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Lead-induced Oxidative Stress and Metabolic Alteration in Seedlings of *Brassica juncea* L.

Pratima H.* and Pratima Mathad
Department of Post-Graduate Studies and Research in Botany, Gulbarga University, Gulabarga, 585106, Karnataka, India
pr_lect@yahoo.co.in

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Abstract

The present study was conducted to assess lead toxicity causes metabolic alteration and oxidative stress in seedlings of Brassica juncea L. by petri-dish experiment. Surface sterilized seeds were germinated with graded concentrations (10, 20, 30 and 40 mM) of lead nitrate in petriplates lined with Whatman no.1 filter paper. Lead stress resulted, a uniform decrease in germination (100-10%), biomass (0.59-0.08mg/fw and 0.05-0.01mg/dw), root (7.20-0.61cm) and shoot (8.12-1.15cm) elongation with increasing concentrations marked as the primary signs of lead injury. An increasing concentration of lead treatment showed a uniform decrease in chlorophylls (16.94-6.10mg/gm) and β-carotene (0.77-0.25mg/g) composition with a significant accumulation of free proline (0.24-1.52mg/100mg) suggesting an osmoprotection from lead. Total peroxide (0.22-0.62 µmol g^{-1} fw) and lipid peroxidation (2.92-5.10 µmol g^{-1} fw) showed uniform increase under metal treatment. The glutathione, ascorbate and polyphenol contents showed a decrease at 30mM and 40mM of lead. The antioxidative of enzymes such as peroxidase (0.13-0.50 unit $min^{-1}mg^{-1}$) and glutathione reductase (0.42-0.70unit mg^{-1}) were markedly enhanced while catalase (1.08-0.14 unit mg^{-1}) and superoxide dismutase (7.38-2.32unit mg^{-1}) decreased prominently with increasing concentration of lead. The finding pointed to the role of oxidative stress in the underlying lead phytotoxicity in Brassica juncea L. seedling.

Keywords: *Brassica juncea,* Lead, Oxidative Stress, Toxicity, Lipid Peroxidation, Antioxidant Enzymes.

Introduction

Lead (Pb) is a toxic heavy metal in the environment widely distributed in soil and waters. The main sources of lead pollution include atmospheric lead paint chips, used ammunition, fertilizers, pesticides and lead-acid batteries or other industrial products. Soils are major sink of lead which might be absorbed and bio-accumulated by plants and animals. The presence of metal bioelements in plant and animal organisms has different effects depending on their level. High lead concentration in soil decreases the germination and has a harmful effect on growth and metabolism in plants.[1,2] Low concentration of lead can inhibit some vital plant processes such as photosynthesis, mitosis, root growth and water absorption[3,4] .

Toxic effects of lead on plants grown on lead contaminated soils include inhibition of photosynthesis, deficient mineral nutrition uptake and the problem of water imbalance, which considerably reduce both the vegetative and reproductive growth of plants[5,6]. In addition, lead is reported to produce reactive oxygen species (ROS) and enhance antioxidant enzyme activity in plants[7]. The ROS produced as a result of oxidative stress causes a variety of harmful effects in plant cells, such as inhibition of photosynthetic activity, inhibition of ATP production, lipid peroxidation, and DNA damage[8]. Production of excess ROS in heavy metal stressed plants may be a consequence of the distribution of the balance between their production and the antioxidative enzyme activity, composed of enzymic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) [9,10].

Brassica juncea L. is commonly called mustard belongs to the family Brassicaceae. The chief use of *Brassica* seeds in India as oil and condiments for the preparation of pickles and vegetables. Lead phytotoxicity studies have been conducted on various plants such as *Phaseolus vulgaries*[11], *Cicer aerietinum*[12], Pea root cells[13], radish[14], Water hyacinths[7], *Abutilon indicum*[15] and rice plant[16] but there are no reports about the lead toxicity on *Brassica juncea* L. The present investigatation was undertaken to study the phytotoxic role of lead causing oxidative stress in *Brassica juncaea* L. seedlings.

Materials and Methods

Brassica Juncea L. (Mustard) seed were used for Petri-dish experiment. Seeds were surface sterilized by rinsing in 0.1% of $HgCl_2$ followed by rinsing with distilled water for 2–3 times. Surface sterilized seeds were germinated in petriplates lined with Whatman No. 1 filter paper (25 seed per plate). Further, the filter paper was moistened with solution of lead nitrate of different concentrations (10, 20, 30 and 40 mM). Similar experiment without lead nitrate was conducted as control.These petriplates were maintained in laboratory conditions at 28 ± 2°C. Seed germination, seedling growth in terms of root-shoot length,

biomass and biochemical content was determined after 7 days of treatment. The dry mass was determined after keeping them in an oven at 70°C for 48hrs.

The biochemical parameters such as chlorophylls were estimated by the method of Arnon[17], β-carotene content was estimated by Jensen[18], proline content was estimated the method of Bates *et al.*[19], peroxide content by Sagisaka[20], lipid peroxidation (or thiobarbituric acid reactive substances (TBARS)) was estimated by Heath and Packer[21]. Further the analysis of ascorbate, glutathione and polyphenols was made by the methods of Klein and Perry[22], Griffith[23], Malick and Singh[24] respectively.

For enzyme extraction the plant material was homogenized with 5 ml of phosphate buffer (pH 6.8) in a pre-chilled mortar and pestle. The extract was centrifuged at 4°C for 15 min at 15000 rpm. The supernatant obtained was used as the enzyme extract for the analysis of enzymes viz., catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione reductase (GR). The CAT activity was estimated by the method of Teranishi *et al.*[25]. The SOD activity was estimated by Dhindsa *et al.*[26]. The POD and GR activity was estimated by the method of Malick and Singh[24], Foyer and Halliwell[27].

Statistical analysis: All experimental results are means from three replications. Statistical analysis was performed by one way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) by using SPSS package version 10.

Results and Discussion

Effect of different concentrations of lead on germination, root and shoot lengths, biomass of 7 days seedlings of *Brassica juncea* L. are shown in Table-1. The seeds in the control and in 10 mM lead showed 100% germination, further increase in lead concentrations led to reduction in seed germination. Higher concentration of lead inhibited seed germination and seedling growth has been reported in *Vigna radiate*[28]. It is evident from our results that the root length was adversely affected as compared to shoot length at higher concentrations of lead. Lead treatment showed greater toxic effects on root growth of *L. leucocephala* and rice plant[29,30]. The reduction in root length in metal treatments could be due to reduced mitotic cells in meristematic zone of root[31]. The fresh weight and dry weight of seedlings were higher at 10 mM but it was lowered with increasing concentrations of lead. Similar results were reported in *Sesamum indicum* plant treated with lead[32].

The changes in pigments, proline, lipid peroxidation, total peroxide glutathione, ascorbate and polyphenol contents in different concentrations of lead on 7 days old seedlings of *Brassica juncea* L. are described in Table-2. The chlorophyll content decreased with increasing concentrations due to the inhibitory effect of the metal in chlorophyll biosynthesis or plant specific effect, as reported for other metals in different plants[33]. The β-Carotein content increased at lower concentration compared to control but it declined in increasing concentrations. It could probably due to carotenoids degradation or inhibition of biosynthesis[34]. The proline content increased with increasing concentrations of lead may be due to a decrease in water potential with the imposition of heavy metal treatment[35]. The results show an increase in the level of lipid peroxides with increasing concentrations of lead, indicating that lead induces oxidative stress in *Brassica juncea* seeedlings. The results are in conformity with the observations of other workers who reported lead-induced oxidative stress in pea root cells and rice plant[36,30]. Similarly increased total peroxide content was recorded with increasing concentration of lead in *Brassica juncea* seedlings. The ascorbate and glutathione content were decreased at 30-40mM lead concentration, whereas lower lead concentrations did not affect it. A slight increase in polyphenol content was seen at 10mM concentration compared to control but higher concentrations it decreased. Similar to our observation decreased polyphenol, ascorbate, glutathione have been reported under Al toxicity in Green gram[37] and nitrogen fertilizer in *Labisia pumila* Blume[38].

Table-1
Effect of Different Concentrations of Lead on Growth of 7 Days Old Seedlings of *Brassica juncea* L

Treatment Pb (mM)	Germination %	Root Length (cm)	Shoot Length (cm)	Fresh Weight (mg)	Dry Weight (mg)
Control	100	$7.20^{a} \pm 0.01$	$8.12^{a} \pm 0.05$	$0.59^{b} \pm 0.10$	$0.05^{a} \pm 0.13$
10	100	$5.96^{b} \pm 0.05$	$7.6\ 0^{b} \pm 0.80$	$0.64^{a} \pm 0.11$	$0.05^{b} \pm 0.09$
20	80	$3.18^{c} \pm 0.02$	$7.01^{c} \pm 0.11$	$0.31^{c} \pm 0.14$	$0.03^{b} \pm 0.03$
30	30	$1.90^{c} \pm 0.05$	$5.41^{d} \pm 0.50$	$0.16^{d} \pm 0.10$	$0.02^{cd} \pm 0.08$
40	10	$0.61^{d} \pm 0.10$	$1.15^{d} \pm 0.05$	$0.08^{c} \pm 0.04$	$0.01^{d} \pm 0.05$

Each value is expressed as mean ± S.D. (n=3) and statistically significant at $P < 0.05$

A uniform increase in peroxidase, glutathione reductase activities and decrease catalase, superoxide dismutase activity was detected with increase in lead concentrations (Table-3). The peroxidase and glutathione reductase activities significantly increased with increasing concentration of lead in *Brassica juncea* seedlings suggests their role in detoxification of H_2O_2. Similar, findings for lead and other metals in different plants have been reported by other workers[39,40]. The superoxide dismutase activities increased at lower concentration (10 mM and 20 mM) but it declined at higher concentrations (30 and 40 mM) of lead. A decrease in superoxide dismutase activity in response to lead suggests an inactivation of superoxide dismutase enzyme by free radicals has also been reported by Panda and Patra[33] in other metals. The CAT activity decreased may be due to increase in H_2O_2 in turn inactivated the enzyme. A decline activity of catalase with an increase lead concentration has also been observed water hyacinths[7].

Table-2
Effect of Different Concentrations of Lead on Pigments, Proline, Lipid, Peroxidation, Total Peroxide, Glutathione, Ascorbate and Polyphenol Content of 7 Days Old Seedlings of *Brassica juncea* L

Parameters	Treatment Pb (mM)				
	Control	10	20	30	40
Total chlorophyll (mg/gm)	$16.94^{a} \pm 0.10$	$16.00^{b} \pm 0.09$	$13.03^{c} \pm 0.15$	$11.12^{d} \pm 0.08$	$6.10^{cd} \pm 0.14$
β- carotene (mg/g)	$0.77^{a} \pm 0.15$	$0.60^{b} \pm 0.50$	$0.50^{c} \pm 0.02$	$0.39^{c} \pm 0.12$	$0.25^{d} \pm 0.30$
Proline (mg/100mg)	$0.24^{a} \pm 0.10$	$0.28^{b} \pm 0.30$	$0.60^{c} \pm 0.14$	$1.20^{d} \pm 0.50$	$1.52^{d} \pm 0.15$
Lipid Peroxidation (µmol g^{-1} fw)	$2.92^{a} \pm 0.12$	$3.00^{b} \pm 0.20$	$4.60^{c} \pm 0.15$	$6.12^{d} \pm 0.10$	$5.10^{d} \pm 0.30$
Total Peroxide (µmol g^{-1} fw)	$0.22^{a} \pm 0.13$	$0.31^{b} \pm 0.05$	$0.51^{b} \pm 0.03$	$0.55^{c} \pm 0.08$	$0.62^{d} \pm 0.10$
Glutathione (µmol g^{-1} fw)	$2.63^{a} \pm 0.005$	$2.20^{b} \pm 0.01$	$2.03^{c} \pm 0.09$	$1.50^{d} \pm 0.02$	$1.12^{d} \pm 0.10$
Ascorbate (µmol g^{-1} fw)	$2.14^{a} \pm 0.007$	$1.90^{b} \pm 0.003$	$1.95^{c} \pm 0.008$	$0.80^{c} \pm 0.014$	$0.75^{d} \pm 0.12$
Polyphenol (µmol g^{-1} fw)	$1.53^{a} \pm 0.03$	$1.85^{b} \pm 0.09$	$0.80^{c} \pm 0.01$	$0.75^{c} \pm 0.02$	$0.64^{d} \pm 0.30$

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P < 0.05$.

Table-3
Effect of Different Concentration of Lead on Antioxidative Enzyme Activity of 7 Days Old Seedlings of *Brassica juncea* L

Treatment Pb (mM)	Peroxidase (unit $min^{-1}mg^{-1}$)	Glutathion reductase (unit mg^{-1})	Superoxide dismutase (unitmg^{-1})	Catalase (unit $mg^{-}1$)
Control	$0.13^{a} \pm 0.05$	$0.42^{a} \pm 0.04$	$7.38^{a} \pm 0.13$	$1.08^{a} \pm 0.01$
10	$0.28^{b} \pm 0.10$	$0.50^{b} \pm 0.04$	$9.14^{b} \pm 0.09$	$0.74^{b} \pm 0.20$
20	$0.58^{c} \pm 0.12$	$0.64^{c} \pm 0.12$	$5.72^{c} \pm 0.09$	$0.60^{c} \pm 0.08$
30	$0.67^{c} \pm 0.02$	$0.75^{d} \pm 0.08$	$2.34^{d} \pm 0.07$	$0.38^{cd} \pm 0.14$
40	$0.50^{d} \pm 0.05$	$0.70^{d} \pm 0.10$	$2.32^{d} \pm 0.30$	$0.14^{d} \pm 0.05$

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P < 0.05$

Conclusion

The study revealed that lead toxicity in *Brassica junceae* L seedlings causes oxidative stress, as evidenced by increasing lipid peroxidation and that increase peroxidase and glutathion reductase activity perhaps to help in detoxifying the H_2O_2 produced in response to lead treatment.

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