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Effect of Cd and Pb on growth, certain antioxidant enzymes activity, protein profile and accumulation of Cd, Pb and Fe in *Raphanus sativus* and *Eruca sativa* seedlings

Amal A H Saleh

Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

ABSTRACT

Seedlings of *Raphanus sativus* and *Eruca sativa* were grown in pots containing acid-washed soil and irrigated with every 3 days for 4 weeks nutrient solution based on one-fifth Long Ashton solution containing 50, 100 and 200 ppm of Cd and Pb concentrations. Measurements of plant height, fresh and dry weights, chlorophyll contents, electrophoteric patterns of proteins, oxidative enzymes activity (Ascorbic acid oxidase and peroxidase) and accumulation of Cd, Pb and Fe were evaluated in seedlings of both plants. It was found that, increased supply of Cd and Pb significantly increased fresh and dry weights and significantly decreased plant height in both *Raphanus sativus* and *Eruca sativa*. Total chlorophyll content was increased with increasing Cd and Pb concentration in both two plants studied. Treatment with Cd and Pb at all concentrations increased significantly peroxidase and ascorbic acid oxidase activity. Low concentrations of Cd and Pb decreased polypeptide concentration in both *Raphanus sativus* and *Eruca sativa* compared with the control. Also, increasing two heavy metals concentration resulted in the appearance of new protein bands. It was also noticed that, increasing Cd and Pb concentration in nutrient solution significantly increased uptake and accumulation of these two heavy metals in *Raphanus sativus* and *Eruca sativa*. Accumulation of Fe in the two studied plants was significantly decreased with increasing supply of Cd and Pb. From the above results, it could be concluded that, *Raphanus sativus* and *Eruca sativa* possess the ability to tolerate and accumulate toxic metals. Also, it was suggested that, *Raphanus sativus* and *Eruca sativa* used as metal-scavenging plants for contaminated soils.

KEYWORDS: Cadmium, lead, growth, antioxidant enzymes, protein, heavy metals accumulation.

INTRODUCTION

Heavy metals are present in soils as a consequence of human activity. Many materials contain heavy metals, and the application of fungicides, fertilizers, metal-rich mine tailings, metal smelting, electroplating, battery recycling, fuel burning, intensive agriculture and sludge dumping are the most important human activities that contaminate soils with large quantities of metals (Moffat 1995). A common characteristic of heavy metals is that they exert toxic effects at low concentrations compared with macronutrients. Cadmium and lead have a great mobility in the soil relative to other heavy metals and are taken up to varying degrees by plants (Varo *et al.* 1980). The plants absorb Cd and can transport it to the shoots to different degrees (Brinkhuis *et al*. 1980). The degree of accumulation in the shoots depends more or less on the tolerance of the plant to Cd (Page *et al*.1972; Coughtrey & Martin 1978). Effects on growth can be variable. For example, the dry weight during growth of *Alyssum pintodasilvae* was not influenced by the presence of Cd, Cr, Cu and Zn in the soil (Varennes *et al.* 1996).

The fresh and dry weight of *Betula pendula* seedlings grown in nutrient solution containing 2μM Cd increased (Gussarsson *et al.* 1996). At toxic levels of added Zn, a Zn-sensitive ecotype of *Holcus lanatus* decreased in growth relative to a Zn-tolerant ecotype (Rengel 2000). In maize, radical elongation was depressed at a concentration of 25μg Cd or 250μg Pb (Hassett *et al*.1976), and in *Holcus lanatus* (Symeonidis & Karataglis 1992), the root length declined with increasing Pb concentration in the nutrient solution. Because of the variations, it is difficult to propose a limit for toxic concentrations of Zn, Cu, Cd and Pb in soil. Besides the time of exposure, the degree of toxicity is influenced by the biological availability of the metals and interactions with other metals in the soil, nutritional status, age

* Address for Correspondence

and mycorrhizal infection of the plant (Pahlsson 1989). Loikema & Vooijs (1986) found that chlorophyll content of tolerant plants of *Silene cucubalus* was not affected by Cu, whereas sensitive plants became chlorotic. Also, Sasadhar (1987) found that Pb decreased protein and chlorophyll contents of *Cuscuta reflexa*. Symeonidis & Karataglis (1992) found that the greater chlorophyll content found in tolerant genotypes of *Holcus lanatus* L., in different Pb and Zn concentrations, in comparison with the control. The highest Cd concentration decreased photosynthesis, but substantially increased ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) content in sunflower leaves (Pankovi *et al*. 2000). One of the possible mechanisms for the way elevated concentrations of heavy metals may damage plant tissues is by the stimulation of free radical production imposing oxidative stress (Foyer *et al*. 1997).

Plants possess a number of antioxidant molecules and enzymes that protect them against oxidative damage. Superoxide dismutase (SOD), the first enzyme in the detoxifying process, convert O_2 radicals to H_2O_2 at a very fast rate (Polle & Rennenberg 1994). Sulphahydryl-containing enzymes are very sensitive to heavy metals, whereas peroxidase is relatively resistant in all plant populations studied (Ernst 1976). Ascorbate oxidase activity increased in *Trifolium subterranean* with increasing Cu supply (Delhaize *et al*. 1985). Cu increased antioxidative enzymes such as ascorbate peroxidase, glutathione reductase and peroxidase in rice seedling (Chen *et al*. 2000). An increase in peroxidase activity is a common response to various oxidative stress factors (Gasper *et al.* 1985).

The protein concentration increased up on treatment with 0.05 mM Cd and decreased at low Cd concentration (Wichmann *et al*. 1983; Lozano-Rodriguez *et al*.1997). It was found that, the concentrations of Cd and Pb in root of *Phaseolus vulgaris* increased in response to soil concentration (Hardiman *et al*. 1984). Cd uptake by *Beta vulgaris* showed a linear relationship to the concentration of Cd in nutrient solution (Maria & Sylvia 1986). The addition of heavy metals to the soil resulted in increased uptake by *Alyssum pintodasilvae* (Varennes *et al*.1996; Pilegaard1987). High heavy metals concentration reduced the iron (Fe) content of the tissues and total uptake of Fe in Sorghum (Kue & Mikkelsen 1981). Moreover, the mobilization of Fe by phytosiderophores (non-protein amino acids released by the roots of grasses) is slightly inhibited by Zn and strongly inhibited by Cu (Zhang *et al*. 1991).

The present study was carried out to investigate the effect of different concentrations of Cd and Pb on plant height, fresh and dry weights, chlorophyll content, antioxidant enzyme activity, electrophoteric patterns of proteins and heavy metal uptake by *Raphanus sativus* and *Eruca sativa* seedlings.

MATERIALS AND METHODS

Plant culture: Seeds of Raphanus sativus and Eruca sativa were obtained from the Field Crop Institute, Agricultural Research Center, Egypt. Seeds were germinated in pots half-filled with acid-washed sandy soil. The pots were irrigated with one-fifth Long Ashton solution every three days for two weeks.
Exposure to Cd and Pb: Seedlings were then irrigated every three days with nutrient solution containing additional 50, 100 and 200 ppm Cd or Pb, replicated three times. The plants were grown under these conditions for 4 weeks, and then, harvested.
Growth parameters: Six plants from each treatment (6- weeks- old) were chosen randomly for the measurement of plant height, fresh and dry weights. The plants were dried to constant weight at 70 °C to estimate the elements content (Cd, Pb and Fe).
Estimation of chlorophyll content: The concentration of pigments as μg/g was determined spectrophotometrically in fresh leaves according to Metzner *et al.* (1965).

Antioxidant enzymes (Ascorbic acid oxidase & Peroxidase): The activity of ascorbic acid oxidase was measured in the leaves of both by using the method of Oberbaker and Vines (1963), and peroxidase activity by using the method of Malik & Singh (1980).

Protein Electrophoresis: Samples were prepared for electrophoresis by solubilization in equal volumes of SDS buffer (0.0625 M Tris-Hl pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol and bromophenol blue 0.001%). Six reference proteins differing in their molecular weights were used as markers and run in parallel with the protein under study. The method was used as recommended by King & Laemmli (1971).

Determination of minerals in plant material: The wet ashing method described by Westerman (1990) was used for the estimation of Cd, Pb and Fe concentrations. Analysis was carried out using a Perkin-Elmer 3100 atomic absorption spectrophotometer. The data were recorded in mg/kg. dry weight.

Statistical analysis: Standard analysis of variance was performed with all data using regression analysis for estimation of minerals and 2-way ANOVA for other parameters.

RESULTS

Plant height, Fresh weight and dry weight: Plant height was significantly decreased at 50 and 100 ppm Cd in *Raphanus sativus* and in *Eruca sativa* at 50 ppm Cd only by 40.6, 31.1, and 14.7 % respectively compared to the control. Fresh weight was significantly increased at 100 and 200 ppm Cd and at 50 and 100 ppm Pb in *Raphanus sativus* by 17.2, 27.6, 31.0 and 10.3 % respectively in comparison with the control. In *Eruca sativa,* fresh weight was significantly increased by addition of Cd and Pb at 200 ppm by 53.8 and 61.5 % respectively in comparison with the control. Dry weight increased in *Raphanus sativus* at all concentrations of Cd and Pb and at all concentrations of Pb in *Eruca sativa.*

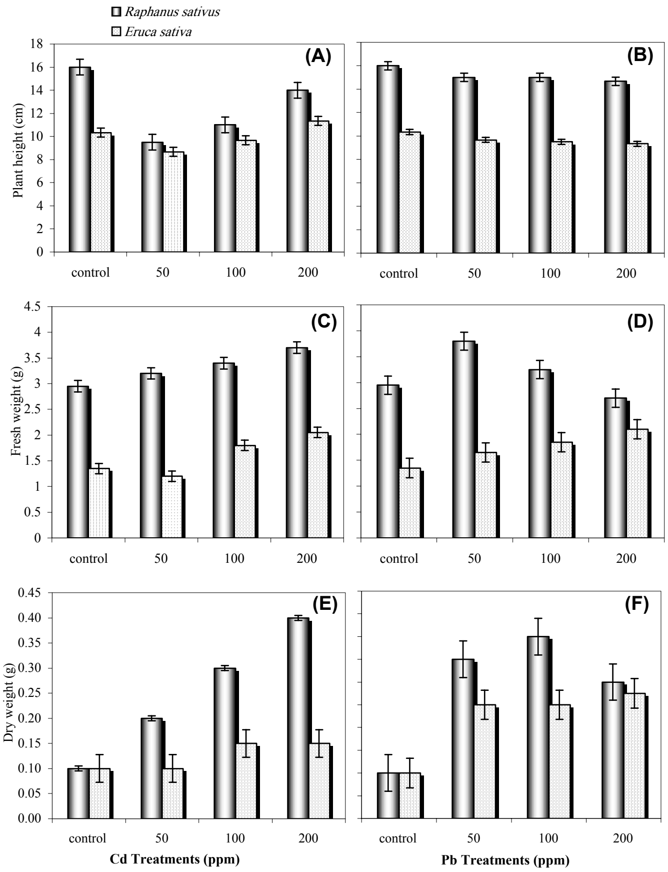
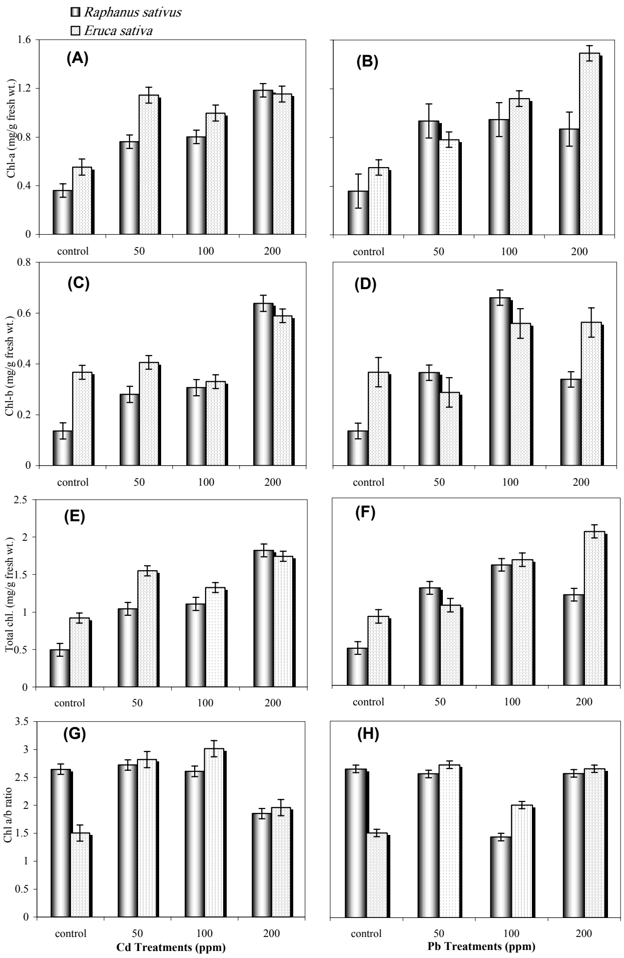


Figure 1: Effect of Cd (A, C, E) and Pb (B, D, F) supply in irrigation solution on some growth parameters (plant height, fresh and dry weights) of *Raphanus sativus* and *Eruca sativa* seedlings.

Chlorophyll content: The chlorophyll content in *Raphanus sativus* and *Eruca sativa* is shown in Figure 2. Increasing Cd and Pb concentration increased chlorophyll (a) and total chlorophyll in both plant species. Chl.a:b ratio decreased significantly in *Raphanus sativus* at all Pb concentrations and 200 ppm Cd by 32.5 % compared to the control, but increasing Cd and Pb concentrations significantly increased the chl a:b ratio in *Eruca sativa.*



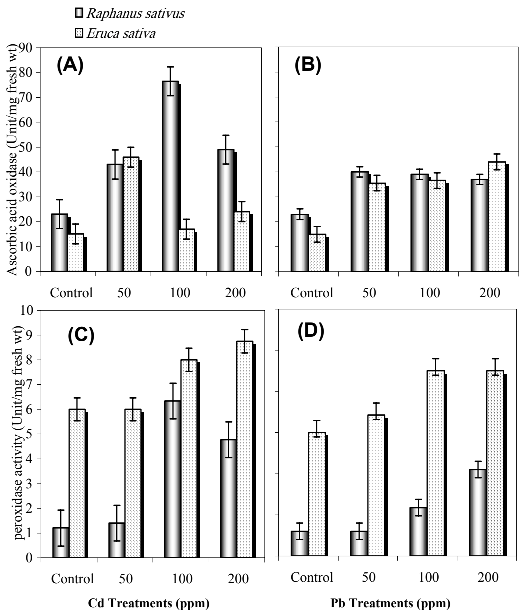


Figure 3: Effect of Cd (A, C) and Pb (B, D) supply in irrigation solution on Ascorbic acid oxidase and peroxidase activity in *Raphanus sativus* and *Eruca sativa*.

Antioxidant enzyme activity

a. Ascorbic acid oxidase activity: Figure 3 show that ascorbic acid oxidase activity in *Raphanus sativus* and *Eruca sativa* significantly increased with increasing Cd and Pb concentrations, for Pb greater *Eruca sativa* than in *Raphanus sativus*. In *Raphanus sativus*, the percentage of change in ascorbic acid oxidase activity was higher at some Cd concentrations than *Eruca sativa*

b. Peroxidase activity: Results from Figure 3 show that peroxidase activity significantly increased at all Cd and most Pb concentrations in *Raphanus sativus*. The same was true in *Eruca sativa*, where peroxidase activity also significantly increased for most treatments, but less than in *Raphanus sativus*.

Protein electrophoresis: The electrophoretic pattern of protein analysis of *Raphanus sativus* and *Eruca sativa* plants are shown in Fig. (4) and (5). It was noticed that, low concentration of the two heavy metals (50 ppm) in *Raphanus sativus* induced a degradation of protein polypeptide bands as compared with control (Fig. 4). Increasing Cd and Pb concentrations resulted in the appearance of new polypeptides bands between 94,000 and 67,000 kD and between 43,000 and 20,000 kD

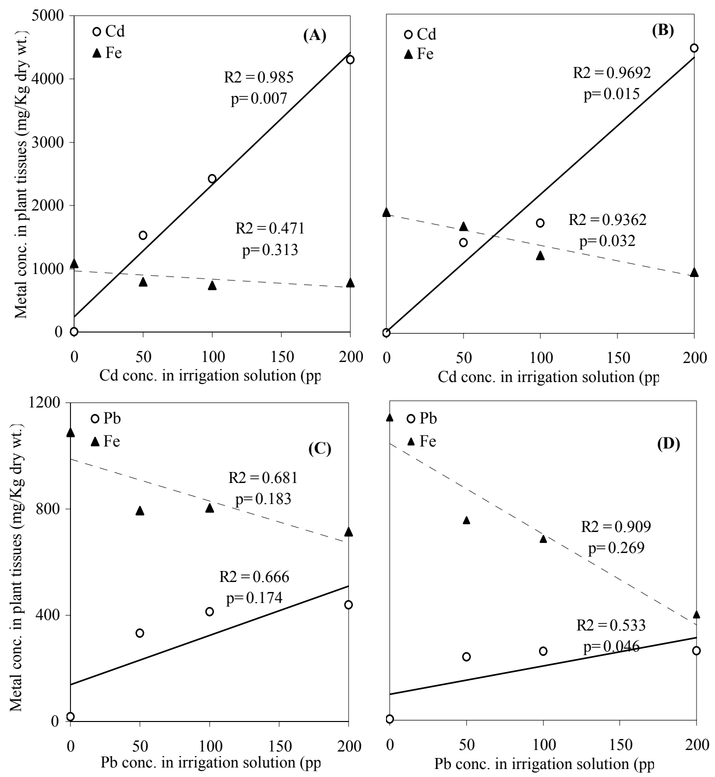
In respect to *Eruca sativa,* Fig. 5 shows that the three Cd concentrations and the low Pb concentration induced a decrease in polypeptide concentrations as compared to control.

However, application of 100 ppm Cd resulted in the appearance of polypeptide bands between 30,000 and 20,000 kD.

On the other hand, application of 100 ppm Pb increased the concentration of polypeptides in the plants, so did the staining intensity of most bands. The same treatment resulted in the appearance of new polypeptide bands with molecular weights lies between (67,000 and 43,000), (43,000 and 30,000), (30,000 and 20,000) kD. However, Increasing Pb concentration (200 ppm) decreased the concentration of these new bands so did the decrease in staining intensity.

(4) (5)

Figures 4 and 5: Electrophoretic profiles of *Raphanus sativus* and *Eruca sativa* protein patterns in response to Cd and Pb with three different concentrations (50, 100 and 200 ppm). (M is biomarkers with different molecular weights).



Heavy metals and Fe content: It was shown from Fig. 6 that as Cd and Pb increase in irrigation solution their uptake and accumulation increase in *Raphanus sativus* and *Eruca sativa* tissues. It was also noticed that, *Raphanus sativus* accumulated Cd more than *Eruca sativa* while Pb accumulation in *Eruca sativa* more than that of *Raphanus sativus*.

The increase of Cd concentration in irrigation solution decreased the uptake of Fe in both plants under investigation. However, the effect of Cd was more pronounced in *Raphanus sativus* than *Eruca sativa.* It was also noticed that, increasing Pb concentration decreased Fe uptake in *Raphanus sativus* to nearly the same limit. While, in *Eruca sativa* it was found that as Pb concentration increased Fe uptake decreased.

DISCUSSION

The plant height of *Raphanus sativus* and *Eruca sativa* was significantly decreased at all Cd and Pb concentrations. These results are in agreement with those of Hassett *et al*. (1976) who observed that radical elongation was depre
(1992) noticed that the height of *Holcus la*
nutrient solution. Also, This could be due
and specially their effect on enzyme activity. Root growth is the result of two different mechanisms; cell division; at root tip and cell elongation in the extension zone; (Woolhouse 1993). Both cell elongation and division are affected by the presence of heavy metal (Balsberg 1989). Bonnet *et al*.(2000) suggested that the decrease in growth of ryegrass plant height might be due to an accumulation of heavy metals in leaves. On the other hand, Rengel (2000) showed that at toxic level of added Zn, the Zn-sensitive ecotype of *Holcus lanatus* decreased in growth than the tolerant ecotype. Fresh and dry weight of *Raphanus sativus* and *Eruca sativa* increased significantly with the addition of Cd and Pb in irrigation solution. This result is agreement with Gussarsson *et al*. (1996) who suggested that fresh and dry weight of *Betula pendula* seedling grown in nutrient solution containing Cd increased because phytochelatins participate in protecting the root against Cd interference with growth, possibly by restricting Cd-induced changes in the nutrient composition of the plant. From the results of growth parameters of this experiment, it was concluded that *Raphanus sativus* and *Eruca sativa* have the ability to grow in soil contaminated with heavy metals without exhibiting toxicity symptoms, thus *Raphanus sativus* and *Eruca sativa* termed as tolerant plants.
The results obtained with photosynthetic pigments, in the present work (Figure 2) showed that chlorophyll a and total chlorophyll in *Raphanus sativus* and *Eruca sativa* were increased with increasing Cd and Pb concentration in irrigation solution. These results are in agreement with Loikema & Vooijs (1986) and Symeonidis & Karataglis (1992) who found that the greater chlorophyll content found in tolerant genotypes of *Holcus lanatus L.* grown under different Pb and Zn concentrations.

There are two tolerance mechanisms in plant against heavy metal toxicity, external and internal. Internal tolerance mechanism is immobilizes and compartmentalizes or formation of heavy metal-citrate or oxalate complex, a non-phytotoxic form (Shao *et al.* 1998). Another mechanisms for heavy metal detoxification in plants is the chelation of the metal by a ligand, in some cases, the subsequence compartmentalization of the ligand-metal complex and chelation by organic acids such as citrate and malate (Christopher 2000; Metch *et al*. 2000). Results in Figure (3) show that the antioxidant enzymes (ascorbic acid oxidase and peroxidase) activity in *Raphanus sativus* and *Eruca sativa* increased significantly with increasing Cd and Pb supply in nutrient solution. This result is agreement with Delhaize *et al*.

(1985) who found that ascorbate oxidase activity increased in *Trifolium subterranean* with increasing Cu supply. An increase in peroxidase activity is a common response to various oxidative stress factors (Gasper *et al*. 1985). It was found that Cu increased the activities of antioxidative enzymes in rice seedling (Chen *et al.* 2000). Cellular damage and oxidative damage caused by free radicals (as a result of high heavy metal concentration, and demonstrated in isolated chloroplast) might be reduced or prevented by antioxidative enzymes. The protective mechanisms and adapted by plants to scavenge free radicals and peroxides include several antioxidative enzymes as glutathione reductase, peroxidase and ascorbic acid oxidase (Chen *et al*. 2000).

The low Cd and Pb concentrations decreased protein concentration in *Raphanus sativus* and *Eruca sativa* compared to control. . Wichmann *et al*. (1983) found that, the protein content was decreased at low Cd concentration. In contrast, Sasadhar (1987) found that, Pb decreased protein content of *Cascuta reflexa*. This decrease in protein revealed that soluble protein may be leaked or diffused out of the plant material or possibly the catabolic enzymes were induced and destroyed the proteins. Also, increasing two heavy metal concentrations resulted in the appearance of new protein bands. This result are in agreement with Lozano-Rodriguez *et al*. (1997) who showed that the protein concentration of pea shoot increased upon treatment with 0.05 mM Cd due to Cd treatment. From the results of this experiment, It was suggested that the protein content were higher in tolerant plants such as *Raphanus sativus* and *Eruca sativa* than sensitive plants. Increasing Cd and Pb concentrations in irrigation solution caused a gradual increase of accumulation by the two plants under investigation. The degree of accumulation of these heavy metals differed between the two plants. This might be due to plant genotype or could be attributed to individual plant characteristics. Hardiman *et al*. (1984) suggested that genetically controlled features and morphological and anatomical differences might be responsible for the resulting deviation. Low *et al.,* (1984) reported that metal enrichment was found to be dependent on the plant species and metal type. Similarly, Pilegaard (1987) and Varennes *et al.,* (1996).found a linear correlation between root heavy metals of *Daucus carota, Raphanus sativus* and *Alyssum pintodasilvae* and soil Cd, Pb and Cu concentrations. Fig. 3 & 4; show that increase supply of Cd and Pb in nutrient solution decreased significantly the uptake and accumulation of Fe in *Raphanus sativus* and *Eruca sativa* seedlings. Kue & Mikkelsen (1981) reported that the total uptake of Fe in sorghum was reduced by high levels of heavy metals. This reduction of Fe in shoot resulting from coating occurred on root surface and intensified with increasing heavy metals concentrations in the substrate or could be attributed to the formation of high valent heavy metals oxides on the root surfaces which may certain Fe and reduce its absorption by sorghum. Also, Zhang *et al*. (1991) and Nicolaus *et al*. (1999) showed that mobilization Fe by phytosiderophores slightly inhibited by Zn and strongly inhibited by Cu. Nicotinamine (NA) occurs in all plants and chelates metal cations include FeII and have an important role in scavenging Fe and protecting cell from oxidative damage

It could be concluded that, increasing heavy metals by pollution induced increased uptake of these elements by the studied plants without exhibiting toxicity symptoms. *Raphanus sativus* and *Eruca sativa* termed as hyperaccumulator and tolerant to heavy metals. Also, it was suggested that, continued growth and harvest of these plants to detoxify Cd and Pb-contaminated soil.

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الملخص العربي

تأثير الكادميوم والرصاص على نمو ونشاط بعض الأنزيمات المضادة للأكسدة وصورة البروتين وتراكم كل من الكادميوم و الرصاص والحديد في بادرات الفجل والجرجير

أمال احمد حسن صالح

قسم النبات-كلية العلوم-جامعة قناة السويس-الإسماعيلية

تم زراعة بذور الفجل والجرجير وعوملت بمحلول لونج اشتون (5/1 تركيز) بالإضافة إلى تركيزات مختلفة من الكادميوم والرصاص (50، 100، 200جزء في المليون) وذلك كل ثلاثة أيام لمدة أربعة أسابيع. وقد تم قياس طول النبات والوزن الغض والجاف وكذلك محتوي كل من الكلوروفيل ا ,ب والكلوروفيل الكلى بالإضافة إلى نشاط إنزيمات الأكسدة مثل إنزيمي البيروكسيديز و أوكسيديز حمض الاسكوربيك وكذلك مدى امتصاص وتراكم كل من الكادميوم والرصاص والحديد في بادرات الفجل والجرجير. أوضحت الدراسة أن زيادة تركيز كل من الكادميوم والرصاص أدت إلى زيادة معنوية في كل من الوزن الغض والجاف ونقص في طول النباتات محل الدراسة كما تسببت أيضا في زيادة معنوية في كلوروفيل ا,ب والكلوروفيل الكلى . وجد أيضا أن زيادة تركيز عنصري الكادميوم والرصاص أدي إلى زيادة الإنزيمات المضادة للأكسدة (البيروكسيديز و أوكسيديز حمض الاسكوربيك) في النباتين. و قد أوضحت الدراسة أن التركيزات المنخفضة من الكادميوم و الرصاص قللت تركيز الببتيدات العديدة في النباتين محل الدراسة، كما أوضحت أيضا أن زيادة تركيز كل من العنصرين أدى إلى ظهور مناطق جديدة من البروتين. كما تسببت زيادة تركيزات الكادميوم والرصاص إلى زيادة تجمع هذين العنصرين في كل من الفجل والجرجير وان كان تجمع وتراكم الكادميوم في الفجل اكثر منه في الجرجير وتجمع الرصاص في الجرجير اكثر منه في الفجل أما بالنسبة لعنصر الحديد فقد أدت زيادة تركيزات الكادميوم والرصاص إلى نقص مستوى الحديد. من النتائج السابقة يمكن استنتاج أن نباتي الفجل والجرجير لهما القدرة على النمو بكفاءة عالية ومقاومة التركيزات السامة من العناصر الثقيلة وتراكمهما داخل أنسجتهما وكذلك يمكن استخدامهما لإزالة هذه العناصر السامة من التربة الملوثة بهما.