CATRINA (2010), 5 (1): 1 - 7

Physiological and Biochemical Aspects of Tolerance in *Lepidium sativum* (cress) to Lead Toxicity

Sameera O. Bafeel
Biology Department, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia

ABSTRACT

The present study was undertaken to assess the toxicity of Pb^{2+} on photosynthetic pigments, total soluble protein and malondialdehyde (MDA) contents as well as the activities of superoxide dismutase SOD (EC 1.15.1.1), catalase CAT (EC 1.11.1.6) and peroxidase POD (EC 1.11.1.7) in *Lepidium sativum* L. leaves after 10-days of supplying Lead (0-600 ppm) in the nutrient solution. The results indicated that Lead treatment adversely affected plant growth and disturbed the cell metabolism seriously. The development of toxic symptoms, corresponded to a high accumulation of Pb^{2+}, was due to the increase in H_2O_2 and MDA contents decrease in protein content and to the much elevated SOD and POD activities in leaves. In addition, the results demonstrated that the high concentration of lead (Pb^{2+} > 400 ppm) could result in a disintegration of the antioxidant system in *Lepidium* seedlings. Also, the significant decrease in the contents of photosynthetic pigments was related to high-level of metal stress. High concentrations of Pb^{2+}, especially 400 and 600 ppm, resulted in a great variation in protein pattern distribution. It can be concluded that *Lepidium sativum* can tolerate low levels of lead in contaminated soil.

Keywords: Lead; *Lepidium sativum* L.; Protein; oxidative stress, Superoxide dismutase, Catalase, Peroxidase.

INTRODUCTION

Millions of tons of trace elements continue to be produced and released in the biosphere through air, water and soil. Many of them including lead can not be degraded and therefore accumulates in organisms living in contaminated areas. Hence lead particularly, has become a strong environmental pollutant (Sharma and Dubey, 2004).

Acid soils are found throughout the world, about 40% of the arable soils and 12% of the land used in crop production have a pH below 5.5 (Von Uexkull and Mutert, 1995). Moreover, soil acidification is increasing world-wide. Increasing acidity of soil solution leads, in general, to an increase in the availability of heavy metals (Punz and Sieghardt, 1993). Plants growing in acid soils suffer lead toxicity stress, where crop production is markedly reduced. The toxicity of Pb^{2+} has been recognized as a major factor that limits plant growth in acidic soil (Paivoke, 2002). Lead accumulating plants grow well in strongly acidic soils experiencing cumulative Pb^{2+} toxicity damage (Ahmed and Tajmir-Riahi, 1993).

Disturbance of the metabolism by excessive Pb^{2+} or heavy metals appears to happen in several ways including, reduction of chlorophyll content, inhibition of plant growth and respiration, changing the ultrastructure of the cell organelles, and alteration in the activity of many enzymes involving in various metabolic pathways (Frankart *et al*., 2002; Yamamoto *et al*., 2003). Lead has also been shown to affect the protein and starch mobilization adversely, this affect could be used to assess the qualitative and quantitative effects of a biotic stresses on the structural organization of plant cells (Schützendübel and Polle 2002; Polle and Schützendübel, 2003). It has been proposed that heavy metals can lead to oxidative stress resulting in inhibition of photosynthesis, respiration rate and other metabolic processes in plants. Many evidences suggest that these metabolic disturbances were closely related to the accumulation of heavy metals and the subsequent excessive production of reactive oxygen species (ROS) in plants, including superoxide radical ($O2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Gardea-Torresdey *et al*., 2004; Ibrahim, 2006; Hou *et al*., 2007).

High concentrations of heavy metals in plant tissues can induce disorder in nutrient metabolism, as a consequence, leading to abnormal growth. Moreover, ROS can cause oxidative damage to the bio-molecules such as lipids. This can lead to membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage. To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense antioxidative system, including low-molecular mass antioxidants, as well as antioxidative enzymes, such as superoxide dismutase (SOD) and peroxidase (POD) (Halliwell and Gutteridge, 1984; Han, 1999). SOD is the most effective antioxidative enzyme in preventing cellular damage that catalyzes the conversion of the superoxide anion to H_2O_2, while POD utilizes H_2O_2 in the oxidation of various inorganic and organic substrates. Many evidences suggest that high levels of Al can affect protein metabolism and induce oxidative stress in plants. On the other hand, great differences in Pb^{2+} tolerance have been found among plant species and genotypes (Guo *et al*., 2007). Therefore, it is very important to identify the antioxidative defense system, the adaptive capacity and the toxicity of *Lepidium* plant exposed to lead.

* *eMail: sbafil@kau.edu.sa*

In the present study, concentrations of Pb^{2+} were chosen according to preliminary test in order to examine the inhibitory effect of acidic soil on *Lepidium sativum* seedlings with reference to: (1) changes in protein content; (2) changes in photosynthetic pigments (chlorophyll *a*, chlorophyll *b,* and carotenoids) and (3) changes in the activities of antioxidant enzymes (peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD)), as well as the content of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Therefore, this can determine the concentration extent to which *Lepidium sativum* is suitable to tolerate the lead polluted soils.

MATERIALS AND METHODS

Plant Materials and Treatments

Seeds of *Lepidium sativum* L. collected from different locations of Saudi Arabia were screened for germination responded to Pb^{2+} (data not shown). *Lepidium sativum* obtained from Abha was the most lead tolerant species. Seeds were surface sterilized by immersing in 0.1 % $HgCl_2$ for two min, washed with five changes of sterile distilled water and soaked in continuously aerated distilled water for 24 h in darkness. Thirty seeds were sown in each pot (15 cm diameter x 20 cm height), filled with pre acid washed sand. All pots were placed in a growth chamber under 70-80% RH with 16/8h day/night cycle and controlled temperature of 28/25^{o}C. Light intensity was 500 µmol m^{-2} s^{-1} at the top of plants supplied by a mixture of fluorescent and incandescent lamps. Each pot was irrigated with 250 ml distilled water at first, then occasionally with a certain amount of distilled water in order to keep the soil water content constant. Pots were irrigated with half strength Hoagland solution every two days to reach 80% of water holding capacity throughout the whole experimental period (Hoagland and Arnon, 1950).

Experimental Design

Twenty five-days-old *Lepidium sativum* seedlings of a uniform size were carefully taken from the pots with care to avoid any injury to the roots and placed in sponge support collars. Collars were then fitted into holes in the tops of glass bottles containing 500 ml continuous aerated Hoagland's solution supplemented with various concentrations of Pb^{2+} for 10 days. Individual Pb^{2+} treatments were a control, with Hoagland nutrient solution (0 ppm Pb^{+2}), and four Pb^{2+} concentrations 100, 200, 400 and 600 ppm using lead acetate. These concentrations were chosen on the basis of preliminary experiments, the lowest one being was below the toxicity threshold and the highest one was above. The pH of the nutrient solution was buffered to pH 5.0 and kept constant during the experiment. All solutions were changed every 3 days during the 10 experimental days to maintain the metal concentrations. All bottles were placed in a growth chamber under the same conditions previously indicated. After ten days of exposure to Pb^{2+}, soluble protein, photosynthetic pigments, POD, CAT, SOD, and MDA of leaves were determined. A minimum of three replicates was performed in each treatment.

Photosynthetic Pigments Determination

Chlorophyll *a*, *b,* and carotenoids were extracted in 80% ethanol and measured colorimetrically following the method of Lichtenthaler (1987).

Protein Analyses

Proteins of control and Pb^{+2} treated leaves plants were extracted with an equal volume of 20 mM Tris-HCl buffer (pH 8.6) containing 5 % β-mercaptoethanol (v/v), at 4°C. Each extract was filtered through the three layers of gauze and centrifuged at 10,000 g for 30 min. The supernatant was collected and assayed for total proteins according to the Bradford method (1976) using bovine serum albumin (BSA) as standard.

SDS-PAGE of proteins

Various electrophoretic techniques were applied to investigate the banding patterns of the purified enzyme and proteins in leaves of *Lepidium sativum* L. plant treated with different concentrations of lead. Electrophoresis was performed exclusively in vertical 3 mm slabs in PANTA-POHR apparatus (Labor Muller, D-3510 Hann Munden, Germany).

The extract of leaf proteins was mixed with an equal volume of SDS sample buffer [10 % glycerol (w/v), 5 % 5 *β*-mercaptoethanol (v/v), 2.3 % SDS, 62.5 mM Tris-HCl (pH 6.8) and 0.01 Bromophenol Blue] and boiled for 5 min prior to putting it in 15 % polyacrylamide slab gel for SDS-PAGE. The gel was run at a constant current of 20 mA for 4 h (Laemmli, 1970). The gel was stained with 0.25 % Coomassie Brilliant Blue R-250 (CBB R-250) in a mixture of ethanol, acetic acid and distilled water (9: 2: 9, v/v) for 1 h and distained in several changes of a solution containing ethanol, acetic acid and distilled water (25: 8: 165, v/v).

Quantitative difference between protein spectra were evaluated by the intensity of staining of bands while qualitative differences were estimated by the number and molecular weight values of protein bands. Electrophoretic patterns were scanned with a Gel-Pro. Analyzer V 3. 1. using media cybernetics 1993-1997 V 3.1 for Windows 95 NT (USA). After that, the determination of the molecular weight of the proteins was made.

Extraction of Antioxidant enzymes

Leaves were lyophilized and powdered in liquid nitrogen. The powder was extracted for in buffer (100 mM potassium phosphate). The extract was centrifuged a Sephadex G-25 column was used and

then, the supernatant was used for the enzyme assays as describrd by Schwanz *et al*. (1996).

Enzymes Assay

Superoxide dismutase SOD (EC1.15.1.1) activity was assayed using the photochemical nitroblue tetrazolium (NBT) method according to Stewart and Bewely (1980). Catalase (CAT) activity was determined according to Aebi (1983). Peroxidase (POD) activity was estimated according to Adam *et al*. (1995).

Determination of MDA Content

The level of lipid peroxidation was expressed as MDA content and was assayed by the method of Hodgson and Raison (1991).

Determination of H_2O_2 Content

Hydrogen peroxide was measured in the leaves colorimetrically as described by Jana and Choudhuri (1982) and Okuda *et al.* (1991).

Statistical Analysis

All data presented are the means of at least five replicates. Significance of differences of samples was calculated by Student's t-test. Results of testing were considered significant if the calculated P-values were ≤ 0.05.

RESULTS

Effects of lead on Photosynthetic Pigments

A concentration-dependent decline of Chl *a* occurred as a consequence of exposure to Pb^{2+} concentrations of 0- 600 mg L^{-1}, where the greatest decrease 75% of control was reached at the highest Pb^{2+}concentration (Table 1). On the other hand, the content of Chl *b* decreased at a relatively lower rate where its value was 69% of the control at 600 ppm Pb^{2+} (Table 1).

Figure (1B) showed that the total chlorophyll content steadily declined with the increase of Pb^{2+} in the growth medium. Moreover, the ratio of Chl *a/b* under Pb^{2+} exposure declined regularly with the increasing Pb^{2+} concentration (Fig. 1C). The changing trends of carotenoids content in *Lepidium sativum* leaves exposed to a range concentration of Pb^{2+} were similar to those of Chl *a* (Table 1), where its content reduced steadily with the increased Pb^{2+} concentration.

Effects of Different Concentrations of Lead on Protein Patterns

Changes in protein content of *Lepidium sativum* treated with different concentrations of Pb^{2+} were shown in Figure (1A). The loss in total protein content under lead treatment was notable as compared with control. During the exposure time, the loss under Pb^{2+} treatment was ranged from 12.90 to 6.10 mg g^{-1} DW according to

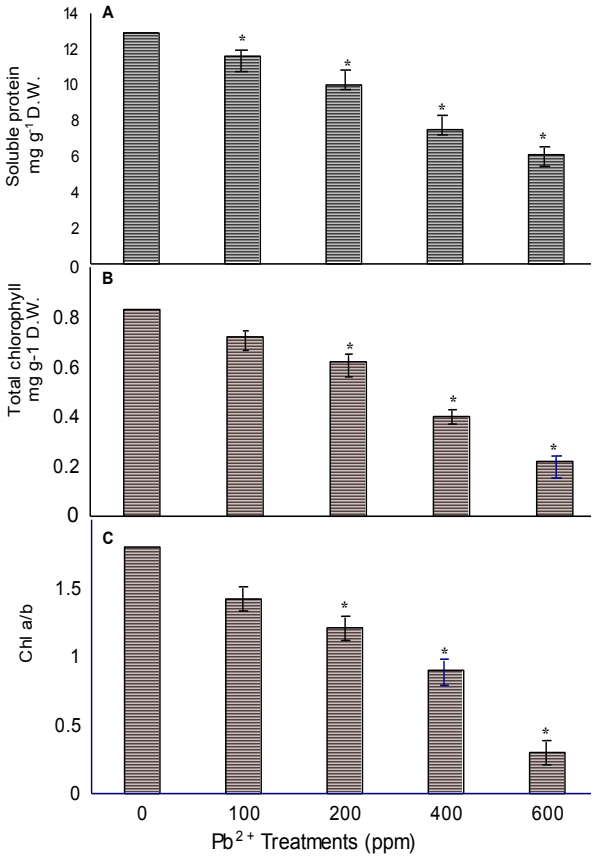

Figure (1): Soluble Protein (A), Total Chlorophyll (B) and Chlorophyll *a/b* ratio (C) of *Lepidium sativum* leaves subjected to various concentration of Pb^{2+}. Each value represents the mean ±SE of five replicates. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

the concentrations used (100-600 mg g^{-1}). This indicates that the rate of protein hydrolysis was very much impaired under heavy metal treatment particularly at higher concentrations. Exposure to 100 ppm Pb^{2+} for 10 days resulted in a slight decrease in protein content (about 10%) whereas, 600 ppm Pb^{2+} resulted in a significant reduction in protein content, reaching about 53% decrease relative to control.

It is notable that 100 and 200 ppm Pb^{2+}concentration participate in the induction of protein (61.4 KDa) which has not been presented previously in control plants as presented in plate (1). On the other hand, treatment with higher concentrations of Pb^{2+} resulted in the disappearance of these polypeptides, which means that Pb^{2+} induces proteins with approximately the same molecular weight but with different amounts peculiar to metal concentrations. Some proteins disappeared completely due to the treatment; for example, 400 and 600 ppm Pb^{2+} causes the disappearance of proteins (82.4 KDa and 43.6 KDa).

Table (1): Changes in Chl *a, b* and total carotenoids contents in leaves of *Lepidium sativum* leaves treated with various concentrations of Pb^{2+} for 10 days. Values in parentheses were expressed as the percent of reduction relative to the control (100%). Each value is the mean of three replicates (± SD). Values carrying asterisk are significantly different at $P \leq 0.05$.

Pigment mg g^{-1} D.W.	Pb^{2+} ppm				
	Control	100	200	400	600
Chl *a*	0.57±0.049 (100)	0.48±0.02 (16)	0.42±0.021* (26)	0.24±0.013* (58)	0.14±0.010* (75)
Chl *b*	0. 26±0.014 (100)	0.24±0.017 (8)	0.20±0.025* (23)	0.16±0.019* (39)	0.08± 0.009* (69)
Carotenoids	0.195±0.022 (100)	0.18±0.020 (8)	0.12±0.016* (38)	0.095±0.014* (51)	0.03±0.001* (85)

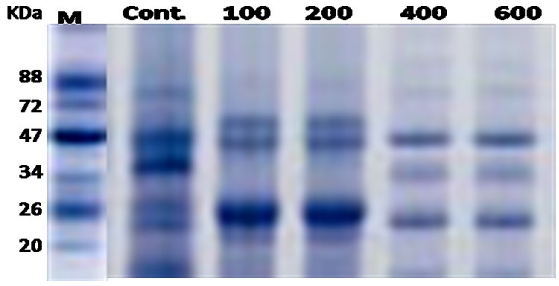

Plate (1): SDS-PAGE (10%) shows the variation of protein pattern in leaves of *Lepidium sativum* treated with various concentrations of Pb^{2+} up to 600 mg L^{-1}) Gels were scanned for their banding patterns. MW= molecular weight in kilo Dalton (kDa), M = protein marker.

Effects of Lead on Antioxidant Enzymes, H_2O_2 and MDA

The lowest Pb^{2+}concentration (100 ppm) produced a significant stimulation (25%) in the POD activity (Fig. 2A). This activity increased up to a maximum value of 1479 U mg protein^{-1} min^{-1} as the concentration of Pb^{2+} increased to 400 ppm. However, the POD activity at the highest Pb^{2+} concentration was less than other Pb^{2+} levels but still (5%) higher than control.

The presence of Lead in growth medium stimulated the SOD activity, and reached a maximum value of 198% over the corresponding control at the 200 ppm Pb^{2+} concentration (Fig. 2B). On the other hand, the high concentrations of lead resulted in a progressive decrease in SOD activity especially at 600 ppm.

As the concentration of Pb^{2+} increased to 400 ppm, the CAT activity reached a maximum value of 105 U mg protein^{-1} min^{-1}, however the CAT activity decreased with the increment of Pb^{2+} (Fig. 2C). The concentration of H_2O_2 in the control leaves declined progressively with the growth of plants. However, the level of H_2O_2 in leaves of Pb-treated plants was gradually increased from 22 % (at 100 mg g^{-1}) to 206 % (at 400 ppm) and 352% (at 600 ppm) over a period of 10 days.

The malondialdehyde content increased gradually with the increased concentration of lead. Significant stimulation (276%) of MDA appeared after 10 days at 600 ppm Pb^{2+} treatment (Fig. 3).

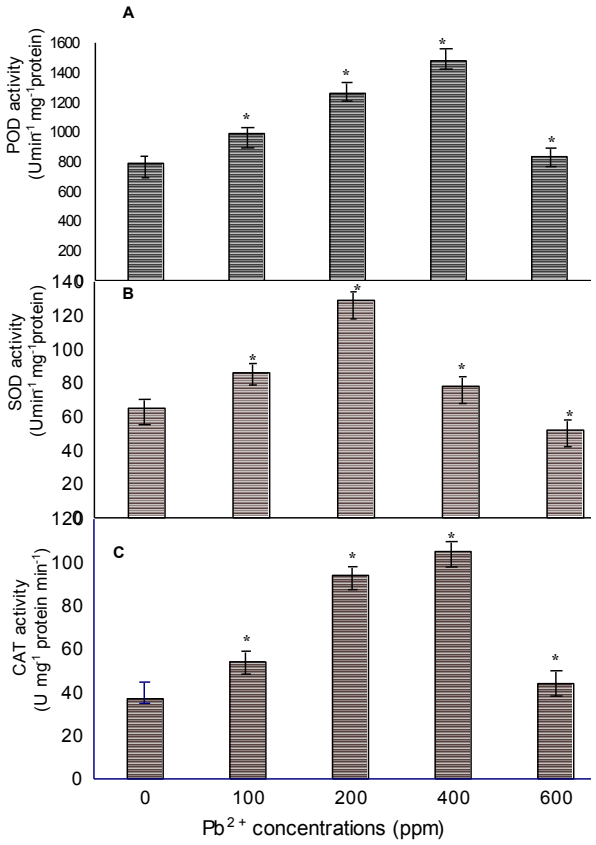

Figure: (2): Antioxidant enzymes activities POD (A), SOD (B) and CAT(C) of *Lepidium sativum* leaves subjected to various concentration of Pb^{2+}. Each value represents the mean ±SE of five replicates. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

DISCUSSION

Effects of Lead on Soluble Protein and Photosynthetic Pigments

The present results clearly indicate that lead ion brought about the toxicity to *Lepidium sativum* plants due to its strong inhibitory effects on the contents of soluble protein and photosynthetic pigments. It was

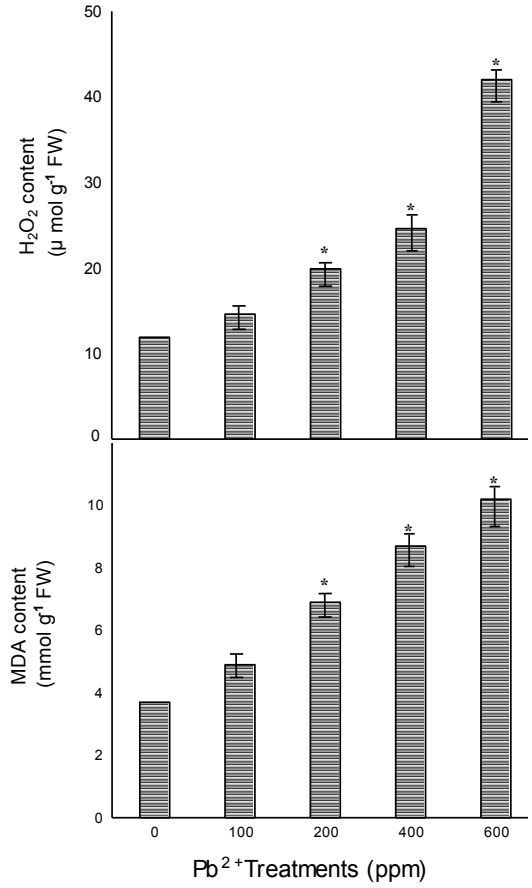

Figure (3): H_2O_2 and MDA contents in *Lepidium sativum* leaves subjected to different concentration of Pb^{2+}. Each value represents the mean ±SE of five replicates. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

reported that Pb^{2+} and Cd^{2+} resulted in a significant inhibition of protein in *Brassica juncea* L. and root tips of barley seedlings (Singh and Tewari, 2003; Liu *et al*., 2005; Ibrahim, 2003). Similarly, changes in the soluble protein content of *Lepidium sativum* leaves exhibited irreversible relationships with increased Pb^{2+} concentrations, where high concentration of Pb^{2+} significantly reduced the soluble protein content more than low Pb^{2+}concentration. The inability of *Lepidium sativum* leaves to synthesize protein after lead treatment may be due to acute oxidative stress induced by excess Pb^{2+} in plant cells. The presence of various protein bands, which differ in migration position and bands' intensities, proves the significant effect of Pb^{2+} on the protein content of leaves through the induction of new stress protein bands. This difference could be explained by the reduction in protein set synthesized during the experimental period due to the effect of Pb^{2+} or by partial protein degradation and this may be the initial cause of growth reduction under metal treatment. However, additional proteins were also induced by Pb^{2+} treatment. The results are consistent with other previous studies by Ibrahim (2003).

The low Pb^{2+} concentration (100 ppm) caused a slight inhibition of photosynthetic pigments, but the 400 and 600 ppm Pb^{2+} were sufficient to induce a decrease in the pigments, indicating that excess lead could be a strong inhibitor to photosynthesis (Parys *et al*., 1998). The loss of chlorophyll could be due to peroxidation of chloroplast membranes mediated (Mal *et al*., 2002).

It has been reported by Nishihara *et al*. (2001) that Pb^{2+} reduced the activity of delta- aminolevulinic acid dehydratase (ALA) in mouse blood. Thus, lead may inhibit the formation of chlorophyll by interfering with protochlorophyllide and the synthesis of aminoevulinic acid. Such an effect may influence the different steps of Calvin cycle, resulting in the inhibition of photosynthetic CO_2 fixation. Also, lead could do great harm to chloroplast envelopes and thylakoids via increased production of free radicals (Muhammad *et al*., 2008).

Moreover, the degradation rate of Chl *b* under lead stress was slower than that of Chl *a* and carotenoids, suggesting that the damage of lead on Chl *a* is greater than that on Chl *b*. It is well known that Chl *a* is one of the most important center pigments in photosynthesis and therefore the decrease of Chl *a* can greatly inhibit photosynthesis. Carotenoids play a role in protecting the chlorophyll, and serve as an antioxidant to quench or scavenge the free radicals and reduce the cell damage, and its main genetic composition (Ahmed and Tajmir-Riahi, 1993).

Effects of Lead on Antioxidant Enzymes, H_2O_2 and MDA

The activity of POD and CAT of *Lepidium sativum* treated with lead mainly displayed biphasic responses due to an increase in Pb^{2+} concentration. At low-level metal stress, leaves could activate POD and CAT activities, which led to strengthening the plants, to scavenge ROS responsible for lipid peroxidation (Kosobrukhov *et al*., 2004). However, the activities of both enzymes decreased distinctly under acute stress which overloaded cellular defense system of the plants (Guecheva *et al*., 2003). The decline in the activities of POD and CAT might be due to the formation of protein complex with metals that change the structural integrity of proteins (Kollmeier *et al*., 2000; Ibrahim, 2006). For plants treated with an increased Pb^{2+} concentration, POD and CAT activities appeared to be inhibited when exposed to 600 ppm (Fig 2A and C). The results suggested that lead was toxic to *Lepidium sativum* because at relatively high concentrations, the antioxidant system appeared to be in disorder.

In the experiment, SOD activity under elevated lead stress was steadily stimulated with the increasing metal ion value in medium up to 600 ppm. The results showed that, under high metal stress, POD and CAT activities were inhibited, while SOD activity was stimulated, indicating that those enzymes were located at different

cellular sites, which had different resistances to heavy metals. Therefore, the deterioration of cellular system functions by high metal stress might result in the inactivation of enzyme activity (Mittler, 2002; Dewez *et al*., 2005).

The current study with *Lepidium sativum* plant indicated that Pb^{2+} treatment increased H_2O_2 level in the leaf tissue (Fig. 3A) and was concentration dependent. Accumulation of H_2O_2 is a general stress response, which has been observed in plants exposed to low temperature, heat, pathogens, and chilling (Levine *et al*., 1994; Mehdy, 1994; Bafeel and Ibrahim, 2008) as well as after exposure of plants to excess levels of Cu^{2+} and Cd^{2+} (Schützendübel and Polle, 2002). Furthermore, H_2O_2 can also be produced by a number of non-enzymatic and enzymatic processes in cells such as SOD as well as CAT which are responsible, respectively, for H_2O_2 production and its scavenging (Jiménez *et al*., 1997). However, it should be noted that although H_2O_2 takes part in several important functions in plant cells (Foyer and Noctor, 2005), control of its build-up is essential to prevent oxidative damage to membranes and proteins.

MDA is the decomposition product of polyunsaturated fatty acids of biomembranes that increased under high-level of oxidative stress. Cell membrane stability has been widely used to differentiate between stress tolerant and susceptible cultivars of many crops and in some cases higher membrane stability could be correlated with better performance (Premachandra *et al*., 1991). The present results showed that increasing lead concentration increased the MDA content of *Lepidium sativum* leaves due to the enhancement of lipid peroxidation (Fig. 3). Finally, the present study showed that *Lepidium sativum* could tolerate low level of lead stress less than 400 ppm, the low-level toxic Pb^{2+} soil was quite efficient for *Lepidium sativum* growth.

ACKNOWLEDGMENTS

This research was supported through the Deanship of Scientific Research at King Abdul-Aziz University, project no. 428/524. And I would like to thank Dr. Mohamed Ibrahim for his contribution.

REFERENCES

ADAM, A.L.; C.S. BESTWICK, AND R. MANSFIELD. 1995: Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* cv. Phaseolicola. Planta **197:** 240-249.

AEBI, H. 1983. Catalase. In: "Methods of Enzymatic Analysis". Bergmeyer (Ed). Vol 3. Verlag Chemie, Weinheim, Germany pp. 273-277.

AHMED, A., AND H.A. TAJMIR-RIAHI. 1993. Interaction of toxic metal ions $Cd^{2+,}$ Hg^{2+} and Pb^{2+} with light-harvesting proteins of chloroplast thylakoid membranes. An FTIR spectroscopic study. J. Inorg. Biochem. **50:** 235-243.

BAFEEL, O.S., AND M.M. IBRAHIM. 2008. Activity of antioxidant enzymes and accumulation of αtocopherol in *Medicago sativa* seedlings subjected to dark and chilling conditions. Int. J. Agric. Biol. **10(6):** 593-598.

BRADFORD, M. 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248-254.

DEWEZ D.; L. GEOFFROY, AND G. VERNET. 2005. Determination of photosynthetic and enzymatic biomakers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliquus*. Aquatic Toxicol. **74:** 150-159.

FOYER, C. H., AND G. NOCTOR. 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell. **17:** 1866–1875.

FRANKART, C.; P. EULLAFFROY. AND G. VERNET. 2002. Photosynthetic responses of *Lemna minor* exposed to xenobiotics, heavy metals and their combinations. Ecotoxicol. Environ. Saf. **53:** 439-445.

GARDEA-TORRESDEY, J.L.; J.R. PERALTA-VIDEA, AND M. MONTES. 2004. Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis*. impact on plant growth and uptake of nutritional elements. Bioresource Technol. **92:** 229-235.

GUECHEVA, T.N.; B. ERDTMANN, AND M.S. BENFATO. 2003. Stress protein response and catalase activity in freshwater planarian Dugesia (Girardia) schubarti exposed to copper. Ecotoxicol. Environ. Saf. **56:** 351-357.

GUO, T.R.; ZHANG, G.P. AND ZHANG Y.H. 2007. Physiological changes in barley plants under combined toxicity of aluminum, copper and lead. Colloids and Surfaces B: Biointerfac. **57:** 182–188.

HALLIWELL, B., AND J.M.C. GUTTERIDGE. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1-14.

HAN, Y.S. 1999. Advances of the function of Beta-carotene and carotenoids. J. China Agric. Univ. **4:** 5-9.

HOAGLAND, D.R. AND D.R. ARNON. 1950. The Water-culture Method for Growing Plants without Soil. California Agriculture Experiment Station, Circular. **374:** 1-32.

HODGSON, R.A. AND L. RAISON. 1991. Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light. Planta. **85:** 215-219.

HOU, W., X. CHEN, G. SONG, Q. WANG AND C. CHI CHANG. 2007. Effects of copper and cadmium on heavy metal polluted water body restoration by duckweed (Lemna minor). Plant Physiol. Biochem. **45:** 62-69.

IBRAHIM, M.M. 2003. Response of antioxidant defense

systems and photosynthetic capacity in *Glycine max* L. leaves to the treatment with copper element. Ph.D. Thesis, Alexandria University. pp 150

IBRAHIM, M.M. 2006: Oxidative metabolism dysfunctions caused by cadmium in *Zea mays* L. seedlings. EGYPT. J. BOT. **46:** 29-45.

JANA S. AND CHOUDHURI M.A. 1982. Senescence in submerged aquatic angiosperms: Effects of heavy metals. New Phytol. **90:** 477-484.

JIMÉNEZ, A., J.A. HERNÁNDEZ, L.A. DEL RÍO, AND F. SEVILLA. 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol.**114:** 275–284.

KOLLMEIER, M.; H.H. FELLE, AND W.J. HORST. 2000. Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? Plant Physiol. **122:** 945–56.

KOSOBRUKHOV, A.; I. KNYAZEVA; AND V. MUDRIK. 2004. *Plantago major* plants responses to increase content of lead in soil: growth and photosynthesis. Plant Grow. Regul. **42:** 145–51.

LAEMMLI, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(259): 680-685.

LEVINE, A.; TENHAKEN, R.; DIXON, R. AND LAMB, C. 1994. H_2O_2 from oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell. **79:** 583-593.

LICHTENTHALER, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology. **148:** 350–382.

LIU, W.; P.J. LI, AND X.M. QI. 2005. DNA changes in barley (*Hordeum vulgare*) seedlings induced by heavy metal pollution using RAPD analysis. Chemosphere **61:** 158-167.

MAL, T.K.; P. ADORJAN, AND A.L. GORBETT. 2002 Effect of copper on growth of an aquatic macrophytes *Elodea Canadensis*. Environ. Pollut. **120:** 307-311.

MEHDY, M.C. 1994. Active oxygen species in plant defense against pathogens. Plant Physiol. **105:** 467-472.

MITTLER, R. 2002. Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. **17:** 405-410.

MUHAMMAD, S.A. A.1, MUMATZ, H., SAMINA, I. AND AMBREEN, K.A. 2008. Photosynthetic Performance of Two Mung Bean (*Vigna radiata*) Cultivars Under Lead and Copper Stress. Int. J. Agric. Bio. **07–277(10–2):** 167–172

NISHIHARA, E.; K. TAKAHASHI, N. TANAKA, K. TANAKA, AND K. WATANABE. 2001. Effect of 5-aminolevulinic acid (ALA) on photosynthetic rate, hydrogen peroxide content, antioxidant level and active oxygen-scavenging enzymes in spinach (*Spinacia oleracea* L.). J Jpn Soc Hort Sci **70:** 346–352.

OKUDA, T.; Y. MATSUDA, A. YAMANAKA, AND S. SAGISAKA. 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. Plant Physiol. **97:** 1265-1267.

PAIVOKE, A.E.A. 2002. Soil lead alters phtase activity and mineral nutrient balance of *Pisum sativum*. Environ. Exp. Bot. **48:** 61-73.

PARYS, E.; E. ROMANOWASKA, M. SIEDLECKA, AND J. POSKUTA. 1998. The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of *Pisium sativum*. Acta Physiol. Plant. **20:** 313-322.

POLLE, A. AND A. SCHÜTZENDÜBEL. 2003. Heavy metal signalling in plants: linking cellular and organismic responses. In: Plant Responses to Abiotic Stresses (Hirt H, Shinozaki K, Eds), Vol 4: Topics in Current Genetics. Springer Verlag, Berlin, pp 167-215.

PREMACHANDRA, G.S.; H. SONEOKA, AND M. KANAYA. 1991. Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize, J. Exp. Bot., **42:**167-171.

PUNZ, W.F., AND H. SIEGHARDT. 1993. The response of roots of herbaceous plants species to heavy metals. Environ. Exp. Bot., **33:** 85-98.

SCHÜTZENDÜBEL, A., AND A. POLLE. 2002. Plant responses to a biotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. **53:**1351-1365

SCHWANZ, P.; C. PICON, P. VIVIN, E. DREYER, J. GUEHL AND A. POLLE. 1996. Responses of antioxidative systeme to drought stress in *Pendunculate oak* and *Maritim pineas* modulated by elevated CO_2 Plant Physiol., **110:** 393-402.

SHARMA, P., AND R.S. DUBEY. 2004. Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms of stresses and protective roles of osmolytes. Plant Sci. **167:**541-550.

SINGH, P.K., AND R.K. TEWARI. 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants, J. Environ. Biol., **24:** 107-112.

STEWART, R.R.C., AND J.D. BEWLEY. 1980. Lipid peroxidation associated with accelerated ageing of soybean axes-Plant Physiol., **65:** 245-248.

VON UEXKULL, R.A. AND E. MUTERT. 1995. Global Extent, Development and Economic Impact of Acid Soils. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 5–19.

YAMAMOTO, Y.; Y. KOBAYASHI, S.R. DEVI, S. RIKIISHI, AND H. MATSUMOTO. 2003. Oxidative stress triggered by aluminum in plant roots. Plant Soil. **255:** 239–243.

Received October 10, 2009
Accepted November 23, 2009