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

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**ABSTRACT**

The present study was undertaken to assess the toxicity of Pb²⁺ 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 (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 Pb²⁺, was due to the increase in H₂O₂ 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 (Pb²⁺ > 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 Pb²⁺, 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 Pb²⁺ 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 Pb²⁺ toxicity damage (Ahmed and Tajmir-Riahi, 1993).

Disturbance of the metabolism by excessive Pb²⁺ 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 (Schüttendübel and Polle 2002; Polle and Schüttendübel, 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⁻), hydroxyl radical (’OH) and hydrogen peroxide (H₂O₂) (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 H₂O₂, while POD utilizes H₂O₂ 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 Pb²⁺ 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.

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In the present study, concentrations of Pb\textsuperscript{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\textsubscript{2}O\textsubscript{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\textsuperscript{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\textsubscript{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°C. Light intensity was 500 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) 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\textsuperscript{2+} for 10 days. Individual Pb\textsuperscript{2+} treatments were a control, with Hoagland nutrient solution (0 ppm Pb\textsuperscript{2+}), and four Pb\textsuperscript{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\textsuperscript{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 Lichtenhaler (1987).

**Protein Analyses**

Proteins of control and Pb\textsuperscript{2+} treated leaves plants were extracted with an equal volume of 20 mM Tris-HCl buffer (pH 8.6) containing 5 % \(\beta\)-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 \(\beta\)-mercaptoethanol (v/v), 2.3 % SDS, 62.5 mM Tris-HCl (pH 6.8) and 0.01 Bromphenol 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 destained 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...
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 H₂O₂ 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 Pb²⁺ concentrations of 0-600 mg L⁻¹, where the greatest decrease 75% of control was reached at the highest Pb²⁺ 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 Pb²⁺ (Table 1).

Figure (1B) showed that the total chlorophyll content steadily declined with the increase of Pb²⁺ in the growth medium. Moreover, the ratio of Chl a/b under Pb²⁺ exposure declined regularly with the increasing Pb²⁺ concentration (Fig. 1C). The changing trends of carotenoids content in Lepidium sativum leaves exposed to a range concentration of Pb²⁺ were similar to those of Chl a (Table 1), where its content reduced steadily with the increased Pb²⁺ concentration.

Effects of Different Concentrations of Lead on Protein Patterns
Changes in protein content of Lepidium sativum treated with different concentrations of Pb²⁺ 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 Pb²⁺ treatment was ranged from 12.90 to 6.10 mg g⁻¹ DW according to 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 Pb²⁺ for 10 days resulted in a slight decrease in protein content (about 10%) whereas, 600 ppm Pb²⁺ resulted in a significant reduction in protein content, reaching about 53% decrease relative to control.

It is notable that 100 and 200 ppm Pb²⁺ 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 Pb²⁺ resulted in the disappearance of these polypeptides, which means that Pb²⁺ 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 Pb²⁺ causes the disappearance of proteins (82.4 KDa and 43.6 KDa).
Physiological and biochemical aspects of tolerance in *Lepidium sativum* to lead toxicity

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 ≤ 0.05.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>mg g$^{-1}$ D.W.</th>
<th>Pb$^{2+}$ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Chl <em>a</em></td>
<td>0.57±0.049</td>
<td>0.48±0.02</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(16)</td>
</tr>
<tr>
<td>Chl <em>b</em></td>
<td>0.26±0.014</td>
<td>0.24±0.017</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(8)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.195±0.022</td>
<td>0.18±0.020</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

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$_2$O$_2$ 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$_2$O$_2$ in the control leaves declined progressively with the growth of plants. However, the level of H$_2$O$_2$ 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
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 \textit{et al.}, 1998). The loss of chlorophyll could be due to peroxidation of chloroplast membranes mediated (Mal \textit{et al.}, 2002).

It has been reported by Nishihara \textit{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 aminolevulinic 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 \textit{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\(_2\)O\(_2\) and MDA**

The activity of POD and CAT of \textit{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 \textit{et al.}, 2004). However, the activities of both enzymes decreased distinctly under acute stress which overloaded cellular defense system of the plants (Guecheva \textit{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 \textit{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 \textit{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\(_2\)O\(_2\) level in the leaf tissue (Fig. 3A) and was concentration dependent. Accumulation of H\(_2\)O\(_2\) 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\(_2\)O\(_2\) 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\(_2\)O\(_2\) production and its scavenging (Jiménez et al., 1997). However, it should be noted that although H\(_2\)O\(_2\) 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.

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**REFERENCES**


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


