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# Oxidative Stress Induction by Lead in Leaves of Radish (*Raphanus sativus*) Seedlings

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**Abstract**

Oxidative stress was induced by lead acetate (Pb) in *Raphanus sativus* seedlings grown in a hydroponic system using sand as substrate. Thirty day old acclimated seeds were treated for 7 days with five Pb levels (0 as control, 100, 200, 500 and 1000 mg $l^{-1}$). Parameters such as growth, oxidative damage markers (lipid peroxidation, protein oxidation and hydrogen peroxide contents) and enzymatic activities of catalase (CAT) and peroxidase (POD) were investigated. Lead concentration in plant tissues increased with increasing of Pb levels. Shoot fresh weight, chlorophyll and carotenoid concentration were significantly decreased at 100 mg $l^{-1}$ Pb. Lipid peroxidation, protein oxidation and $H_2O_2$ levels were increased at 500 and 1000 mg $l^{-1}$ Pb compared to control treatment, in shoots. Peroxidase activity showed a straight correlation with $H_2O_2$ concentration, whereas CAT activity decreased only in shoots. These changes in enzymatic and non-enzymatic antioxidants showed that the Pb exposition had a significant disturbance on *Raphanus sativus* plantlets and affect the biochemical and physiological processes.

***Keywords:*** hydrogen peroxide, lead, lipid peroxidation, oxidative stress, protein oxidation, *Raphanus sativus*

## Introduction

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems on world scale. According to the Environmental Protection Agency (EPA), Pb is the most common heavy metal contaminant in the environment (Watanabe, 1997). It is a nonessential element in metabolic processes and may be toxic or lethal to organisms even when absorbed in small amounts (Walker *et al.*, 1996).

Lead contamination in the plant environment is known to cause highly toxic effects on processes such as depression on seed germination (Wierzbicka and Obidzinska, 1998), the disturbance in mitosis (Liu *et al.,* 1994; Wierzbicka, 1994), induction of leaf chlorosis (Johnson and Proctor, 1977), toxicity of nucleoli (Liu *et al.,* 1994), inhibition of root and shoot growth (Fargasova, 1994; Liu *et al.,* 1994), reduction in photosynthesis (Poskuta *et al.,* 1988; Poskuta and Waclawczyk-Lach, 1995) transpiration (Rolfe and Bazzaz, 1975), DNA synthesis (Gabara *et al.,* 1992 ) and inhibition and activation of enzymatic activities (Van Assche and Cliisters, 1990). Lead not only affects plant growth and productivity but also enters into the food chain causing health hazards to man and animals (Seaward and Richardson, 1990).

lead toxicity is also known to induce oxidative stress through over production of reactive oxygen species (ROS) including superoxide radicals ($O_2^-$), hydroxyl radicals (OH) and hydrogen peroxide ($H_2O_2$) (Reddy *et al.,* 2005; Ruley *et al.,* 2004; Verma and Dubey, 2003). These free radicals and hydrogen peroxides cause a variety of harmful effects in plant cells, such as inhibition of adenosine triphosphate (ATP) production, lipid peroxidation, and DNA damage (MacFarlanc, 2003). All these eventually lead to cell death. To combat oxidative damage, plants have antioxidant defense system comprising of enzymes of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and the non-enzymic constituents such as reduced glutathione (GSH) and ascorbate (As) that remove, neutralize, and scavenge ROS (Foyer *et al.*, 1997; Lee *et al.*, 1976; Navari-Lazzo and Quartacci, 2001). SOD dismutates $O_2^-$ to $H_2O_2$, and this is decomposed to $H_2O$ by POD and CAT, so that the accumulation of $O_2^-$ and $H_2O_2$ is effectively prevented (Liu *et al.*, 2002).

Radish in considered to be a model cropand is widely used for studies related to heavy metal pollution (Khan and Frankland, 1983; Kostka-Rick and Manning, 1993). The advantage of using radish and other members of cabbage (*Brassicaceae*) family for heavy metal studies are well described by Mathe-Gaspar and Anron (2002) and it has economic and nutritional value, also is a rich source of two important medicinal compounds: peroxidases and isothiocyanates (Curtis, 2003).

Lead was recognized as causing oxidative stress in plants, so radish may have a strong resistance to Pb, but

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little is known about the effects of Pb on the physiological processes of radish seedlings. The objective of the present investigation is to study the effects of different concentrations of Pb on leaf of radish including growth, physiological and biochemical processes such as the different pigments, soluble proteins, lipid peroxidation, protein oxidation and hydrogen peroxide contents; and the activities of some antioxidant enzymes (CAT and POD). The possible mechanisms of radish seedlings tolerance of Pb stress are briefly discussed in the present study.

**Materials and methods**

*Plant material*

Radish (*Raphanus sativus*, 'Early Menu') seeds were used for Petri-dish experiment. Seeds were surface sterilized with 0.1% $HgCl_2$ for the prevention of fungal and bacterial contamination (Young, 1926); and were grown hydroponically in aerated diluted (1: 4) Hoagland's nutrient solution containing various concentrations of Pb (0, 100, 200, 500 and 1000 mg $l^{-1}$) were supplied exogenously as lead acetate [$Pb(CH_3COOH)_2$] for 24°C, 16/8 h light/dark photoperiod and light intensity of 175 µM $m^{-2}$ $s^{-1}$. After 7 days of Pb exposure, the seedling fresh weight was determined and the shoots sample were kept at -80°C for further analyses.

*Chlorophyll and carotenoid determination*

Fresh biomass (leaves) was homogenized in 80% ice-cold acetone in the dark and then centrifuged at 10000g for 10 min at 4°C and the supernatant was used for the immediate determination of pigments. Absorbance of the solution was determined spectrophotometrically at 663, 645 and 480 nM the contents of chlorophyll a, b, and carotenoid, respectively; with the following equations help of Arnon's formulae (Arnon, 1949), for quantification of the total chlorophyll, chlorophyll a and chlorophyll b content in an 80% acetone extract:

Total chlorophyll = 20.2 ($A_{645}$) + 8.02 ($A_{663}$)
Chlorophyll a = 12.7 ($A_{663}$) - 2.69 ($A_{645}$)
Chlorophyll b = 22.9 ($A_{645}$) - 4.68 ($A_{663}$)
And Carotenoids = (1000$A_{480}$ - 3.27[chl a] - 104[chl b])/227

Chlorophyll and carotenoid concentrations were expressed as mg $g^{-1}$fresh weight.

*Estimation of lipid peroxidation*

The level of peroxidation was measured in terms of malondialdehyde (MDA) (a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Frozen shoot was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 15 min and 4.0 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then quickly cooled on ice bath. The contents were centrifuged at 10000 g for 15 min and the absorbance of the supernatant was measured at 532 nM and the value for the non-specific absorption at 600 nM was subtracted. The concentration of MDA was calculated using coefficient of absorbance of 155 $mM^{-1}$ $cm^{-1}$. MDA content expressed as nM $g^{-1}$ fresh weight.

*Estimation of protein oxidation*

The reaction of carbonyls with 2, 4-dinitrophenylhydrazine (DNPH) was used to determine the amount of protein oxidation, as described by Levine *et al.* (1990). The shoots of radish seedlings was homogenized in a 25 mM K-phosphate buffer (pH 7.0), containing 10 ml $l^{-1}$ Triton X-100 at a proportion of 1:5 (w: v). The homogenate was centrifuged at 9000 g for 30 min at 4°C. After the DNPH-reaction, the carbonyl concentration was calculated by absorbance at 370 nm, using the molar extinction coefficient 21 $M^{-1}cm^{-1}$ and expressed as nM carbonyl $mg^{-1}$protein.

*Determination of hydrogen peroxide*

The $H_2O_2$ concentration was determined according to Loreto and Velikova (2001). Approximately 0.1g of shoots was homogenized at 4°C in 2 ml of 0.1% trichloroacetic acid (TCA) (w: v). The homogenate was centrifuged at 12000 g for 15 min at 4°C. Then, 0.5 ml of the supernatant was added to 0.5 ml of 10 mM K-phosphate buffer (pH 7.0) and 1 ml of 1M KI. The $H_2O_2$ concentration of the supernatant was evaluated by comparing its absorbance at 390 nM with a standard calibration curve. Hydrogen peroxide concentration was expressed as µM $g^{-1}$ fresh weight.

*Catalase and peroxidase assay*

The frozen shoots material was homogenized in 50 mM Tris-HCl buffer (pH 7.0). The supernatant solution was used to measure the activity of the enzymes, and the protein content was determined according to Bradford (1976).

**Catalase (EC.1.11.1.6)** activity was assayed by the method of Barber (1980). The reaction mixture consisted of enzyme extract, 5 mM $H_2O_2$ and 50 mM Tris-buffer (pH 7.0). After 1 min incubation at 25°C, the reaction was stopped by adding 1.0 ml of 2.5 N $H_2SO_4$. The residual $H_2O_2$ was titrated with 0.01N $KMnO_4$ and measured spectrophotometrically at 240 nm. Catalase activity was expressed as ml $H_2O_2$ oxidized $g^{-1}$ fresh weight $min^{-1}$.

**Peroxidase (EC.1.11.1.7)** activity was assayed by the method of Kar and Mishra (1976). The reaction mixture contained 100 mM Tris-buffer (pH 7.0), 10mM pyrogallol and 5 mM $H_2O_2$. The reaction was started by adding 25µl enzyme solution and stopped after 5 min incubated at 25°C by adding 1.0 ml 2.5 N $H_2SO_4$. The amount of purpyrogallin formed was measured spectrophotometrically at 425 nm. The enzyme activity was expressed as change in absorbance units $g^{-1}$ fresh weight $min^{-1}$.

*Data analysis*

All data were analyzed in three replications and the obtained data were evaluated statistically using Student's t-test, and least significant difference (LSD) was calculated at $p < 0.05$.

## Results

*Growth and fresh weight*

The results after 7th days of aqueous exposure of different lead levels in Radish seedlings showed considerable reduction in growth in respective doses of lead. The fresh weight of seedlings decreased on increasing the concentration of lead, the fresh weight was observed 92, 67, 36 and 20 mg/seedling in respective concentration of lead (100, 200, 500 and 1000 mg $l^{-1}$) in comparison to 125 mg per seedling of control.

*Chlorophyll and carotenoid*

Increased lead exposure was noted in total chlorophyll as for example, they were 0.368, 0.299, 0.121 and 0.031 mg $g^{-1}$ fresh weight of tissue in different concentration of lead (100, 200, 500 and 1000 mg $l^{-1}$ Pb) in comparison to 0.406 mg $g^{-1}$ fresh weight of control. Total carotenoids were 0.155, 0.114, 0.069 and 0.032 mg $g^{-1}$ fresh weight of tissue in 100, 200, 500 and 1000 mg $l^{-1}$ of lead in comparison with 0.198 mg $g^{-1}$ fresh weight of control (Tab. 1). Effect of Pb on Chlorophyll content Chl a, Chl b, total chlorophyll, and total carotene contents in radish seedlings were significantly ($p < 0.05$) lower than control in all treatments (Tab. 1); although decrease in chlorophyll 'b' was more marked than chlorophyll 'a'.

Tab. 1. Effect of lead on different pigments in radish (*Raphanus sativus*) seedlings

| | Chlorophyll (mg $g^{-1}$ Fresh tissue weight) | | | Total carotenoid (mg/g) |
|---|---|---|---|---|
| Treatments | a | b | Total | |
| Control | 0.260 ±0,007 | 0.119 ±0.009 | 0.406 ± 0.005 | 0.198 ± 0.004 |
| Pb 100 mg $l^{-1}$ | 0.256 ± 0.003 | 0.110 ± 0.005* | 0.368 ± 0.003 | 0.155 ± 0.003* |
| Pb 200 mg $l^{-1}$ | 0.205 ± 0.006* | 0.083 ± 0.007* | 0.299 ± 0.008* | 0.114 ± 0.008* |
| Pb 500 mg $l^{-1}$ | 0.077 ± 0.008* | 0.041 ± 0.004* | 0.121 ± 0.004* | 0.069 ± 0.006* |
| Pb 1000 mg $l^{-1}$ | 0.016 ± 0.002* | 0.015 ± 0.008* | 0.031 ± 0.002* | 0.032 ± 0.007* |

The averages of three replicates ± SE and (*) statistically significant at $p < 0.05$ level

Tab. 2. Effect of lead on lipid peroxidation (nM $g^{-1}$ fresh weight), protein oxidation (nM carbonyl $mg^{-1}$protein) and hydrogen peroxide (µM $g^{-1}$ fresh weight) in radish (*Raphanus sativus*) seedlings

| Treatments | Lipid peroxidation | Protein oxidation | Hydrogen peroxide |
|---|---|---|---|
| Control | 1.42 ± 0.39 | 2.22 ± 0.47 | 3.38 ± 0.54 |
| Pb 100 mg $l^{-1}$ | 3.80 ± 0.85 | 2.58 ± 0.63* | 5.88 ± 0.78* |
| Pb 200 mg $l^{-1}$ | 4.65 ± 0.77* | 3.42 ± 0.77* | 7.19 ± 1.88* |
| Pb 500 mg $l^{-1}$ | 5.85 ± 1.15* | 4.62 ± 0.82* | 9.31 ± 1.69* |
| Pb 1000 mg $l^{-1}$ | 7.44 ± 1.02* | 5.10 ± 1.19* | 12.53 ± 1.74* |

The averages of three replicates ± SE and (*) statistically significant at $p < 0.05$ level

*Lipid peroxidation, protein oxidation and hydrogen peroxide*

The lipid peroxidation were increased from 3.80, 4.65, 5.85 and 7.44 nM MDA $mg^{-1}$ protein, the protein oxidation were increased from 2.58, 3.42, 4.62 and 5.10 nM carbonyl $mg^{-1}$ protein and the hydrogen peroxide were increased from 5.88, 7.19, 9.31 and 12.53 µM $g^{-1}$ fresh weight of tissue, as compared to control where their values were 1.42, 2.22 and 3.38, respectively (Tab. 2).

*Soluble proteins, CAT and POD*

The soluble proteins were decreased from 46.5, 38, 27.5 and 16.44 µg/g in 100, 200, 500 and 1000 mg $l^{-1}$ of lead respectively as compared to control when it was 51.27 µg/g fresh weight of tissue. The catalase (CAT) was decreased from 28, 24, 22 and 20 ml $H_2O_2$ hydrolysed $g^{-1}$ fresh weight of tissue and peroxidase (POD) activity was increased from 6.88, 11.19, 14.31 and 15.32 Δ.O.D. $g^{-1}$ fresh weight of tissue was noticed in all lead treatments as compared to control where their values were 37 and 2.38 respectively (Tab. 3).

Tab. 3. Effect of lead on soluble proteins (µg/g fresh tissue weight), catalase (ml $H_2O_2$ hydrolysed $g^{-1}$ fresh tissue weight) and peroxidase (Δ.O.D. $g^{-1}$ fresh tissue weight) activities in radish (Raphanus sativus) seedlings

| Treatments | Soluble proteins | Catalase | Peroxidase |
|---|---|---|---|
| Control | 51.27 ± 2.59 | 37.0 ± 0.57 | 2.38 ± 0.34 |
| Pb 100 mg $l^{-1}$ | 46.50 ± 1.55* | 28.0 ± 0.53* | 6.88 ± 0.38* |
| Pb 200 mg $l^{-1}$ | 38.00 ± 0.57* | 24.0 ± 0.88* | 11.19 ± 0.88* |
| Pb 500 mg $l^{-1}$ | 27.50 ± 0.54* | 22.0 ± 0.84* | 14.31 ± 0.69* |
| Pb 1000 mg $l^{-1}$ | 16.44 ± 1.08* | 20.0 ± 1.20* | 15.32 ± 0.74* |

The averages of three replicates ± SE and (*) statistically significant at $p < 0.05$ level

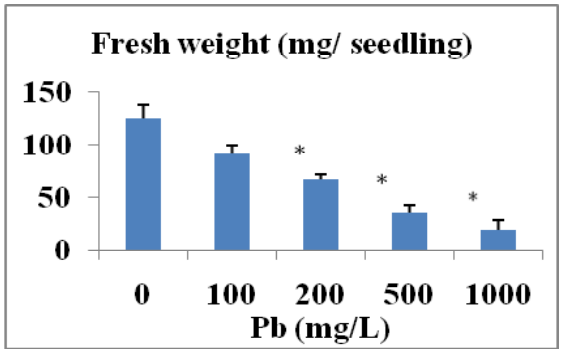


Fig. 1. Effect of lead on seedling growth in radish (Raphanus sativus).
The averages of three replicates ± SE and (*) statistically significant at $p<0.05$ level.

## Discussion

*Pb effects on growth, fresh and dry weight*

Lead is not generally considered an essential element for the growth of plants, but may stimulate growth of some plants in small amounts (Dou, 1988). The results from the present study show inhibitory effect of Pb on *Raphanus sativus* in seedling growth and shoot weight, exposed to

100, 200, 500 and 1000 mg $l^{-1}$ Pb during the whole experiment (Fig. 1). Apparently, radish is more tolerant to Pb on an equimolar basis compared to *Brassica. juncea* (Liu *et al.*, 2000) and *Allium cepa* (Liu *et al.*, 1994). Gopal and Rizvi (2008) were showed that excess Pb reduce the fresh and dry weight in leaves of radish at 30 day (0.5 mM). The decrease of growth were shown in radish stressed with Cd (Anuradha and Rao, 2007) and in cucumber stressed with Al for 10 days (Perira *et al.*, 2010). The results from Wierzbicka (1994) indicated that lead ions cause water deficit by disturbing water balance, which is one of the main factors which causes a poorer growth and development of plants. Seregin and Ivanov (1998) also observed 50% inhibition of root growth in maize at $10^{-4}$ M lead nitrate and browning of roots treated with Pb ions.

*Lead effect on chlorophyll and carotenoid*

As a visible symptom, the reduced chlorophyll and carotenoid concentrations can be used to monitor Pb induced damage in radish leaves (Tab. 1). In the present study, the reduction in chlorophyll concentration observed indicates oxidative damage induced by Pb exposure, possibly due to the inhibition of aminolevulinic acid dehydratase, an important enzyme in chlorophyll biosynthesis (Pereira *et al.*, 2006). Similar to our observations, the chlorophyll level was reduce in wheat treated with Ni (Gajewska and Sktodowska, 2007), in maize treated with Cd (Jain *et al.*, 2007), in radish treated with Pb (Gopal and Rizvi, 2008) and in cotyledons cucumber treated with Aluminum (Perira *et al.*, 2010). Although the principal recognized role of carotenoids is to act as photoreceptive antenna pigment for photosynthesis, collecting wavelengths of light that are not absorbed by chlorophylls, their protective function against oxidative damage has also been recognized for several decades (Larson, 1988). Perhaps the most important function of carotenoid is the dissipation of excess energy of excited chlorophyll and the elimination of ROS (Lawlor, 2001).

*Lead effect on MDA*

Malondialdehyde is an oxidized product of membrane lipids, and accumulates when plants are exposed to oxidative stresses. Malondialdehyde concentration is commonly considered a general indicator of lipid peroxidation as well as stress level (Chaoui *et al.*, 1997; Ding *et al.*, 2004). An increase in MDA content in radish seedlings grown under Pb stress was observed (Tab. 2), indicating a high level of lipid peroxidation. it is possible that the increase in MDA concentration in the radish seedling may be due to a increase in polyunsaturated fatty acid concentration relative to saturated fatty acids, which has also been reported in radish under stressful conditions (Kramer *et al.*, 1991). Our results show the conformity with the observations of Verma and Dubey (2003) in rice shoots and elevated the oxidative stress. Similar to our observations, lipid peroxidation is reported to be induced under Cd toxicity in radish (Anuradha and Rao, 2007), under Ni toxicity in wheat (Gajewska and Sktodowska, 2007), and under Al toxicity in cucumber (Perira *et al.*, 2010). This suggests that the toxic effect of heavy metals is probably exerted through free radical generation.

*Lead effect on carbonyl*

Despite being a non-redox metal, and thus not directly producing ROS (Benavides *et al.*, 2005), Pb can interfere with antioxidant defense systems. Under stressful conditions the protective system can be over ridden by a rapid production of large amounts of ROS, leading to various structural modifications in proteins (Cargnelutti *et al.*, 2006). These oxidative modifications are characterized by the formation of carbonyl derivatives on side chains of histidine, arginine, lysine, and proline residues (Shacter *et al.*, 1994). Halliwell and Gutteridge (1999) suggested that the oxidation of proteins to form carbonyls occurs via the hydroxyl radical, since neither $H_2O_2$ nor superoxide is reactive enough to provoke oxidation. Carbonyl content is a sensitive indicator of oxidative damage to proteins (Levine *et al.*, 1990), and levels of carbonylated proteins in plants demonstrate oxidative stress associated with heavy metals (Boscolo *et al.*, 2003).

The data from the present study indicate an increase in protein oxidation levels in radish seedlings treated with Pb (Tab. 2).

The accumulation of carbonyls in the shoot of radish studied indicates that the quantity of radicals generated exceeded the capacity of the antioxidant defensive system.

Our data demonstrated that the seedling exposure to 1000 mg $l^{-1}$ of Pb caused a remarkable increase in carbonyl formation, indicating that Pb promoted a high protein oxidation. This result is in agreement with that reported by Arvind and Prasad (2005) and Rellán-Álvarez *et al.* (2006) who noticed carbonyl accumulation in *Ceratophyllum demersum* and *Zea mays* plants exposed to Cd. In another study, Cargnelutti *et al.* (2006) and Pereira *et al.* (2010) an increase in protein oxidation was showed in cucumber exposure to Hg and Al, respectively.

*Effect of lead on hydrogen peroxide*

Hydrogen peroxide also appears to play an important role in signal transduction during plant abiotic stress. $H_2O_2$ produced from oxidative burst functions, such as a local trigger of programmed cell death of challenged cell, causes a rapid cross-linking of cell wall proteins (Levine *et al.*, 1994). However, $H_2O_2$ is a signaling molecule, it must have regulated synthesis, specific responses and cellular targets, and there must be mechanisms for its metabolism or removal subsequent to signaling events (Neill *et al.*, 2002). A possible component of a systemic signal is $H_2O_2$, whichst up an acclimatory response in unstressed regions of plant (Bhattacharjee, 2005). In the present study, the level of hydirogen peroxide was increased significantly, in the shoots of radish in all treatment with Pb compared

with the control (Tab. 2). This result is similar to Tecklić *et al.* (2008) and Perira *et al.* (2010) who suggests an increased level of $H_2O_2$ in radish leaves treated with Pb and in cucumber treated with Al.

Increased level of $H_2O_2$ is produced either due to action of SOD on superoxide radicals or by direct formation in biochemical pathways, photorespiration (Mishra *et al.*, 2006).

*Pb effects on antioxidant enzyme*

To explore the mechanism how plants tolerated Pb-induced oxidative stress, an examination of various antioxidant enzymes was performed. Activities of CAT and POD induced significantly at all Pb concentrations (Tab. 3).

This results show an increase of soluble protein content in shoots of radish seedlings in all treatment with lead. This increase is possible due to de novo synthesis of stress proteins provoked by metal exposure (Verma and Dubey, 2003). Cargnelutti *et al.* (2006) showed that Hg-treated cucumber presented increased in total soluble protein content.

CAT is one of the key enzymes in the removal of toxic peroxides. This is mostly universal oxide reductase that scavenges $H_2O_2$ via a two-electron transfer producing $O_2$ and $H_2O$ (Lin and Kao, 2000). The major function of CAT is to metabolize the peroxide liberated in the peroxisome following the conversion of glycollate during photorespiration (Qureshi *et al.*, 2007). In the present study, in shoots of *Raphanus sativus*, the decrease in CAT activity under Pb concentration indicating removal of $H_2O_2$ and toxic peroxides and, in turn, the reduction in the free radical mediated lipid peroxidation under Pb toxicity. Lead decreased the activity of CAT in the shoot only in the *Raphanus sativus* at lower Pb concentrations.

However, with increasing Pb level, reduction in CAT activity was observed, which has been attributed to the activation of the enzyme protein (Feieraband and Engel, 1986) and the decrease in enzyme synthesis (Mittler, 2002). The decrease in CAT activity could indicate its inactivation by accumulation of $H_2O_2$ induced by lead in *Cassia angustifolia* (Qureshi *et al.*, 2007).

POD is also an important enzyme, able to scavenge $H_2O_2$, which is a major substance degraded by SOD. The role of POD as a stress enzyme in plants has been widely accepted. POD activity has been suggested as a potential biomarker for metal toxicity in plant species (Agawal and Pandey, 2004). Induction of POD activity has been documented under many stress conditions such as NaCl stress (Agawal and Pandey, 2004), high temperature (El-Shintinawy *et al.*, 2004) and toxic concentrations of Cu, Pb, Zn, Cd and Al (Cakmak and Horst 1991; Guo *et al.*, 2004; Radotic *et al.*, 2000).

In the present investigation, radish exposed to 100, 200, 500 and 1000 mg $l^{-1}$ Pb showed a significant increase in POD activity in shoot compared with the control. POD activity was stimulated by the accumulation of peroxide in the plants. Enhancement of POD activity in radish reflects its great capacity to acclimate to Pb stress by rapidly engaging an antioxidative defense system.

## Conclusions

Thus, the finding that *Raphanus sativus* shows that it is good plant material for studying other aspects of abiotic stress resistance mechanisms. Based on the present work, it can be suggested that toxic concentrations of Pb cause oxidative stress, as evidenced by increased $H_2O_2$ formation, lipid peroxidation and oxidation of proteins in shoot of radish. In this study, a significant reduction in different parameters such as growth of shoot and roots, chlorophyll and carotenoid concentrations coupled with lipid peroxidation, protein oxidation and hydrogen peroxide indicated that high Pb levels in nutrient solution produced toxic effects. It was proposed that the reduced growth in Pb of radish exposed to toxic levels of Pb might be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation. Moreover, it was possible to observe that Pb-tolerant plants developed some defense mechanisms against oxidative stress. Further studies are required to investigate whether the oxidative stress caused by Pb toxic levels is an early symptom that can trigger shoot growth inhibition.

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