Pb 200 ppm + VAM.
7. Cd 100 ppm.
8. Cd 200 ppm.
9. Cd 100 ppm + VAM.
10. Cd 200 ppm + VAM.

Three weeks after sowing the plants were thinned to leave 3 uniform young plants per pot. Roots of radish plants were stained with trypan blue (Phillips and Hayman, 1970) and microscopically investigated to ensure that the fungal infection was occurred.

At harvesting (45 days after sowing) length of both root and shoot systems and their fresh and dry weight (g) as well as leaf area (cm^2) per plant using Watson (1958) method were recorded.

3. Chemical analysis:

Photosynthetic pigment concentrations (mg/g fresh weight) were determined in the 3rd leaf (Mackinny, 1941).

The plants were separated to root and shoot systems and dried, ground digested (Peterburgski, 1968) to determine total soluble carbohydrate concentrations in the shoots by method described by (Dubois *et al.*, 1956).

Pb^{++} and Cd^{++} cations were determined in the root and shoot systems by atomic absorption spectrophotometer (BHF 80 B).

Endogenous plant hormones:

Extraction purification and separation of plant hormones were carried out according to (Gazit and Blumenfeled, 1970). Methanolic extracted of radish shoot system was used for endogenous hormones (IAA, GA and ABA) estimation by high performance liquid chromatography (HPLC) according to the methods described by Shindy and Smith (1975). The technique used to assay the activity of cytokinin was followed as in Esashi and Leopold (1969) using sunflower cotyledonary leaf section test.

All the chemical analysis except photosynthetic pigments and carbohydrate concentrations were carried out during the second season only.

4. Anatomical studies:

For anatomical studies samples were taken (At harvesting) during the second season from the middle part of the main root and the midrib region of the 3rd leaf. Killing and fixing of samples were done in FAA solution, dehydrated in alcohol series and embedded in paraffin wax 52-54°C m.p.). Cross sections at 15-20 µm thick were prepared using a rotary microtome, stained in saffranin-light green combination, cleared in clove oil and mounted in canada balsam (Gerlach, 1977) and examined microscopically.

Statistical analysis:

Data were statistically analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Growth parameters:

Generally, severe reduction of the plant growth with young leaves and chlorosis of leaves were the most remarkable symptoms due to both lead and cadmium toxicity. Moreover, in soils contaminated with cadmium, early visible symptoms, including witting with roll downward of leaves and interveinnals areas of leaves were observed throughout the experimental period during the two growing seasons. There is also evidence that mycorrhizal infection improved plant performance under heavy metals stress as represented by plant growth.

Data in Table (1) indicated that contaminated soils with both lead and cadmium at two concentrations used decreased significantly plant growth expressed by length of both root and shoot systems, their fresh and dry weights and leaf area per plant in both seasons. Cadmium had more deleterious effects on plant growth compared with lead. The deleterious effects of heavy metals including lead and cadmium on plant growth may be attributed to its inhibition effect on both cell division and cell elongation through the reduction of meristem size and reduction of cell differentiation leading to decrease the number of mature cells (Obraucheva *et al.*, 1998 and Sresty and Madhova Rao, 1999).

Results in the same table show that inoculation with VA-mycorrhizae improved plant growth through an increase in all the above mentioned growth parameters in both polluted and non-polluted soils with the two heavy metals. The beneficial effect of VA-mycorrhizae seems likely to be related to an enhanced supply on nutrients particularly phosphorus and nitrogen for the host plant (Abdel-Fattah and El-Katony, 1996 and Houka, 2000). Nitrogen is known to increase the meristimatic activity of plant tissues (Saleh *et al.*, 1982). Moreover, mycorrhizae infection may decrease heavy metals accumulation in plants growing in polluted soils. Thus, protect the host plants against phytotoxic metal effects and improve the plant growth (Leyval *et al.*, 1991).

The inhibitory effect of heavy metals could be a consequence of its interference with a number of metabolic processes associated with normal development such as water and nutrient uptake (Abo-Hamed *et al.*, 1987 and Suzan A. Sayed, 1999); the balance of internal phytohormones (Table 3) Hassan and Wahdan (1991) and photosynthesis (Sheoran *et al.*, 1990).

Photosynthetic pigments:

The effects of lead, cadmium and VA-mycorrhizae and their interactions on photosynthetic pigment concentrations (mg/g fresh weight) are presented in Table (2). It is clear that chlorophylls a, b and carotenoids tended to be sharply decreased in the radish leaves with increasing the two heavy metals concentrations. Cadmium had a severely harmful effect in this respect compared with lead ions. On the other hand, inoculation with VA-mycorrhizae increased all the above mentioned aspects in non-polluted soils with the applied two heavy metals. Moreover, it particularly improved these parameters in polluted soils. The beneficial effect of VA-mycorrhizae was greatest under pollution with lead. The deleterious effect of lead on photosynthetic pigments may be attributed to an inhibition of the biosynthesis of aminolevulinic acid (ALA), a precursor of chlorophyll (Tomas and Singh, 1996), damaging of photosynthetic apparatus (Ahmed and Tajmir-Riahi, 1993), enhancement of chlorophyll degradation by chlorophyllase (Drazkiewicz, 1994) and it can alter chlorophyll biosynthesis by inhibiting protochlorophyllide reductase through interfering the sulfhydryl site on the enzyme (Lagriffoul *et al.*, 1998). Moreover, it decreases the carotenoids that prevent chlorophyll photodestruction and inhibits Fe uptake and transport to the plant leaves (Fodor *et al.*, 1998).

Table (1): Effects of lead, cadmium, VA-mycorrhizae and their interactions on length of root and shoot systems, their fresh and dry weights (g) and leaf area (cm^2) of radish plants during the two growing seasons of 2000/2001 and 2001/2002.

Treatments	Root length (cm)		Shoot length (cm)		Fresh weight of root system (g)		Fresh weight of shoot system (g)		Dry weight of root system (g)		Dry weight of shoot system (g)		Leaf area per plant (cm^2)	
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Control	9.73	8.70	16.70	17.30	4.20	4.40	13.30	14.33	0.73	0.80	1.12	1.20	344.43	362.67
VA-mycorrhizae (VAM)	13.40	13.53	25.77	25.83	4.60	5.13	17.57	18.67	0.84	0.90	1.80	1.93	415.20	458.33
Pb 100 ppm	7.53	8.03	15.83	16.20	2.57	2.83	8.40	8.86	0.60	0.63	0.91	1.01	150.63	163.00
Pb 200 ppm	5.37	6.93	14.60	15.97	2.00	2.70	7.40	7.97	0.59	0.60	0.78	0.80	144.03	158.00
Pb 100 ppm+VAM	7.70	8.53	16.77	17.50	4.32	4.63	13.57	14.40	0.71	0.79	0.97	1.10	352.47	359.66
Pb 200 ppm+VAM	7.20	7.40	14.30	14.45	3.50	2.30	12.46	13.57	0.65	0.70	0.93	0.97	219.30	236.33
Cd 100 ppm	5.00	5.17	10.80	11.33	0.91	0.93	5.67	7.03	0.44	0.48	0.55	0.58	141.83	156.00
Cd 200 ppm	4.33	4.40	6.76	7.76	0.57	0.70	4.23	5.40	0.33	0.40	0.37	0.45	137.07	142.67
Cd 100 ppm+VAM	6.97	7.10	14.23	15.30	1.60	1.80	8.30	9.47	0.51	0.57	0.69	0.63	155.50	230.67
Cd 200 ppm+VAM	4.27	5.63	8.00	11.63	1.20	1.62	7.70	8.56	0.38	0.53	0.58	0.60	141.40	164.00
L.S.D. at 5%	1.20	0.89	1.70	1.90	0.22	0.25	0.56	0.71	0.02	0.02	0.01	0.03	66.50	34.16

Table (2): Effects of lead, cadmium, inoculation with VA-mycorrhizae and their interactions on both photosynthetic pigments (mg/g fresh weight) and total soluble carbohydrates (mg/g dry weight) concentrations in the shoot system during the two growing seasons of 2000/2001 and 2001/2002.

Treatments	Chlorophyll A		Chlorophyll B		Carotenoids		Total soluble carbohydrates (mg/g dry weight)	
	2001	2002	2001	2002	2001	2002	2001	2002
Control	1.25	1.36	0.50	0.51	0.46	0.48	2.98	3.11
VA-mycorrhizae (VAM)	1.35	1.43	0.94	0.95	0.57	0.61	3.55	3.73
Pb 100 ppm	1.17	1.22	0.45	0.51	0.41	0.44	1.41	1.47
Pb 200 ppm	1.10	1.16	0.43	0.45	0.39	0.40	1.19	0.87
Pb 100 ppm+VAM	1.26	1.39	0.51	0.54	0.47	0.46	3.12	3.13
Pb 200 ppm+VAM	1.18	1.33	0.45	0.49	0.37	0.39	2.74	2.80
Cd 100 ppm	1.14	1.17	0.40	0.42	0.33	0.34	0.60	0.66
Cd 200 ppm	0.59	0.63	0.19	0.21	0.21	0.23	0.54	0.58
Cd 100 ppm+VAM	1.12	1.16	0.32	0.33	0.30	0.32	0.83	0.90
Cd 200 ppm+VAM	0.91	1.10	0.24	0.30	0.26	0.29	0.80	0.84
L.S.D. at 5%	0.30	0.14	0.05	0.05	0.05	0.05	0.30	0.47

The reduction of chlorophyll concentrations in the leaves of cadmium treated plants may be due to reduction in the development of chloroplasts and changes in chloroplast ultrastructure by disorganized grana stacking with severe disruption of grana and increased intrathylakoid irregularly spaces (Ghoshroy and Nadakavkaren, 1990). Moreover, reduction in chlorophyll under Cd^{++} may be due to the inhibition of its biosynthesis and the activation of its enzymatic degradation (Somashekaraiah *et al.*, 1992). Sheoran *et al.* (1990) noted that chlorophyll reduction might be the major cause of decreased photosynthesis. Suppression of photosynthetic activity by cadmium occurs through various ways, i.e. stomata closure (Lamoreaux and Chaney, 1978); decreased the stomatal conductance (Sheoran *et al.*, 1990) and inhibition of photosynthetic electron transport by interfering with the function of PS II system (Clijsters and van Assche, 1985). Moreover, cadmium inhibited the formation of photosynthetic apparatus (Fodor *et al.*, 1996) and decreased CO_2 fixation due to its effect on either light or dark reactions of the CO_2 assimilatory process (Sheoran *et al.*, 1990).

The stimulating effect of mycorrhizae on photosynthetic pigments may be due to an increase of leaf area and cytokinin content as well as other stimulating phytohormones (Tables 1 and 3). Cytokinin is known to delay senescence of plant tissues through its effect on reducing the loss of chlorophyll (Gadallah, 1995 b) and inhibition of chlorophyll degradation through the inhibition of chlorophyllase activity (Drazkiewicz, 1994)

Carbohydrate concentrations:

Data in Table (2) show that similar performance was found in both photosynthetic pigments and total carbohydrates in the shoot system under heavy metals conditions. It is clear that total soluble carbohydrate concentrations were decreased significantly with lead and cadmium treatments. However, inoculation with mycorrhizae led to an increase in this respect.

Reduction in soluble carbohydrate concentrations induced by Pb^{++} treatments may be due to its inhibitory effect on photosynthetic activities (Fodor *et al.*, 1996), and photosynthetic pigment concentrations (Table 2) and ribulose diphosphate carboxylase (the key enzyme for carbohydrates synthesis) leading to decrease in all sugar fractions (Stibrova *et al.*, 1986). Moreover, the reduction effect of cadmium on carbohydrate concentration may be attributed to a reduction of leaf area, photosynthetic pigment contents and consequently reduction in the photosynthesis and accumulation of carbohydrates (Fouda and Arafa, 2002).

The positive role of inoculation with VA-mycorrhizae on carbohydrates may be due to its stimulatory effect on photosynthetic pigments (Table 2) and increased the production of cytokinins (Table 3), which enhanced the accumulation of soluble sugars.

Endogenous plant hormones:

Generally, data in Table (3) showed that there is an antagonistic effect between the heavy metals and inoculation with mycorrhizae on plant hormones concentrations in the shoot system of radish plant.

Table (3): Effects of lead, cadmium, inoculation with VA-mycorrhizae and their interactions on endogenous plant hormones concentrations (µg/g fresh weight) during the second season 2001/2002.

Treatments	IAA	GA_3	Cytokinins	ABA
Control	41.13	53.20	35.44	91.80
VA-mycorrhizae (VAM)	37.47	62.47	37.94	81.63
Pb 100 ppm	26.80	44.03	29.85	104.73
Pb 200 ppm	22.87	34.33	22.34	113.00
Pb 100 ppm+VAM	38.20	46.87	31.87	72.60
Pb 200 ppm+VAM	32.40	39.17	25.82	84.93
Cd 100 ppm	17.17	25.67	13.43	120.60
Cd 200 ppm	7.03	17.90	10.03	150.90
Cd 100 ppm+VAM	21.20	30.63	20.67	113.17
Cd 200 ppm+VAM	16.03	21.07	17.15	127.47
L.S.D. at 5%	1.39	1.42	0.71	3.91

Data in Table (3) indicate that polluted soil with lead and cadmium at two concentrations decreased all growth stimulators, i.e. IAA, GA_3 and cytokinins, while ABA concentration was increased. On the other hand inoculation with VA-mycorrhizae increased the concentrations of the above mentioned growth stimulators and at the same time reduced the accumulation of ABA compared with heavy metals treated plants. Similar results were obtained by Duke *et al.* (1986); Dixon (1990) and Torelli *et al.* (2000).

The depressive effect of both lead and cadmium treatments on endogenous auxin concentration may be attributed to that heavy metals not only reduce both the releasing of tryptophane, a precursor of IAA (Van Overbeek, 1966) and auxin transport within the plants (Hess, 1981), but also inhibit the conversion of tryptophane to IAA (Letham *et al.*, 1978). Additionally, El-Saied (2001) noted that the decrease in auxin concentration as a result of cadmium treatment may probably due to its effect that stimulate the formation of IAA oxidase and peroxidase leading to destruction of IAA or due to decrease in IAA biosynthesis. He added that the increase in ABA concentration caused by cadmium treatment may probably due to the effect on increasing its biosynthesis or by preventing the ABA catabolism.

The noticeable decline in gibberellins caused by Cd^{++} may result from conversion of free active gibberellins into bound inactive gibberellins as well as cadmium treatment may interfere with the metabolism of gibberellins; thus causing deactivation of gibberellins or inhibiting their biosynthesis (El-Saied, 2001).

The reduction in cytokinins concentrations by heavy metals may probably due to inhibiting the root growth (Table 1). Roots are considered to be the predominate site of cytokinins synthesis. In addition, heavy metals application may inhibits the translocation of cytokinins from root to shoot systems.

The role of the inoculation with mycorrhizae on overcoming the depressing effect of heavy metals on endogenous plant hormones may be attributed to its effect on increasing sytokinins and reducing ABA concentration (Table 3). Several studies revealed that the application of exogenous cytokinins alleviated the metal toxicity (Suzan, Sayed, 1999 and Fouda and Arafa, 2002). In addition, Saleh and Hemberg (1980) noted that kinetin may either stimulate the synthesis of auxin or act as an inhibitor of IAA oxidase.

Heavy metals concentrations in the shoots:

Data illustrated in Figure (1) indicates for the accumulation of both lead and cadmium in both root and shoot systems with increasing the heavy metal concentrations in the soil. Moreover, cadmium was more concentrated in the plant shoot and lead in the plant root. These results may be partly due to the lower mobility of lead than cadmium and partly due to restricted lead transport to shoot (Khan and Frankland, 1983 and Epstein *et al.*, 1999). There is an evidence on the relatively low heavy metals in the shoot compared with their concentrations in the soil. This evidence may explain the role of plant internal detoxification system which may have limited translocation of both lead and cadmium (Cieslinski *et al.*, 1996). It is also clear that inoculation with mycorrhizae not only suppressed both lead and cadmium accumulation in the shoots resulted in protection of the plants against the negative effects of heavy metals, but also improved plant performance under heavy metal stress.

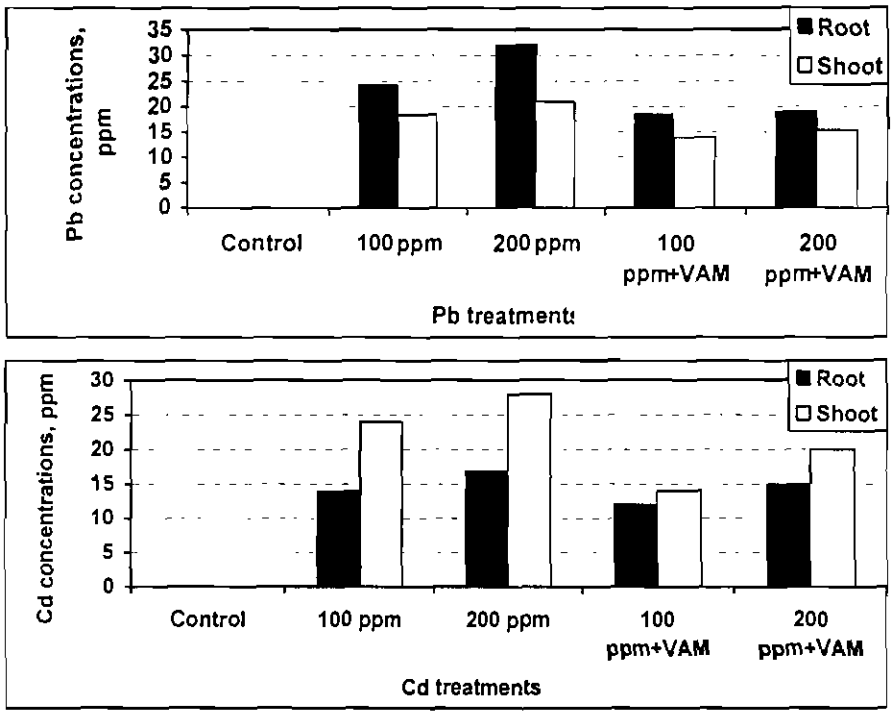

Figure (1): Lead and cadmium concentrations in both root and shoot systems of radish plant as affected by different treatments.

The observed suppression in Pb^{++} and Cd^{++} in the plants growing in polluted soils by inoculation with mycorrhizae may be attributed to hindered access of heavy metals to the root surface caused by the fungal sheath around the root surface (Jentschke and Godbold, 2000), or by preventing access of heavy metals to sensitive extra- or intra-cellular sites (Schützendüble and Polle, 2002). Cadmium was found to be bound to negative charged sites associated with the cell wall components such as cellulose and cellulose derivatives (Galli *et al.*, 1994). Another important role of mycorrhizae is improvement of plant performance under heavy metal stress and limiting heavy metal toxicity through binding of cadmium onto cell walls and accumulation of cadmium in the vacuolar component (Blaudez *et al.*, 2000).

Anatomical structure:

A. Root structure:

The most remarkable anatomical feature in radish roots is early formation of secondary roots, which often woody and massive in size (Figure 2).

Data in Table (4) and illustrated in Figure (2) show that treatments with both lead and cadmium at two concentrations decreased root diameter, cortex thickness, diameter of vascular cylinder and diameter of metaxylem vessels. While, inoculation with mycorrhizae increased all the above mentioned anatomical parameters (Figure 2 D). The inhibiting effect of the

two heavy metals on root structure may be attributed to their inhibition effect on meristimatic region of roots through reduction of both cell division and differentiation. Moreover, heavy metals caused a death of the root meristem and consequently reduction of cytokinins synthesis (Table 3).

Table (4): Measurements of some anatomical characters (µm) in the root of radish plant as affected by lead, cadmium and inoculation with VA-mycorrhizae as well as their interactions during the second growing season.

Treatments	Root diameter	Cortex thickness	Vascular cylinder diameter	Metaxylem vessels diameter
Control	3310	1040	2270	58
VA-mycorrhizae (VAM)	3550	1200	2350	62
Pb 100 ppm	3145	1000	2145	48
Pb 200 ppm	3100	920	2180	44
Pb 100 ppm+VAM	3220	990	2230	50
Pb 200 ppm+VAM	3040	860	2180	42
Cd 100 ppm	2540	820	1820	36
Cd 200 ppm	2370	600	1770	30
Cd 100 ppm+VAM	2790	820	1970	40
Cd 200 ppm+VAM	2620	740	1850	36
L.S.D. at 5%	71.0	36.0	59.0	3.5

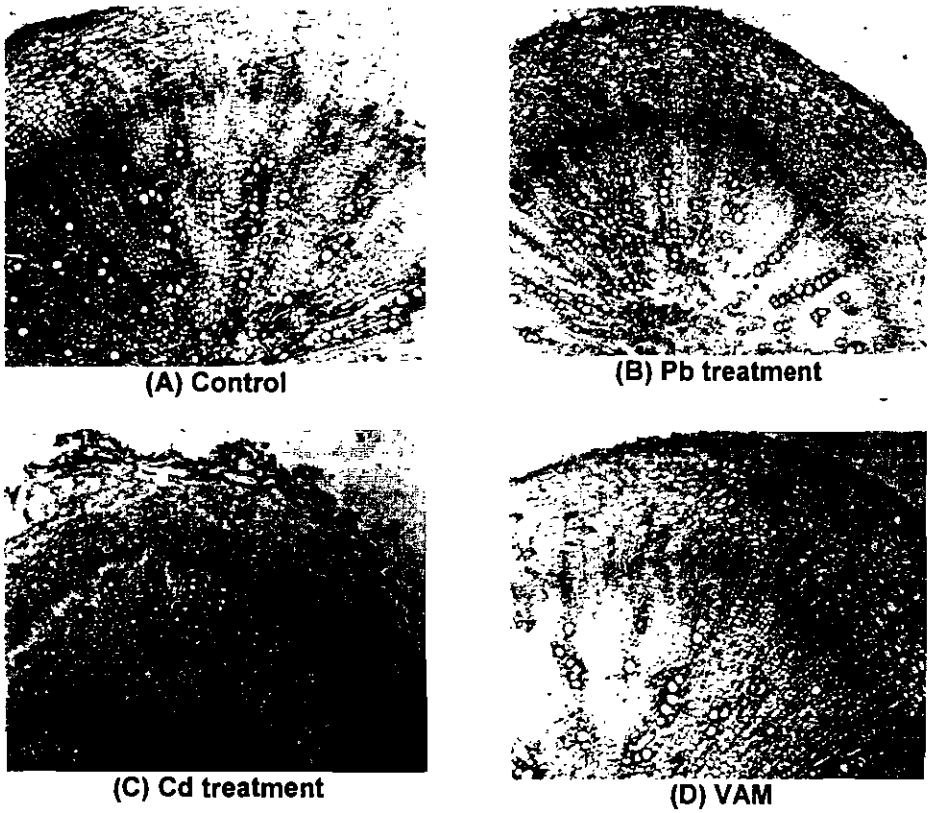

Figure (2): Cross sections of radish roots affected by lead, cadmium and VA-mycorrhizal fungi (Obj. x4. Oc. X15)

(A): Control, (B): Lead at 200 ppm, (C): Cadmium at 200 ppm and (D): VA-mycorrhizae

From the previous results, it could mentioned that inoculation with VA-mycorrhizal fungi led not only to alleviation of heavy metals toxicity, but also to improve plant performance in soils contaminated with both Pb and Cd. The beneficial effects of mycorrhizae depend on the type of metals and their concentrations. Moreover, inoculation with VA-mycorrhizal fungi alleviated the adverse effects of lead at all concentrations used, while it partially alleviated the adverse effect of cadmium, especially with the low concentration used.

B. Leaf structure:

The leaf blade internal structure of radish plant as seen in Figure (3) consists of upper and lower epidermis and mesophyll tissue. Mesophyll tissue consists of a single or two layered palisade parenchyma cells and the spongy parenchymatous cells are loosely arranged with numerous large intercellular spaces. The medvein vascular bundle is an open collateral bundle having a narrow cambial zone.

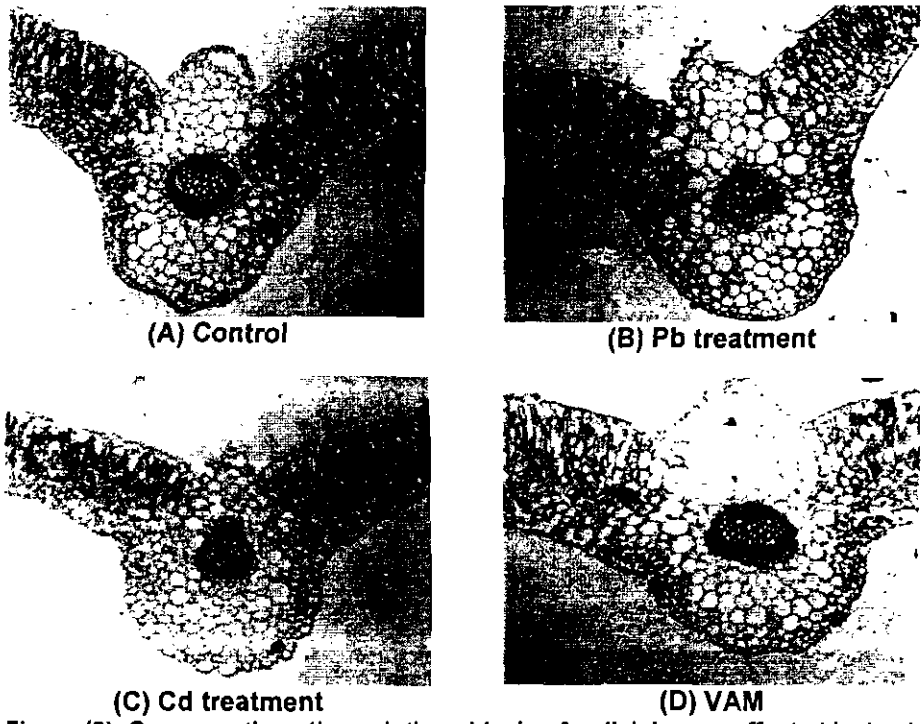

Figure (3): Cross sections through the midvein of radish leaves affected by lead, cadmium and inoculation with VA-mycorrhizal fungi (Obj. x4. Oc. X15)

(A): Control, (B): Lead at 200 ppm, (C): Cadmium at 200 ppm and (D): VA-mycorrhizae

Data in Table (5) and illustrated in Figure (3) indicated that treatments with both lead and cadmium at two concentrations used decreased leaf blade thickness due to a decrease in both palisade and spongy tissues thickness. The size of the midvein vascular bundle was also decreased as indicated by its dimensions as well as xylem and phloem tissues thickness. Metaxylem vessel diameter was also decreased. On the other hand, all the above mentioned anatomical features were increased with inoculated VA-mycorrhizae.

Table (5): Measurements of some anatomical characters (µm) in the 3rd leaf of radish plant as affected by lead, cadmium and inoculation with VA-mycorrhizae as well as their interactions during the second growing season.

Treatments	Leaf thickness in the midrib	Mesophyll tissue thickness	Palisade tissue thickness		Spongy tissue thickness	Midrib V.B. dimension	Phloem tissue thickness	Xylem tissue thickness	Metaxylem vessel diameter
					Length	Width			
Control	2400	360	250	180	230	270	70	160	60
VA-mycorrhizae (VAM)	2640	520	300	220	260	360	75	185	70
Pb 100 ppm	2000	345	230	165	200	220	50	150	40
Pb 200 ppm	1840	360	210	150	160	210	60	120	28
Pb 100 ppm+VAM	2120	470	260	210	236	200	62	174	44
Pb 200 ppm+VAM	1960	400	220	180	215	280	60	155	36
Cd 100 ppm	1320	250	140	110	160	190	45	115	20
Cd 200 ppm	1240	220	120	100	150	180	50	100	16
Cd 100 ppm+VAM	1840	390	165	125	210	195	90	120	27
Cd 200 ppm+VAM	1600	255	145	110	180	180	65	115	23
L.S.D. at 5%	77.5	13.7	33.2	26.2	19.7	12.0	13.4	18.0	3.0

The inhibitory effect of heavy metals on leaf structure may be attributed to their inhibiting effect on both cell division and cell elongation (Srestly and Madhava Rao, 1999). The stimulating effect of inoculation with VA-mycorrhizae on the anatomical features may be related to an enhancement supply of nutrients particularly nitrogen and phosphorus for host plants (Fouda and Arafa, 2002). Nitrogen led to an increase in the meristimatic activity (Saleh *et al.*, 1982). Torelli *et al.* (2000) concluded that mycorrhizae or phosphorus increased both cytokinins and auxin contents in the host plants, which promote cell division and cell enlargement.

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تأثير الرصاص والكادميوم وفطر الميكوريزا والتفاعل بينهم على النمو لنبات الفجل

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يهدف هذا البحث إلى دراسة تأثير عنصري الرصاص والكادميوم على نمو نبات الفجل وإمكانية التغلب على تأثيرهما الضار باستخدام فطر الميكوريزا. وأدي استخدام كلا من الرصاص والكادميوم بتركيز ١٠٠ ، ٢٠٠ جزء في المليون إلى نقص أطوال المجموع الجذري والخضري والوزن الغض والجاف لهما وكذلك مساحة الورقة لكل نبات بالإضافة إلى نقص محتوى الأوراق من صبغات البناء الضوئي ، وكذلك محتوى المجموع الخضري من الأكسينات ، والجبريلينات ، والسيتوكينينات ، بينما أدى إلى زيادة حمض الأبسيسك ، إضافة إلى زيادة تراكم عنصري الرصاص والكادميوم في كل من المجموع الجذري والخضري للنبات وكان عنصر الكادميوم أشد ضررا من الرصاص.

- على الجانب الأخر أدي التلقيح بفطر الميكوريزا إلى زيادة كل الصفات السابقة مع نقص في محتوى المجموع الخضري من حمض الأبسيسك وكذلك تراكم العناصر الثقيلة في كل من المجموع الجذري والخضري. وتشريحيا: أدت المعاملة بكلا العنصرين إلى نقص قطر الجذر ، وسمك القشرة ، وقطر الأسطوانة الوعائية ، وأوعية الخشب التالي ، بالإضافة إلي نقص سمك نصل الورقة والنسيج المتوسط نتيجة نقص كلا من النسيج العمادي والإسفنجي ، كما نقص حجم الحزمة الوعائية معبرا عنه بنقص أبعادها ، إلا أن التلقيح بفطر الميكوريزا أدى إلى زيادة كل الصفات السابقة ، كما أدى إلى تخفيف التأثير الضار لعنصري الرصاص والكادميوم وتحسين نمو النبات في الأراضي الملوثة بكلا العنصرين خاصة تحت التركيزات المنخفضة.