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RESEARCH ARTICLE

LEAD UPTAKE AND ITS EFFECTS ON ANTIOXIDANT DEFENCE SYSTEM IN *SANSEVIERIA ROXBURGHIANA* SCHULT & SCHULT. F.

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Abstract

The effects of lead (Pb) stress on plant growth, lipidperoxidation and on the activity of antioxidant enzymes were studied in *Sansevieria roxburghiana* Schult. & Schult. F., grown under hydroponical conditions in the absence and in the presence of various concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 g/l) of lead nitrate. The activity of antioxidant enzymes Ascorbate peroxidase (APX), Guiacol peroxidase (GPX), and Glutathione-s-hydrogenase (GSH) was increased in plants by lead treatment in a dose-related manner. The relative increase in enzyme activities demonstrated that *Sansevieria roxburghiana* is more tolerant to Pb. Lipid peroxidation was enhanced in stressed *Sansevieria roxburghiana* with the increased Pb concentration. The maximum accumulation of Pb (142.6 mg/kg DW, 322.0 mg/kg DW , 323.3 mg/kg DW, 385.3 mg/kg DW and 536.6 mg/kg DW) occurred in plants followed by increase in enzyme activities proved this plant can be effectively useful in removal of Pb from Pb contaminated sites.
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Keywords: Antioxidant enzymes, Lead, *Sansevieria roxburghiana* and dose-related.

Introduction

Environmental pollution has become a burning problem throughout the world over the past several decades. Many industrial processes are major contributors to extensive environmental pollution (Cunningham *et al*., 1995). Clean-up of Pb-contaminated soils is difficult. Existing methods such as mechanical removal and chemical engineering are expensive, and are often incompatible with maintaining soil structure and fertility. Phytoremediation, i.e. the use of plant systems to remove toxic elements from the soils, has recently attracted a great deal of attention as an alternative means of soil decontamination, since it is a cost-effective, environmentally-friendly approach, applicable to large areas. Among all pollutants lead plays a predominant role and it exists in many forms in natural sources throughout the world. According to the USA Environmental Protection Agency, Pb is one of the most common heavy metal contaminants in aquatic and terrestrial ecosystems and can have adverse effects on growth and metabolism of plants, due to its direct release into the atmosphere (Watanabe, 1997).

The effect of lead depends on the concentration, type of salts, soil properties and plant species involved (Patra *et al*., 2004). There have been many reports of Pb toxicity in plants (Choudhury & Panda, 2005), including disturbance of mitosis (Wierzbicka, 1998; Jiang & Liu, 2000), inhibition of root and shoot growth (Liu *et al*., 2009), induction of leaf chlorosis (Pandey *et al*., 2007), reduction of photosynthesis (Xiao *et al*., 2008) and inhibition or activation of several enzyme activities (Verma & Dubey, 2003; Sharma & Dubey, 2005; Liu *et al*., 2009). Plants use a diverse array of enzymes like superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.1), as well as low molecular weight

antioxidants like cysteine, nonprotein thiol, and ascorbic acid to scavenge different types of reactive oxygen species (ROS), thereby protecting cell injury and tissue dysfunction (Halliwell, 1987).

There is urgency in identifying effective lead phytoremediating species suitable for the particular ecological condition of lead dumping sites. In the present study, *Sansevieria* was selected as suitable pb accumulator because of its survival in nearby Pb contaminated sites. A number of species such as *Sansevieria cylindrica, Sansevieria ehrenbergii, Sansevieria guineensis, Sansevieria longiflora, Sansevieria roxburghiana, Sansevieria trifasciata* and *Sansevieria zeylanica* are grown as ornamental plants (USDA.2008). *Sansevieria roxburghiana* Schult. & Schult. F. (Agavaceae), called murva in Sanskrit and Hindi, Indian bowstring hemp in English, is a herbaceous perennial plant with short fleshy stem and stout rootstock, occurring in the Eastern coastal region of India, also in Sri Lanka, Indonesia and tropical Africa (Eggli, 2002; Prakash *et al*., 2008).

The present experiments addressed the effect of lead on the antioxidant activity of ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione-s-hydrogenase (GSH) including accumulative potential of lead in *Sansevieria roxburghiana* grown in hydroponic culture.

Material and Methods

Plant material

Sansevieria roxburghiana plants were collected from Sri Venkateswara University campus and grown in green house. The specimen was identified by Dr. K.Madhavachetty, taxonomist at Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Plants were hydroponically grown for 1 wk without Pb and then exposed to nutrient solutions with 0, 0.5, 0.1, 1.5, 2.0 and 2.5 g/l for 90 days period. The experiment was repeated for three times with five replicates. For the experimental procedure, 90 d-old plants were grown in Hoagland's medium (Hoagland and Arnon, 1950), with 16-h photoperiod (PAR 200 µmol m^{-2} s^{-1}, temperature 25 ±1 °C, relative air humidity 50 - 60 %). The nutritional solution was changed every 7 days and aerated every day.

Preparation of enzyme extracts

Plant samples were homogenized for 30 sec in a chilled mortar with 50 mM potassium phosphate buffer (pH 7.0) with 2% Polyclar AT added. Homogenates were centrifuged at 15,000 g for 30 min at 4°C and the supernatant was used for enzyme assays.

Determination of Ascorbate Peroxidase Activity (EC 1.11.1.11)

The activity of APX was measured according to Nakano and Asada (1987). The reaction mixture contained 50 mM potassium-phosphate buffer, 0.5 mM L-ascorbate (AsA), 0.1 mM H_2O_2 and the enzyme extract. H_2O_2-dependent oxidation of AsA was followed by a decrease in absorbance at 290 nm. APX activity was expressed as the absorbance decrease units mg^{-1} protein.

Determination of Guaiacol peroxidase Activity (EC 1.11.1.7)

GPX activity was estimated according to Hammerschmidt *et al*. (1982). The reaction mixture contained 25 mM potassium phosphate buffer (pH7.0), 0.2 mM guaiacol, 0.09 mM H_2O_2 and the enzyme extract. H_2O_2-dependent oxidation of guaiacol was followed by an increase of absorbance at 470 nm. Enzyme activity was calculated as Mm^{-1} cm^{-1}.

Determination of glutathione-s-hydrogenase

Glutathione-S-Hydrogenase (GSH) content was determined by the recycling method of Anderson (1985). Fresh plant material (0.5 g) was homogenized in 3.0 ml of 5% (w/v) sulfosalicylic acid under cold conditions and was centrifuged at 10,000 rpm for 10 min. Half ml aliquot was taken in a microfuge tube, to which 0.5 ml reaction buffer [0.1 M phosphate buffer (pH 7.0), 3 mM ethylenediaminetetraacetic acid (Na_2EDTA)] and 50µl of 50 dithio-bis-(2-nitrobenzoic acid) (0.15% DTNB) were added. After 5 min, absorbance for determination of GSH was read at 412 nm using UV–Vis spectrophotometer (Shimazdu, Japan). The level of GSH was expressed in nmol g^{-1}fresh weight.

Lipid Peroxidation

The lipid peroxidation products in samples are expressed as 2-thiobarbituric acid-reactive metabolites (TBA-rm) (mainly MDA). MDA was determined in crude extracts with thiobarbituric acid (Peever and Higgins 1989). The absorbance of the

supernatant at 532 nm was measured and corrected for unspecific turbidity by substracting the absorbance at 600 nm. The blank was 0.5% TBA in 20% TCA. The lipidperoxidation levels were expressed as mol g^{-1} FW.

All enzymes were assayed spectrophotometrically (Shimazdu UV-VIS, Japan) by tracing the change in absorbance at 27 °C.

Plant digestion

The roots, stems and leaves were digested in a HNO_3-$HClO_4$ (3:1,v:v) mixture and Pb concentrations were determined by atomic absorption spectrophotometry (AAnalyst 200, Perkin-Elmer, UK).

Statistical analysis

Statistics were analyzed with SPSS version 11.0, and ANOVA was performed, with significance at $p < 0.05$. All values were mean of five independent experiments.

Results

Pb accumulation

Sansevieria roxburghiana plants grown in the presence of 0.5, 1.0, 1.5, 2.0 and 2.5 g/l of lead nitrate showed accumulation of 142.6 mg/kg DW, 322.0 mg/kg DW, 323.3 mg/kg DW, 385.3 mg/kg DW and 536.6 mg/kg DW. Pb accumulation increased according to dose dependent manner. The enhancement of metal accumulation in similar trend observed in *Chromolaena odorata* and *Lespedeza davidii* exposed to lead according to Tanhan *et al*. (2011) and Zheng *et al*. (2011).

Effect of Pb on ascorbateperoxidase activity

Ascorbateperoxidase (APX) was involved in the destruction of H_2O_2. Thus, a rapid increase in APX activity was observed on 30 days (6.06±1.25 units mg^{-1} protein), 60 days (6.58±0.83 units mg^{-1} protein) and 90 days (6.738±1.14 units mg^{-1} protein) compared to controls (3.48±0.074 units mg^{-1}protein) (Fig. 1). Similar increase in the activity of anti-oxidative enzyme APX in *P. vulgaris* was observed (Chaturvedi *et al*. 2009).

Effect of Pb on guiacolperoxidase activity

In the present experiment, the activity of guaiacol peroxidase (GPX) increased in *S.roxburghiana* plants exposed to Pb. The GPX activities ranged higher for a treatment period of 90 days (10.69±0.63 Mm^{-1} cm^{-1}) compared to 30 days (10.49±0.55 Mm^{-1} cm^{-1}) and 60 days (10.52±0.55 Mm^{-1} cm^{-1}). Activities of guaiacol peroxidase (GPX) were not sensitive to Pb treatment and significant correlation was found between GPX activities and lead accumulation (Fig. 2). It can be seen clearly that very low GPX activity at initial concentrations ($p<0.05$). Increased Gpx activity also observed in *Lemna minor* according to Monika *et al*. (2007).

Effect of Pb on glutathione-s-hydrogenase

GSH, another enzyme of AGC (ascorbate-glutathione cycle), showed a significant increase in its activity after treatment with Pb. Enhancement in glutathione -S-hydrogenase (GSH) in all treatments was observed with increasing concentration and time. The increased GSH activity was highest at 2.0 g/l (1.95±0.61 nmol g^{-1} fresh weight) and 2.5 g/l (2.32±0.75 nmol g^{-1} fresh weight) Pb concentrations (Fig. 3). Glutathione also increased showing the active participation in ROS detoxification as reported earlier in case of Pb, Cu and Cd toxicity in tea plant (Rennerberg, 1982; Asada and Takahashi, 1987).

Effect of Pb on lipidperoxidation

The effects of lipidperoxidation increased with increasing lead concentration and treatment time for *S.roxburghiana* species. The concentrations of lipid peroxides on days 30 (37.56±0.46 mol g^{-1} FW), 60 (51.012±0.52 mol g^{-1} FW) and 90 days (65.61±0.57 mol g^{-1} FW) compared to controls (26.83±0.75 mol g^{-1} FW) were observed. There was a positive correlation between Pb-induced stress and lead accumulation (Fig. 4). Similar Pb exposure induced generation of reactive oxygen species (ROS) and increased the level of lipid peroxidation, accompanied by upregulation of antioxidative enzymes in *Zea mays* (Gupta *et al*. 2009) observed. Pb supplied at a toxic level caused a burst of reactive oxygen species (ROS) in root cells of *Sedum alfredii* (Huang *et al*. 2012).

Discussion

In the present experiment, increase in APX followed by GPX clearly indicated that antioxidant enzymes such as APX and GPX can scavenge free radicals in synchrony. GSH and GPX are known to be responsive to biotic and abiotic stresses, but they

Figure 1. Effect of Pb on ascorbateperoxidase activity

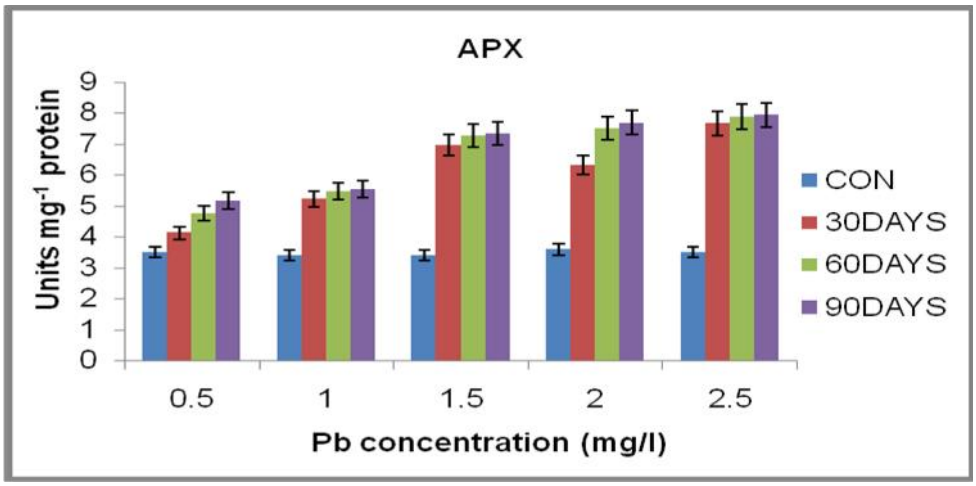


Figure 2. Effect of Pb on guiacolperoxidase activity

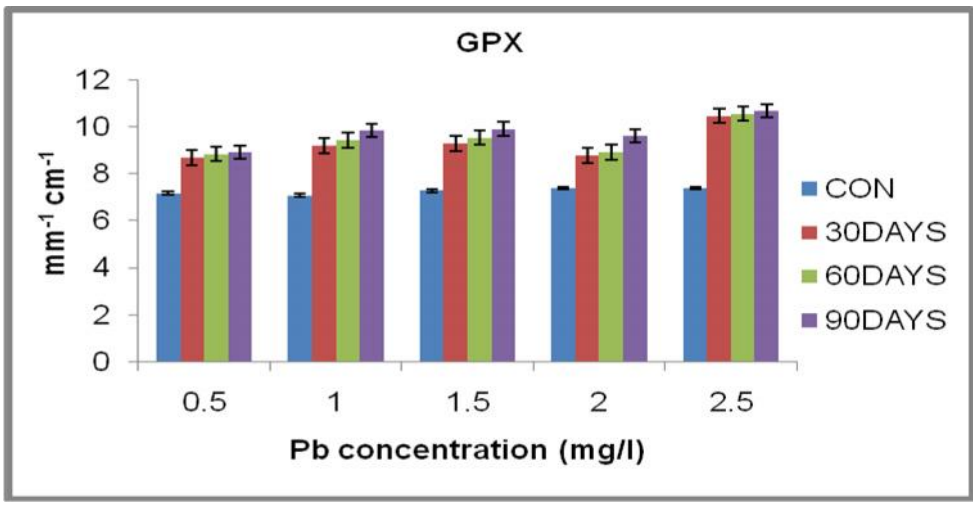


Figure 3. Effect of Pb on glutathione-s-hydrogenase

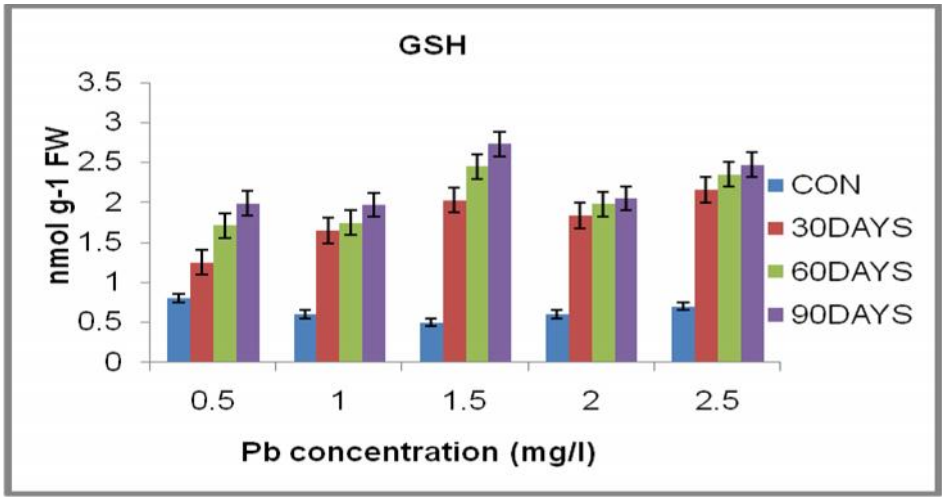
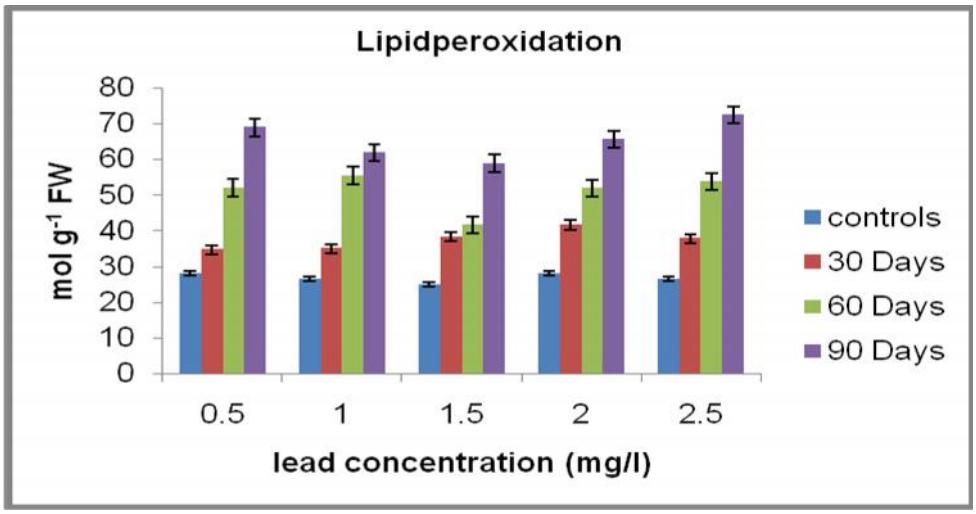


Figure 4. Effect of Pb on lipidperoxidation



are well characterized with respect to their antioxidative roles in plants. This provided evidence of activation of enzymatic defense mechanisms, such as APX and GPX in the *S.roxburghiana*, limited the production of free radicals and H_2O_2. Pb treatment caused a increase in both GSH activity and GPX activity in *S.roxburghiana* plants. The increase in the activities of these enzymes might explain the inhibition of the production of MDA. Since ascorbate is the primary antioxidant and H_2O_2 is the major stable oxidant, the ratio of these redox components is indicative of the redox balance within the tissue. As Kingston-Smith *et al*., (1997) described the relative amounts of ascorbate and H_2O_2 may be used in determining the effectiveness of AGC. GSH content enhanced upon exposure to Pb stress. Elevated GSH concentration is correlated with the ability of plants to withstand a metal-induced oxidative stress (Freeman *et al*., 2004). Mohan *et al*., (1997) suggested that the increase of peroxidase activity may be an effect of accelerated senescence, connected with enhanced formation of hydrogen peroxide (H_2O_2) or secondary metabolites such as phenolic compounds.

We can add that this possibility does not apply to ascorbate peroxidase, an enzyme of the Halliwell-Asada pathway, but only to GPX activity thus we can say increased activity of APX and GPX show that they are competed to remove H_2O_2. In our model conditions the activity of guaiacol peroxidase (GPX) increased in *S.roxburghiana* plants exposed to Pb, but there was no effect on Glutathione-S-hydrogenase (GSH). There is some evidence that modification of membrane structure could activate production of oxygen free radicals and induce lipid peroxidation (Gupta *et al*., 1999; Elstneret *et al*., 1988). Lipid peroxidation mediated by activated oxygen species should be accompanied by changes in activities of antioxidative enzymes involved in oxygen metabolism. Enhancement of lipid peroxidation of membranes through oxygen free radicals by lead is also supported by elevated enzyme activity. One of the most damaging effects of these active oxygen free radicals and their products in cells is the peroxidation of membrane lipids. Here Lead may have shown low phytoavailability and restricted transport within the plants according to Kabata-Pendias and Sharma and Dubey (1999, 2005).

The measured ratios of ascorbate to H_2O_2 are always higher in the treated plants in comparison with the control, which indicated that the cycle is robust and it is not disordered by Pb-stress. The highest dose (2.5g/l) was slightly toxic to *S.roxburghiana*: the plants were chlorotic and fresh weight decreased threefold (results not included); their accumulation capacity probably was exhausted, but according to the Kabata-Pendias *et al*. (1999) reports on the positive effect of lead (II) nitrate (V) on plant growth, although data showing a physiological justification for this phenomenon are lacking. In our study, lead at concentrations lower at 0.5 g/l did not harm the growth of *S.roxburghiana*, and even stimulated it slightly. Here the enzymes GPX, GSH, and APX are involved in the detoxification of $O2^{\bullet-}$, and H_2O_2 respectively by preventing the formation of $^{\bullet}OH$ radicals.

The results suggest that the Pb-induced increase in antioxidative enzymes in the *S.roxburghiana* plants may represent a secondary defensive mechanism against oxidative stress that is not as direct as the primary defensive responses such as phytochelatins and vacuolar compartmentalization (Sanita de Toppi and Gabbrielli 1999). The induction of APX, GPX, and GSH provided effective defense against metal toxicity.

Conclusion

This study established a link between lead accumulation and antioxidant potential in the species of *Sansevieria roxburghiana* in tolerance to metal stress. Lead concentration increased activity of antioxidant enzymes Ascorbate peroxidase (APX), Guiacol peroxidase (GPX), and glutathione - S-hydrogenase (GSH) including MDA content in a dose-related manner. Inspite of high lead concentration a little physiological damage was observed like death of root tips. The increase in the activities of these enzymes explains the inhibition of the production of lipidperoxidation. The plant S.roxburghiana taken in the present experiment is an ornamental plant so this plant will be the better options as phytoremediator will not affect food chain. This evidences suggests that the *Sansevieria roxburghiana* was tolerant towards Pb and be useful for phytoremediation of lead-contaminated soils.

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