

Selenium Alleviates Coleus from Oxidative Damage under Pb Stress by Resource Allocation and Antioxidant Defense System

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Abstract: The role of selenium (Se) against lead (Pb) stress in Coleus (*Coleus blumei* benth.) was investigated by evaluating the allocation plasticity and antioxidant properties of plants cultured hydroponically with lead (1.0 mM Pb) and selenite (0~5.0 mM Se) in this study. It was showed that Pb reduced the root growth and caused serious damage in the roots, which was accompanied by biomass changes and Pb accumulation in the organ. When Se application up to 1.0 mM improved the biomass allocation and Pb accumulation of Coleus organs and significantly decreased Thiobarbituric Acid Reactive Substances (TBARS) accumulation in Coleus treated with 1.0 mM Pb. However, above 1.0 mM, Se induced stress in Coleus grown with Pb. These results indicated that at this concentration, Se can protect Coleus from Pb stress by expressing primarily biomass allocation and metal partitioning. Significant changes of antioxidant defense system in Coleus exposed to Pb stress were also found as a result of the added Se. Se supplied up to 1.0 mM greatly decreased root Superoxide Dismutase (SOD) and Peroxidase (POD) activity in Pb-stressed Coleus ($p<0.05$). In addition, Catalase (CAT) and glutathione peroxidase (GSH-Px) activity was increased with increasing Se concentration ($p<0.05$). The effect of Se on glutathione (GSH) in Coleus Pb-treated was dose-dependent. The GSH content increased up to a concentration of 2.5 mM Se and then decreased. These changes in enzymatic and non-enzymatic antioxidants showed that Se supplied had a significant disturbance on Coleus under Pb stress and affected the biochemical and physiological processes. Furthermore, the obtained results also showed that appropriate Se supplementation may ameliorate Pb-induced oxidative stress by decreasing lipid peroxidation and altering antioxidant defense system and that Se detoxification and accumulation in Coleus might be associated closely with the efficiency of these mechanisms.

Keywords: Antioxidant defense system, *Coleus blumei* benth., Lead, resource allocation, selenium

INTRODUCTION

Lead (Pb), a heavy metal with characteristic toxic action, has attracted considerable attention for its widespread distribution and potential risk to the environment. The plant processes such as the biosynthesis of nitrogenous compounds, carbohydrate metabolism and water absorption are adversely affected by increasing Pb levels in soil and even at every low concentration (John *et al.*, 2009; Hamid *et al.*, 2010). Furthermore, the metals are also accumulated in different plant parts and thereby enter into the food chain. However, the plant response to Pb contamination is a key research problem and a special effort is undertaken in seeking factors which affect the reduction of Pb absorption or toxicity in plants. Selenium (Se) is an important element for human and animal nutrition based on its presence in antioxidative defence systems, but was not considered essential for plants (Kápolna *et al.*, 2009). Nevertheless, several studies reported the

beneficial effects of Se, because it increased the antioxidant activity in plants, leading to better plant yield (Lyons *et al.*, 2009; Cartes *et al.*, 2010). Recent publications indicated that Se addition may alter the total content of heavy metals in plant tissues (Pedrero *et al.*, 2008; Feng *et al.*, 2009). In *Brassica napus* seedlings, Se was found to reverse the Cd-induced decrease in fresh mass and changes in lipid peroxidation as well as changes in the DNA methylation pattern (Filek *et al.*, 2008). Studies on animals had also shown that Se was one of the potential antagonists to Pb, Cr, Hg and Cd and limited the toxic effects of heavy metals (Cerklewski and Forbes, 1976; Ikemoto *et al.*, 2004; Soudani *et al.*, 2010). Coleus (*Coleus blumei* Benth.) is a salinity and humidity-resistance ornamental plant which is widely planted in arid and semiarid urban regions. Although Coleus can remove nitrogen and phosphorus in eutrophication water (Liu *et al.*, 2004) and had strong tolerance and accumulation capacity of aluminum (Panizza de León

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et al., 2011), however, its growth and productivity are frequently threatened by different abiotic stresses such as drought, salinity or heavy metal. To cope with these stresses, Coleus has developed arrays of physiological and biochemical strategies to adapt to the adverse conditions, so it is important to understand the mechanisms that confer tolerance to heavy metal environments. In addition, the facts of Se is intriguing, enigmatic and challenging (even capricious) for researcher. Thus, in this study, we were to investigate that:

- The appropriate concentration of exogenous Se alleviating Pb stress by allocation plasticity and plant-metal partitioning, which were relevant to phytoremediation and representative of distinctive growth strategies
- The occurrence of any antagonistic or synergistic interaction between Pb and Se by membrane stability and antioxidant enzyme activities in Coleus.

MATERIALS AND METHODS

Plant material and growth conditions: Coleus (*Coleus blumei* benth.) used in this study was from the clone continually propagated in the Botanical Garden, Nanchang, Jiangxi. The plants were pretreated with tap water for two weeks, to adapt to the water environment. Next, the seedlings of uniform size were transplanted in 15L pots (16 plants per one pot) with modified full-strength Hoagland's medium (Zhao et al., 2007) containing the following mineral components (mM): $\text{Ca}(\text{NO}_3)_2$ 3.0; KNO_3 4.0; KH_2PO_4 1.0; MgSO_4 1.0; MnCl_2 3.6×10^{-3} ; H_2BO_3 4.5×10^{-2} ; CuCl_2 8×10^{-4} ; ZnCl_2 1.5×10^{-3} ; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 1.4×10^{-5} ; Fe-EDTANa₂ 9.0×10^{-2} . To determine the effect of Pb and Se, the growth medium was supplemented with 1.0 mM Pb in the form of $\text{Pb}(\text{NO}_3)_2$ (Sigma) and the concentrations of Se in the form of Na_2SeO_3 (Sigma) are 0 (control), 0.1, 0.5, 1.0, 2.5 and 5.0 mM. The Pb and Se concentrations were chosen on the basis of preliminary experiments and literature data. Four-week cultivation was performed in the greenhouse and the photon flux density was c. 450/ μmol photon s^{-1} m^{-2} at the height of the upper leaves. The temperatures range were 15-18°C (night) and 22-26°C (day), relative humidity was between 45 and 70%. During the experiment, the nutrient solution was continuously aerated and its losses were supplemented daily with dH₂O. The medium were renewed once a week and its pH was kept at 6.0-6.5. Each treatment contained six individual single pots. At the end of experiment, the fresh weights of the seedlings (g/pot) were recorded. The dry weights of seedlings were measured by drying the seedlings at 75°C, to give a constant weight.

Determination of Pb and Se content: Root, shoot and leaves samples of 0.25 g dry weight were put into 100 mL digestion tubes. Next, a 5 mL acid mixture of HNO_3 and HClO_4 (4:1, v/v) was added and digested at 25°C overnight. Samples were then completely digested at 150~165°C in a digestion oven until the solution became clear. After cooling, a 2.5 mL aliquot of 6 M HCl was added and heated to 100°C until gaseous brown fumes ceased and the solution became clear. After cooling, the digested samples were diluted to 25 ml with Milli-Q water. Pb and Se contents were analyzed by the ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) method in the Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Natural Resources and Environmental Science, Zhejiang University. Calibration curves were made using Pb and Se standard aqueous solutions "Suprapur" pure (Merck chemical).

Antioxidant enzyme extraction and assays: Plant material (about 0.5g) was homogenized in 5 ml solutions containing: 100 mM of potassium phosphate buffer (pH7.0) containing 0.1 mM of EDTA-Na₂ and 1% polyvinylpyrrolidone (W/V) at 4°C. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000×g for 15 min at 4°C. Enzyme activity was measured in the supernatant solution. Protein contents were determined according to Bradford (1976), using bovine serum albumin as a standard. SOD, POD and CAT activities were determined using the method of Gajewska et al. (2006) and GPX activities were determined using the methods of Mishra et al. (2006).

Nonenzymatic antioxidant and Lipid peroxidation measurement: Reduced (GSH) and oxidized (GSSG) glutathione contents were determined by the recycling method described by Anderson (1985). Lipid peroxidation was determined by estimation of the Melondialdehyde (MDA) content following Fu and Huang (2001).

Statistical analysis: All data were analyzed in four replications using one-way analysis of variance (ANOVA) and the LSD test to determine significant differences between treatments. The graphs were done on OriginPro Version 8.5 (OriginLab Corporation, Northampton, USA).

RESULTS

Influence of selenium on resource allocation under Pb stress:

Biomass allocation to root, stem and leaf of Coleus: Biomass is a key factor for phytoremediation practices and it is also an overall measurement of plant health.

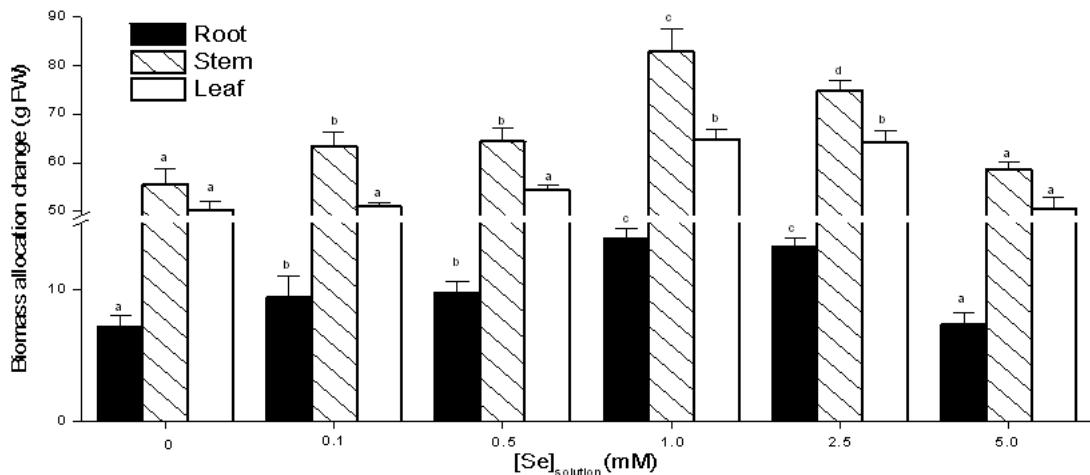


Fig. 1: Influence of Se on biomass allocation of Coleus exposed to Pb stress. The bars indicate mean±SE (n = 6). Values with different letters differ significantly from each other at p<0.05

Table 1: Influence of Se on Pb and Se distribution in the different organs of Coleus exposed to Pb stress

[Se] _{solution} (mM)	Pb accumulation (mg/g DW)			Se accumulation (mg/g DW)		
	Root	Stem	Leaf	Root	Stem	Leaf
0	58.49±1.94a	2.79±0.46a	3.27±0.06b	ND	ND	ND
0.1	69.63±8.99b	4.69±0.12a	2.91±0.07b	0.07±0.001a	0.005±0.000a	0.003±0.000a
0.5	103.99±3.13c	7.69±0.53b	5.20±0.03c	0.20±0.009b	0.01±0.008b	0.01±0.002b
1.0	112.89±9.97c	8.71±0.69b	2.69±0.07b	0.39±0.008b	0.02±0.009b	0.02±0.006b
2.5	107.49±2.96c	4.14±0.31a	2.59±0.09b	0.98±0.004c	0.01±0.004b	0.007±0.00a
5.0	71.86±5.59b	2.69±0.58a	0.47±0.07a	0.80±0.003c	0.01±0.003b	0.003±0.00a

Each value represents means±SE (n = 6). Different letters within columns indicate significant differences between treatments at p<0.05, the same letters are not significantly different between treatments at p>0.05. ND not detectable

Coleus organ biomass varied greatly by different Se concentration exposed to Pb (Fig. 1). The enhancement of Coleus biomass under Pb stress by Se treatments was observed in this study up to 1.0 mM selenium, the root, stem and leaf biomass reached 13.8, 82.9, 64.8 g Fresh Weight (FW), respectively; and then reduced with increasing of Se concentrations, but still enhanced in comparison to the control (Fig. 1). As compared to the control, the root, stem and leaf biomass of Coleus increased by 93.7, 49.6 and 28.9%, respectively (Fig. 1).

Pb and Se distribution in Coleus organs: Pb accumulation varied among Coleus organs and was affected by different Se concentration. The highest Pb contents in the leaf, stem and root of Coleus under Pb stress were found with a low concentration of Se (0.5 and 1.0 mM) (Table 1). A significant increase in Pb accumulation was observed in both root and stem organ (p<0.05) at 1.0mM Se treatment, but in leaf for 0.5 mM Se treatment (Table 1). As expected, a high concentration of Se decreased Pb accumulation in the leaf, stem and root of Coleus, but was higher than those of control treatment. These findings have great implications for optimizing phytoextraction of environmental Pb pollution. When Coleus were grown

in Pb and Se-containing nutrient solutions, Se concentrations increased in Coleus organs (p<0.05). The concentration of Se in different organs differed depending on the Se concentrations in the nutrient solution (Table 1). Concentrations of Se in control Coleus, which were grown without Se, were below the limits of ICP-AES detection. There was significantly difference in Se accumulation in the root organ among treatments and the highest accumulation of Se was found at 2.5 mM Se treatment, but in the stem and leaf was 1.0 mM Se treatment (Table 1).

Influence of selenium on lipid peroxidation and antioxidant defense system under Pb stress:

Lipid peroxidation: The MDA content, one of the major TBARS reactive metabolites, is indicator of the lipid peroxidation of plasma membrane of plant cells and its accumulation is indicative of enhanced production of reactive oxygen species. In this study, the MDA contents under Pb stress in the 2.5 mM Se-treated Coleus root decreased by 32% in comparison to the control (Fig. 2). Moreover, 0.5~2.5 mM Se decreased significantly the MDA level, while 5 mM Se increased the lipid peroxidation by 29.4% in comparison to 2.5mM under Pb stress, but was lower than that of the control (Fig. 2).

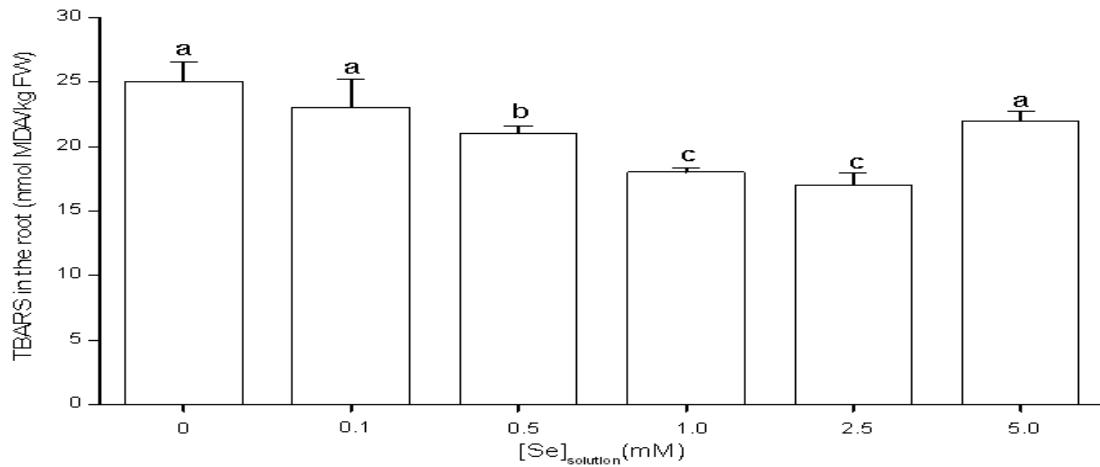


Fig. 2: Influence of Se on lipid peroxidation of Coleus root exposed to Pb stress. The bars indicate mean \pm SE (n = 6). Values with different letters differ significantly from each other at p<0.05

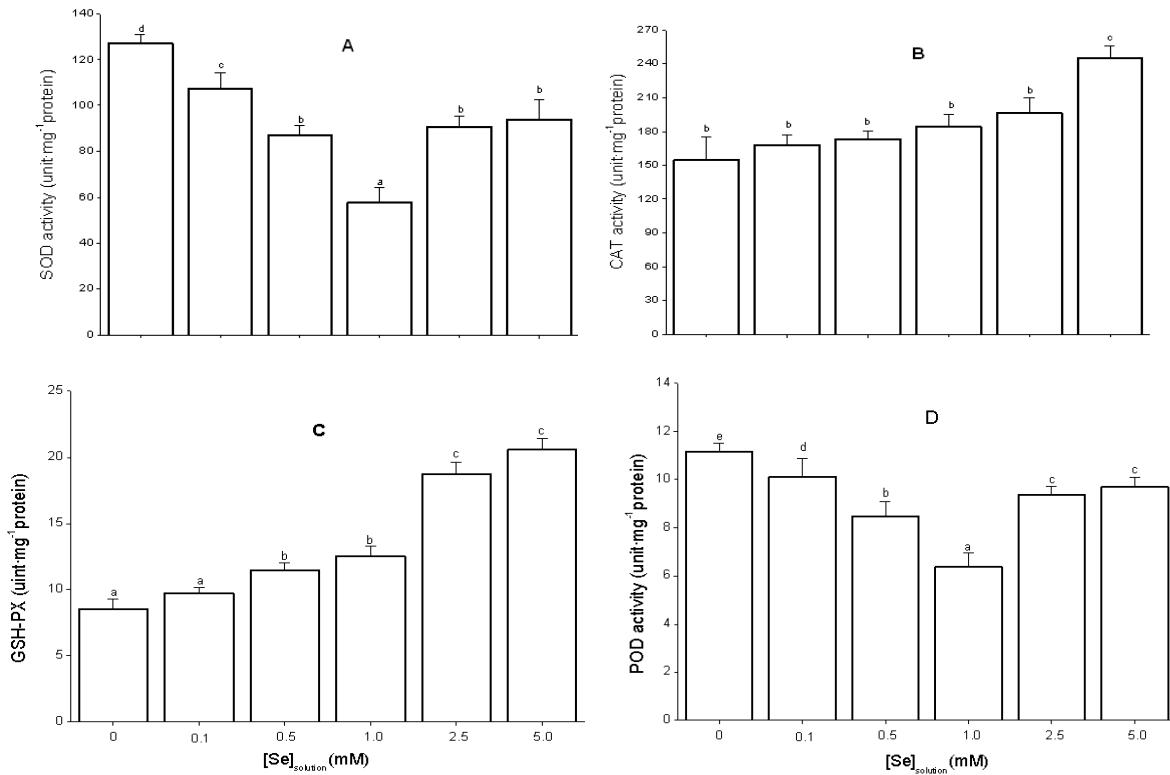


Fig. 3: Influence of Se on antioxidant enzyme of Coleus root exposed to Pb stress. The bars indicate mean \pm SE (n = 6). Values with different little letters differ significantly from each other at p<0.05. The Capital letters of A, B, C and D are SOD, CAT, GSH-Px and POD, respectively

Antioxidant defense system: The activities of CAT, SOD, APX and POD enzymes in Coleus root by Se treatment exposed to Pb stress were given in Fig. 3. The SOD activity decreased significantly at a concentration of 1.0 mM Se treatment and then increased with increasing of Se concentration, but was lower than that of control. Moreover, there was no marked change in SOD activity between 2.5 mM and 5.0 mM treatment

(Fig. 3A). In Se treatment, Coleus exposed to Pb showed a significant increase in the activity of CAT compared to the control, but there was no significant difference among low concentrations of Se. However, the CAT activity was increased by 58.4% at 5.0 mM Se treatment (Fig. 3B). All treatments containing Se had higher GSH-Px activity than the control treatment with the highest activity in the treatment with 5.0 mM

Table 2: Influence of Se on levels of reduced and oxidized glutathione, reduced and oxidized glutathione ratios in Coleus root exposed to Pb stress

[Se] _{solution} (mM)	GSH ($\mu\text{moL/g FW}$)	GSSH ($\mu\text{moL/g FW}$)	GSH+GSSG ($\mu\text{moL/g FW}$)	GSH/GSSG
0	2.19 \pm 0.43a	1.58 \pm 0.02b	4.18a	1.39a
0.1	4.51 \pm 0.70b	1.29 \pm 0.07b	5.97a	3.50b
0.5	5.13 \pm 0.76b	1.08 \pm 0.03a	7.13b	4.75b
1.0	7.04 \pm 0.58c	0.93 \pm 0.02a	9.97b	7.57c
2.5	9.10 \pm 0.72c	0.81 \pm 0.05a	11.02c	11.23d
5.0	8.65 \pm 0.24c	0.97 \pm 0.01a	9.59b	8.95c

Each value represents means \pm SE (n = 6). Different letters within columns indicate significant differences between treatments at p<0.05, the same letters are not significantly different between treatments at p>0.05

(Fig. 3C). Addition of 0.1mM Se Coleus exposed to Pb stress did not alter the GSH-Px activity in the root while 0.5mM Se increased the activity of this enzyme by 34.4%. Whereas GSH-Px activity was approximately 2.4 fold increases than the control at the end of experiment. In the root exposed to Pb stress with Se treatment for 0.1 mM, 0.5 mM and 1.0 mM Se, the POD activity was decreased by 9.1, 24.1 and 42.7%, respectively (Fig. 3D). After addition of Se concentration was above 1.0 mM, a increase in POD activity was found, but still decreased in comparison to the control (Fig. 3D).

The results presented in Table 2 showed that GSH level was significantly increased in Coleus root with increasing of Se concentration under Pb stress than the control. The maximum GSH content in the root was observed at 2.5 mM Se treatment, which was 75.9% higher than in the control (Table 2). A decrease was recorded for GSSG and the GSH/GSSG ratio increased in the root under Se treatment, but no significant difference was observed among low Se concentrations (\leq 1.0 mM) (Table 2), indicating that glutathione pool appeared to be more reduced after Se treatments for Pb stress as compared to the control.

DISCUSSION

Influence of selenium on plant resource allocation under Pb stress: Plants adjust their relative biomass allocation and heavy metal distribution to organ systems (e.g. roots or shoots) when subjected to environmental stress conditions (Gedroc *et al.*, 1996). Although Se was not yet confirmed to be required by higher plants (Terry *et al.*, 2000), several studies demonstrated that at low concentration it may exert diverse beneficial effects, including growth-promoting activities (Djanaguiraman *et al.*, 2005; Cartes *et al.*, 2010). Our results showed that exposed to Pb, Se promoted the each organ biomass of Coleus at concentrations up to 1.0 mM, whereas each organ biomass was greatly depressed when 2.5 or 5.0 μM Se was added (Fig. 1). This was considered to be Se toxicity because retardation of Coleus growth was one of the symptoms noted when Coleus was grown with high levels of Se. However, each organ biomass was reduced by the simultaneous addition of 1.0 mM Pb and 2.5 or 5.0 μM Se (Fig. 1), but it was still increased by

the combined effect of Pb addition and high Se supply levels, compared with no Se addition exposed to Pb. This result indicated that Se had either stimulating or toxic effects on Coleus depending on the Se concentration in the culture media. This was in agreement with a number of recent reports on plants such as *Lolium perenne* L. (Cartes *et al.*, 2010) and *Vicia faba* L. (Mroczek-Zdyska and Wójcik, 2012). According to the results obtained in this study, Coleus could accumulate Pb efficiently during the cultivation and the accumulated amount increased with the concentrations of Se treatments (\leq 2.5 mM) (Table 1). The higher Se concentrations, such as \leq 1.0 mM, led to high Pb accumulation and a significant increase in Pb accumulation was observed in both root and stem (Table 1), which may suggest a greater demand of Se in the root to counteract the toxic effects induced by Pb. Nevertheless, the molecular and physiological mechanisms responsible of this behaviour will require further research. These results for Coleus were similar to those reported by Zembala *et al.* (2010) who found that Se addition significantly decreased the Cd concentration of rape and wheat seedlings exposed to Cd stress. The Coleus Se contents in all treatments differed significantly from each other and roughly proportionally to the Se addition (Table 1). As for the allocation of Se into various organs of Coleus increased effectively with increasing Se dosages (\leq 2.5 mM) (Table 1). Nevertheless, the effects in root and stem can be different, the highest accumulation of Se in the root was found at 2.5 mM Se treatments, but in the stem was 1.0 mM Se treatment (Table 1). This result was in good agreement with Krystofova *et al.* (2010) who had mentioned that a higher amount of Se was determined in the root of *Urtica dioica* L. Our findings indicated that Coleus with Se treatments shifted their biomass and metals distribution more to roots than shoots possibly to circumvent the challenges of Pb conditions.

Influence of selenium on lipid peroxidation and antioxidant defense system under exposed to Pb stress: The benefits of Se on plant growth had been often related with an improvement of plant antioxidant ability. According to our results, the ability of Se to reduce lipid peroxidation was noticeable in Coleus root and the application of 2.5 mM Se decreased the TBARS level by 32% in Coleus root exposed to Pb stress.

Interestingly, we found that Coleus exposed to Pb with high Se concentration (≥ 5.0 mM) exhibited the increase of lipid peroxidation, but was lower than that of the control (Fig. 2). At simultaneous Pb and Se addition the maximal decrease in TBARS was observed at 0.98 mg Se g⁻¹ DW in the root. These findings demonstrated that Coleus not only take up more Se as a consequence of root Pb injury, but also suggested that at low Se addition an extra amount of Se was taken up by Coleus to alleviate the Pb-induced oxidative stress. Indeed, plants possess a complex ROS-scavenging system that includes several antioxidant enzymes and low-molecular-weight antioxidants such as ascorbate, glutathione and phenolic compounds (Noctor and Foyer, 1998). Thus, at low dosages, Se possibly triggered the co-operation of various antioxidative systems to help counteract the oxidative damage induced by Pb in the roots. In contrast, the increase in TBARS accumulation at high selenite levels (5 mM Se) indicated that Se may act as a pro-oxidant in Coleus as reported earlier by Mroczez-Zdryska and Wójcik (2012).

The fluctuations in the level of lipid peroxidation by effect of the added Se were accompanied by significant changes in the activity of the antioxidant enzymes SOD, CAT, GSH-Px and POD in Coleus exposed to Pb stress (Fig. 3). SOD acts as the first line of defense against ROS by catalysing the disproportionation of superoxide radicals (O₂⁻) to H₂O₂ and molecular oxygen (Gratão *et al.*, 2008). In the present study, it was remarkable that selenite additions up to 1.0 mM Se greatly decreased SOD activity in the Coleus root to lower levels compared to those of control (Fig. 3A) and at low selenite levels, the decrease of SOD activity (Fig. 3A) coincided with a reduction of damage of cell membranes (Fig. 2). This fact suggested that, at these concentrations, Se was able to diminish the need of SOD by reducing the level of toxic O₂⁻ in the root of Pb-stressed Coleus. CAT is tetrameric heme-containing enzymes, which directly dismutates H₂O₂ into H₂O and O₂ and is indispensable for ROS detoxification during stress (Garg and Manchanda, 2009). Application of Se, combined with Pb stress, significantly increased the activity of CAT in this study, especially at 5.0 mM Se treatment (Fig. 3B), which indicated a protective role for Se in scavenging H₂O₂ in Coleus root under Pb stress. Similar to our results, increased CAT activity in Se-supplemented plants under cadmium, high temperature and salt stresses were described by other researchers (Filek *et al.*, 2008; Djanaguiraman *et al.*, 2010; Hasanuzzaman *et al.*, 2011). GSH-Px is another enzyme that uses GSH to reduce H₂O₂ and therefore, protects plant cells from damage due to oxidative stress (Gill and Tuteja, 2010). In our study, in comparison to Pb stress alone, the combination of Pb stress and moderate Se resulted in a significant increase in the activities of GSH-Px, but its increase had no significant

different from higher Se concentration (Fig. 3C). This brought the levels down of H₂O₂ and lipid peroxidation in Coleus root (Fig. 3C and 2). Similar increases in GSH-Px activity after Se supplement during stress was observed by other researchers (Filek *et al.*, 2008). Synergistic effect of Se on GSH-Px activity had been demonstrated before (Lobanov *et al.*, 2008). POD is also an important enzyme, able to scavenge H₂O₂, which is a major substance degraded by SOD. In the present study, the POD activity in the root exposed to Pb stress was decreased with increasing of Se concentration (≤ 1.0 mM), but the POD activity for 2.5 or 5.0 mM Se increased noteworthy, which seemed to reflect an increased hydrogen peroxide (H₂O₂) production at higher Se supply levels. In a word, opposite variation trends between SOD-POD activities and CAT-GPx activities were observed during the cultivation. The Se-induced decrease in SOD and POD activities indicated that lower amounts of superoxide anion radicals were produced in cells due to the higher activity of GPX and CAT. On one hand, it can be presumed that the increase in CAT and GPX, which were scavengers of H₂O₂ and lipid hydroperoxides, resulted in reduced formation of superoxide anion radicals through the dynamic inter-transformation among oxygen species. On the other hand, Se increased GPX activities and enhanced the spontaneous disproportion of superoxide radicals, consequently and reduced the need for their scavenger SOD. These results indicated that the prevention of damage to cell membrane of Coleus can be achieved by co-operative effects of the whole system of antioxidant enzymes.

GSH can react chemically with single oxygen, superoxide and hydroxyl radicals and function directly as a free radical scavenger. GSH and its oxidized form, GSSG, maintain a redox balance in the cellular compartments. The conversion of GSSG to GSH by the GR enzyme was correlated with the change in GSH/GSSG ratios, which played an important role in the signal transduction of several transcription and metabolic processes (Namjooyan *et al.*, 2012). Our results demonstrated that the Pb-stressed Coleus with Se supplement showed a higher increase in the level of GSH than did the Coleus subjected to Pb stress alone (without Se). This increase of GSH in Se-treated Coleus root might be due to Se boosting GSH synthesis. This was supported by Anderson and McMahon (2001) who described that Se accelerated efficient recycling of GSH and reported the relationship between Se and GSH synthesis. In our study, the Se and Pb-stressed Coleus showed lower GSSG level than the Coleus treated with Pb alone. Therefore, an increased GSH/GSSG ratio appeared to be an "overcompensation" resulting from intensified recycling of GSH with the aim to keep it in its active, reduced form. This increase in the GSH/GSSG ratio in Se-supplemented Pb stressed Coleus also provided a clear demonstration of the role of Se towards Pb tolerance. Similar results were

obtained by Hasanuzzaman *et al.* (2011) on *Brassica napus* cv. Bina under salt stress supplied with selenium. Based on the above results, Coleus exposed to Pb stress with Se treatment could protect itself from toxicity of Pb by altering various metabolic processes with the involvement of the whole antioxidant systems.

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