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Selenium Treatment Mitigates the Effect of Lead Exposure in *Coleus Blumei* Benth

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Abstract

We examined the effects of selenium (Se) on coleus (*Coleus blumei* Benth.) under lead (Pb) stress, to determine possible mitigating mechanisms of Se. Coleus plants were exposed to 1.0 mM Pb(NO3)2 and varying concentrations of Na₂SeO₃ for 21 d. Application of 1.0 mM Se enhanced biomass allocation and Pb distribution in different organs, decreased superoxide dismutase (SOD) and peroxidase (POD) activity in the roots, and acted as an antioxidant by inhibiting lipid peroxidation via increasing glutathione levels. Root catalase (CAT) and glutathione peroxidase (GSH-Px) activities increased with Se concentration, and changes to root and leaf particles were observed by scanning electron microscopy and X-ray diffraction. Our results indicate Pb is tolerated by coleus plants through allocation plasticity, activation of antioxidant systems, and improvements in particle size and mineralogical effects. *C. blumei* can be useful in phytoremediation of aquatic systems contaminated with Pb, especially with addition of low concentrations of Se.

Keywords: *Coleus blumei*; Biomass; Selenium; Pb tolerance; Phytoremediation

Introduction

Lead (Pb), a heavy metal with characteristic toxic actions, has attracted considerable attention for its widespread distribution and potential risk to the environment. Plant processes such as the biosynthesis of nitrogenous compounds, carbohydrate metabolism and water absorption are adversely affected by increasing Pb levels in soil, even at every low concentration [1,2]. Furthermore, heavy metals also accumulate in different plant organs, and thereby enter the food chain. However, the plant response to Pb contamination is a key research problem, and a special effort is underway to identify factors which can reduce Pb absorption or toxicity in plants. Selenium (Se) is an important element for human and animal nutrition based on its presence in antioxidative defense systems, but it is not considered essential for plants [3]. Nevertheless, several studies have reported the beneficial effects of Se, as it increased antioxidant activity in plants, leading to better yields [4,5]. Recent studies have indicated that Se addition may alter the total heavy metal content in plant tissues [6,7]. 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 [8]. Studies in animals have also shown that Se is one of the potential antagonists to Pb, Cr, Hg and Cd, and Se limited the toxic effects of heavy metals [9,10]. Coleus (Coleus blumei Benth.) is a salt- and humidity-resistant ornamental plant that is widely planted in arid and semiarid urban areas. Although coleus can remove nitrogen and phosphorus from eutrophied water sources [11], and has a strong tolerance and accumulation capacity for aluminum [12], its growth and productivity are frequently threatened by different abiotic stresses such as drought, salinity or heavy metals. To cope with these stresses, coleus has developed an array of physiological and biochemical strategies that enable it to adapt to adverse conditions, so it is important to understand the mechanisms that confer tolerance to heavy metal environments. Due to its antioxidant role, it is hypothesized that Se can counteract the detrimental effects of Pb stress in plants. The objective of this study was to test this hypothesis and investigate whether (a) an appropriate concentration of exogenous Se can alleviate Pb stress by allocation plasticity and plant-metal partitioning, which is relevant to phytoremediation and representative of distinctive growth strategies; (b) any antagonistic or synergistic interactions between Pb and Se by membrane stability and antioxidant enzyme activities occur in coleus; (c) there are changes in scanning electron microscopy (SEM) and X-ray diffraction(XRD) patterns in coleus plants with different concentrations of Se treatments under Pb stress.

Materials and Methods

Plant material, growth conditions, and Se treatments

Coleus (Coleus blumei Benth.) used in this study was from a clone continually propagated in the Botanical Garden, Nanchang, Jiangxi. The plants were washed and pretreated with tap water to adapt to the water environment. After one month, these plants were transferred to 2.5 L pots (16 plants per pot) containing 1/4 strength Hoagland nutrient solution (HNS) [13]. Plants were acclimatized in the hydroponic system for two weeks. Experiments were carried out using the simplest hydroponic system, i.e., a water culture system with slight modification (Modified Hoagland nutrient solution, MHNS) [14]. The platform that holds the plants was made of Styrofoam and floats directly on the nutrient solution. Air was bubbled through the nutrient solution using an air pump connected to an air stone to supply oxygen to the roots of the plants. The experiment was set up as a completely randomized design with six treatments and four replications. The acclimated plants were transferred to full-strength MHNS, which was spiked with 1.0 mmol/L Pb in the form of Pb(NO₃)₂ (Sigma), and the concentrations of Se in the form of Na₂SeO₃ (Sigma) were 0, 0.1, 0.5, 1.0, 2.5 and 5.0 mmol/L. These treatments are expressed as PbSe⁰, PbSe^{0.1}, PbSe^{0.5}, PbSe^{1.0}, PbSe^{2.5} and PbSe^{5.0}, respectively. The solution was aerated

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continuously and replaced twice weekly during the acclimatization period, and the pH was maintained at 6.0-6.5. After the addition of Pb and different concentrations of Se to the nutrient medium, loss of water via transpiration was replenished by frequent additions of deionized water to maintain a constant volume.

Plants were kept in a growth chamber with an 8 h light period at a light intensity of 450 μ molm⁻² s⁻¹, 25°C/20°C day/night temperature, and 60–70% relative humidity. Plants were harvested 21d after Pb and Se treatment. Upon harvest, each plant was separated into root, stem and leaf. Tissue samples were either air-dried for total Pb, Se and plant biomass analysis, or frozen in liquid nitrogen and stored at -80°C for biochemical analyses.

Pb and Se analyses

Air-dried root, stem and leaf samples (0.5 g) were digested with concentrated nitric acid on a temperature-controlled digestion block (Environmental Express, Mt. Pleasant, SC) using USEPA Method 3050B [15]. Pb and Se analyses were performed with a graphite furnace atomic absorption spectrophotometer (Perkin–Elmer SIMAA 6000, Norwalk, CT). A NIST standard reference material (SRM) was used for quality control. Calibration with certified Pb and Se standard solution was included. Matrix spikes were carried out in 10% samples, with an average recovery of 94 \pm 6%. Analytic SRM recovery was within 10% of the true value.

Lipid peroxidation assays

Lipid peroxidation was measured as the amount of thiobarbituric acid reacting substances (TBARs) determined by the thiobarbituric acid (TBA) reaction, following the method of Heath and Packer [16] with slight modifications [17]. Approximately 0.5 g of frozen tissue was cut into small pieces and homogenized with 2.5 ml of 5% (wt/v) trichloroacetic acid (TCA) in a glass homogenizer using a cold mortar and pestle over ice. The homogenates were transferred to 50 ml Nalgene^{*} centrifuge tubes and centrifuged at 10,000 g for 15 min at room temperature (20-22°C). The concentrations of lipid peroxides, together with the oxidatively modified plant proteins, were quantified using an extinction coefficient of 155 mM-1 cm-1 and expressed as total TBARs in terms of μ mol g-1 fresh weight.

Antioxidant enzyme assays

Root tissue that was flash frozen in liquid nitrogen and stored at -80°C was homogenized in 50 mM Tris-HCl buffer (pH 7.0). The supernatant solution was used to measure the activity of the antioxidant enzymes catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX). CAT activity was assayed by the method of Barber [18]. The reaction mixture consisted of enzyme extract, 5 mM H₂O₂ 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 0.01 N KMnO₄ and measured spectrophotometrically at 240 nm. POD activity was assayed by the method of Kar and Mishra [19]. The reaction mixture contained 100 mM Tris-buffer (pH 7.0), 10 mM pyrogallol and 5 mM H₂O₂. The reaction was initiated by adding 25 µl enzyme solution and stopped after 5 min incubation at 25°C by adding ml 2.5 N H₂SO₄. The amount of purpyrogallin formed was measured spectrophotometrically at 425 nm. SOD activity was estimated by following the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT). One SOD unit was defined as the amount of enzyme corresponding to 50% inhibition of the NBT reduction [20]. The reaction mixture contained 400 µL of potassium phosphate buffer (0.1 M pH 7.0), 10 µL of 10 mM EDTA, 50 μ L of 260 mM methionine, 80 μ L of 4.2 mM NBT, 170 μ L of 130 μ M riboflavin, and 300 μ L of supernatant. The absorbance of the samples was measured at 560 nm, after the reaction tubes had been illuminated for 15 minutes. Non-illuminated and illuminated reactions without supernatant were used as controls. GPX activity was assayed by the modified method of Flohe and Gunzler [21], using H2O2 as the substrate. The enzyme was extracted by the protocol described by Hartikainen et al. [22] modified by the addition of 1 mM EDTA and 1% poly(vinylpolypyrrolidone) as protease inhibitors. The absorbance of each sample was measured at 412 nm within 5 min, and the enzyme activity was calculated as a decrease in GSH in the reaction time with respect to a non enzyme-containing reaction. All enzyme activities were calculated on a protein basis. The protein concentration in the enzyme extracts was determined spectrophotometrically by the method of Bradford [23].

Antioxidant assays

Reduced glutathione (GSH) was measured using glutathione reductase (GR) by the method of Gossett et al. [24]. One gram samples of frozen leaf tissue were ground with inert sand and 5 ml of ice-cold 6% (v/v) *m*-phosphoric acid (pH 2.8) containing 1 mM EDTA in a cold mortar and pestle over ice. The homogenate was centrifuged at 22,000 g for 15 min and the supernatant was removed and then filtered through a 0.45 µM ultrafilter. Two solutions were then prepared: Solution A consisted of 110 mM Na,PO, 7H,O, 40 mM NaH,PO, H,O, 15 mM EDTA, 0.3 mM 5,5'-dithiobis-(2-nitrobenzoic acid), and 0.4 mlL⁻¹ BSA; Solution B consisted of 1 mM EDTA, 50 mM imidazole, 0.2 ml L⁻¹ BSA, and an equivalent of 1.5 units GR activity (baker's yeast, Sigma Chemical Company). Total glutathione was measured in a reaction mixture consisting of 400 µl of solution A, 320 µl of solution B, 400 µl of a 1:50 dilution of the extract in 5% (w/v) Na₂HPO₄ (pH 7.5) and 80 µl of NADPH. The reaction rate was measured using a spectrophotometer by following the change in absorbance at 412 nm for 4 min. Oxidized glutathione (GSSG) was determined from the difference between total glutathione (GSH + GSSG) and glutathione (GSH).

Scanning electron microscopy (SEM) and X-ray diffraction (XRD)

XRD measurements were performed on the Bruker D8 Advance diffractometer operating in the reflection mode with Cu-K α -radiation (35kV, 30 mA) and diffracted beam monochromator, using a step scan mode with the step of 0.075° (2 θ) and 4 s per step. Additionally, the morphology of the powdered plant tissue was observed by SEM with a Czech VEGAII-LSU/H high resolution SEM operating at 30 kV.

Statistical data analysis

All results were expressed as an average of four replications. Treatment effects were determined by analysis of variance using OriginPro Version 8.5 (OriginLab Corporation, Northampton, USA). Duncan's test at 5% probability was used for post hoc comparisons to separate treatment differences. All results were expressed as mean \pm standard deviation.

Results

Influence of Se on resource allocation and lipid peroxidation under Pb stress

Biomass allocation: Biomass is a key factor in phytoremediation practices, and it is also an overall indicator of plant health. Coleus biomass varied greatly in response to different Se concentrations when exposed to Pb (Figure 1). The enhancement of coleus biomass under Pb stress by Se treatments was observed in this study up to 1.0 mM Se; the root, stem, and leaf biomass reached 13.8, 82.9 and 64.8 g fresh weight (fw), respectively. Biomass then declined with increasing Se treatment concentrations, but was still enhanced in comparison to the PbSe0 treatment (Figure 1). As compared to the control PbSe0 treatment, root, stem, and leaf biomass of coleus increased by 93.7%, 49.6%, and 28.9%, respectively, for the PbSe1.0 treatment (Figure 1).

Pb and Se distribution: Pb accumulation varied among coleus organs and was affected by treatment with different concentrations of Se. The highest Pb contents in the leaf, stem, and root of coleus under Pb stress were found at relatively low concentrations of Se (PbSe^{0.5} and PbSe^{1.0}; Table 1). A significant increase in Pb accumulation was observed in the root and stem tissues for the $PbSe^{1.0}$ treatment (p<0.05), but in leaf tissue, the highest level was observed for the PbSe^{0.5} treatment (Table 1). As expected, a high concentration of Se decreased the level of Pb accumulation in the leaf, stem and roots of coleus, but the levels were generally higher than those of the PbSe⁰ treatment. These findings have great implications for optimizing phytoextraction of environmental Pb pollution. When coleus plants were grown in Pb- and Se-containing nutrient solutions, Se concentrations increased (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 coleus plants for the PbSe⁰ treatment, which were grown without Se, were below the detection limits. There were significant differences in Se accumulation in the roots between treatments, and the highest accumulation of Se was found in the PbSe^{2.5} treatment; however, in the stem and leaf, the highest level of accumulation was for the PbSe⁰ treatment (Table 1).

Lipid peroxidation: The MDA content, one of the major TBAR

reactive metabolites, is an indicator of lipid peroxidation in the plasma membrane of plant cells, and MDA accumulation is indicative of enhanced production of reactive oxygen species. The MDA content in coleus roots for the PbSe^{2.5} treatment decreased by 32% in comparison to PbSe⁰ treatment (Table 1). Moreover, the MDA level of the roots decreased significantly in the PbSe^{0.5} to PbSe^{2.5} treatments, while the lipid peroxidation increased by 29.4% in PbSe^{5.0} in comparison to PbSe^{2.5}, but was lower than that in the PbSe0 treatment (Table 1).

Influence of Se on the antioxidant defense systems during Pb stress

Antioxidant enzymes: The activities of CAT, SOD, GPX and POD enzymes in coleus roots exposed to Pb stress for the six Se treatments are given in Figure 2. SOD activity decreased significantly in the PbSe1.0 treatment, and then increased with the increasing concentrations of Se, but was lower than in the PbSe0 treatment. Moreover, there was no marked change in the SOD activity for the PbSe^{2.5} and PbSe^{5.0} treatments (Figure 2A). In the Se treatments, coleus exposed to Pb showed a significant increase in the activity of CAT compared to the PbSe⁰ treatment, but there was no significant difference from the other concentrations of Se treatments. However, the CAT activity was increased by 58.4% for the PbSe^{5.0} treatment (Figure 2B). All treatments containing Se had higher GSH-Px activity than did the PbSe⁰ treatment, with the highest activity observed for the PbSe^{5.0} treatment (Figure 2C). Addition of 0.1 mM Se to coleus exposed to Pb stress did not alter the GPX activity in the roots, while the PbSe^{0.5} treatment increased the activity of this enzyme by 34.4%. GPX activity increased by approximately 2.4 fold in the PbSe^{5.0} treatment by the end of the experiment, compared to the PbSe⁰ control. For the PbSe^{0.1}, PbSe^{0.5} and PbSe^{1.0} treatments, the POD activity in the roots decreased by 9.1%, 24.1% and 42.7%, respectively (Figure 2D). At Se concentrations above



b+Se treatment (mM) Pb accumulation (mg/g DW)			Se accumulation (mg/g DW)			Lipid peroxidation (TBARS) in roots (µmol/g fw	
	Root	Stem	Leaf	Root	Stem	Leaf	
PbSe ⁰	58.49 ± 1.94c	2.79 ± 0.46b	3.27 ± 0.06b	ND	ND	ND	25.01 ± 1.507a
PbSe ^{0.1}	69.63 ± 8.99b	4.69 ± 0.12b	2.91 ± 0.07b	0.07 ± 0.001c	0.005 ± 0.000b	0.003 ± 0.000b	23.15 ± 2.18a
PbSe ^{0.5}	103.99 ± 3.13a	7.69 ± 0.53a	5.20 ± 0.03a	0.20 ± 0.009b	0.01 ± 0.008a	0.01 ± 0.002a	21.06 ± 0.62a
PbSe ^{1.0}	112.89 ± 9.97a	8.71 ± 0.69a	2.69 ± 0.07b	0.39 ± 0.008b	0.02 ± 0.009a	0.02 ± 0.006a	18.61 ± 0.37b
PbSe ^{2.5}	107.49 ± 2.96a	4.14 ± 0.31b	2.59 ± 0.09b	0.98 ± 0.004a	0.01 ± 0.004a	0.007 ± 0.00b	17.05 ± 0.91b
PbSe ^{5.0}	71.86 ± 5.59b	2.69 ± 0.58b	0.47 ± 0.07c	0.80 ± 0.003a	0.01 ± 0.003a	0.003 ± 0.00b	22.02 ± 0.76a

Table 1: Influence of Se on Pb and Se distribution in the different tissues of coleus exposed to Pb stress.

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1.0 mM, an increase in POD activity was found, but it was still less than that measured in the control PbSe0 treatment (Figure 2D).

Antioxidants: The results presented in Table 2 show that the GSH level is significantly increased in coleus roots with increasing Se concentrations in plants experiencing Pb stress. The maximum GSH content in the roots was observed at PbSe^{2.5} treatment, and was 75.9% higher than in the PbSe⁰ treatment (Table 2). A decrease was recorded for GSSG, and the GSH/GSSG ratio increased in the roots under Se treatment, but no significant difference was observed at low Se concentrations (\leq 1.0 mM; Table 2), indicating that the glutathione pool appeared to be more reduced after Se treatments for Pb stress as compared to the control.

Influence of Se on SEM and XRD patterns of coleus under Pb stress

SEM and XRD patterns in coleus roots: Treatment with different concentrations of Se had little impact on the particle size of coleus roots under Pb stress, but had a significant influence on their dispersion and degree of aggregation (Figure 3). The particles in the roots showed uneven dispersion with few differences between the various treatments, but the degree of aggregation gradually improved for the PbSe^{0,} PbSe^{0,1} and PbSe^{0,5} treatments. In addition, the particles were distributed into flat, folded shapes in the PbSe^{1,0} treatment, while in the PbSe^{2,5} and PbSe^{5,0} treatments; they were distributed differently and appeared to

be organized in a highly gathered state (Figure 3). The XRD peaks of coleus roots were mainly located at $2\theta=20\sim55^{\circ}$ for the different Se treatments in plants under Pb stress, except for the PbSe^{0.1} treatment, in which there were diffraction peaks at 68.8°, but the peak number, position, and intensity changed with the changes in Se concentrations (Figure 4). There were 19 significant peaks for the PbSe⁰ treatment, eight significant peaks for the PbSe^{0.1} treatment, six significant peaks for the PbSe^{0.5} and PbSe^{2.5} treatments, 22 significant peaks for the PbSe^{1.0} treatment, and five significant peaks for the PbSe^{5.0} treatment (Figure 4). In comparison with the PbSe⁰ treatment, the diffraction angle presented the changes of small angle diffraction boundary to the right and the large angle diffraction boundary to the left, the peak intensity was enhanced for the PbSe^{0.1}, PbSe^{0.5} and PbSe^{2.5} treatments, and the diffraction angle to the right shift and the peak intensity weakened for the PbSe^{1.0} treatment (Figure 4). The peak intensity, height and area increased, and the full width at half maximum (FWHM) decreased for the PbSe^{0.1}, PbSe^{0.5} and PbSe^{2.5} treatments (Table 3), while the peak intensity, height, area and FWHM decreased for the PbSe^{1.0} treatment. Moreover, the peak intensity, height and area increased, the FWHM decreased at 27.21°, and the peak intensity and height decreased, and the area peak and FWHM increased at 21.47° for the PbSe^{5.0} treatment (Table 3).

SEM and XRD patterns in coleus leaves

The morphology and distribution of particles in coleus leaves



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Pb+Se treatment (mM)	GSH (µmol/g fw)	GSSG (µmol/g fw)	GSH+GSSG (µmol/g fw)	GSH/GSSG
PbSe ⁰	2.19 ± 0.43c	1.58 ± 0.02a	3.77c	1.39d
PbSe ^{0.1}	4.51 ± 0.70b	1.29 ± 0.07a	5.80c	3.50c
PbSe ^{0.5}	5.13 ± 0.76b	1.08 ± 0.03b	6.21b	4.75c
PbSe ^{1.0}	7.04 ± 0.58a	0.93 ± 0.02b	7.97b	7.57b
PbSe ^{2.5}	9.10 ± 0.72a	0.81 ± 0.05b	9.91a	11.23a
PbSe ^{5.0}	8.65 ± 0.24a	0.97 ± 0.01b	9.62a	8.92b

^aValues refer to the mean followed by standard deviation. Means followed by the same letter in a column were not significantly different at P<0.05

Table 2: Influence of Se on levels of reduced and oxidized glutathione, and the ratios of reduced and oxidized glutathione in coleus roots exposed to Pb stress.

Pb+Se treatment (mM)	Angle	Intensity	Peak area	Full width at half maximum (FWHM)	Peak height
PbSe ^o	21.33	22.97	14.89	0.60	19.58
	27.12	29.20	15.93	0.53	25.81
PbSe ^{0.1}	21.4	22.94	24.68	0.445	20.61
	27.16	50.83	21.14	0.401	48.50
PbSe ^{0.5}	21.29	37.93	36.82	0.44	34.60
	27.09	72.41	33.37	0.43	69.08
PbSe ^{1.0}	21.36	19.05	11.24	0.59	16.01
	27.19	22.95	11.32	0.51	19.90
PbSe ^{2.5}	21.29	30.26	43.03	0.52	27.55
	27.09	58.14	27.53	0.43	55.43
PbSe ^{5.0}	21.47	12.74	27.06	0.66	10.59
	27.21	30.41	19.77	0.46	28.27

Table 3: XRD data analysis of Coleus blumei roots treated with different concentrations of Se under lead stress.



were greatly influenced by different concentrations of Se under Pb stress (Figure 5). The particle size and dispersion state in the leaves were uneven in the PbSe⁰ and PbSe^{0.1} treatments. The leaf particle morphology became flat in the PbSe^{0.5} treatment, while the flat particles had many folds, and their distribution was more uniform in the PbSe^{1.0} treatment. However, the morphology and particle size distribution were not uniform, and appeared to be in a highly gathered state for the PbSe^{2.5} and PbSe^{5.0} treatments (Figure 5). The peak number, diffraction angle and intensity in coleus leaves changed with the changes in Se concentration under Pb stress (Figure 6). There were 60 significant peaks that were mainly located at about $2\theta=9.22^{\circ}\sim52.1^{\circ}$ for the PbSe⁰ treatment, 63 significant peaks mainly located at about $2\theta = 9.95^{\circ} \sim 55.59^{\circ}$ for the PbSe^{0.1} treatment, 61 significant peaks mainly located at about 2=60.44°, 70.49° and 13.3°~ 55.42° for the PbSe^{0.5} treatment, 57 significant peaks mainly located at about $2\theta=10.27^{\circ}$ ~ 52.93° for the PbSe^{1.0} treatment, 54 significant peaks mainly located at about $2\theta = 10.11^{\circ} \sim 1.19^{\circ}$ for the PbSe^{2.5} treatment, and 72 significant peaks mainly located at about 20 0.86°~ 8.84° for the PbSe^{5.0} treatment (Figure 6). In comparison with the PbSe⁰ treatment, the position of the diffraction angle moved to the right for the different concentrations of Se, and the diffraction angle position moved larger for the PbSe^{0.5} treatment (Figure 6). Compared with the diffraction angle at 21.98° for the PbSe⁰ treatment, the diffraction angle was shifted to the left for the PbSe^{0.1} and PbSe^{0.5} treatments, and it moved to the right for the PbSe^{1.0}, PbSe^{2.5} and PbSe^{5.0} treatments, but the peak area and FWHM were reduced by different concentrations of Se (Table 4). The peak area and FWHM decreased at all concentrations of Se compared with the diffraction angles at 24.90° and 27.16° for the PbSe⁰ treatment (Table 4).

Discussion

Influence of Se on plant resource allocation and lipid peroxidation 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. Although Se has not yet been confirmed to be required by higher plants, some studies demonstrate that at low concentrations it may exert diverse beneficial effects, including growth-promoting activities [5,25]. Our results showed that in plants exposed to Pb, Se promoted the increase in organ biomass of coleus at concentrations up to 1.0 mM, whereas organ biomass was greatly decreased when 2.5 or 5.0 μ M Se was added (Figure 1). This



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Figure 5: SEM images of *Coleus blumei* leaves under Pb stress treated with different concentrations of Se.

Pb+Se treatment (mM)	Angle	Intensity	Peak area	Full width at half maximum (FWHM)	Peak height
PbSe ^o	21.98	16.86	24.14	1.97	13.58
	24.90	14.37	11.50	1.26	11.09
	27.16	14.34	8.08	0.93	11.06
PbSe ^{0.1}	21.87	20.78	11.26	0.65	17.91
	24.87	16.30	15.52	1.27	13.43
	27.09	16.03	9.50	0.88	13.17
PbSe ^{0.5}	21.90	10.27	7.14	1.05	7.62
	24.90	7.59	1.90	0.4	4.94
	27.31	10.73	4.91	0.78	8.07
PbSe ^{1.0}	22.00	16.21	20.63	1.88	12.92
	24.83	12.35	5.93	0.71	9.07
	27.19	13.15	8.21	1.07	9.87
PbSe ^{2.5}	22.02	21.88	11.33	0.66	17.90
	24.71	17.41	11.57	0.92	13.44
	27.06	22.88	18.80	1.46	18.91
PbSe ^{5.0}	22.2	8.26	4.57	0.81	6.08
	24.65	6.84	2.73	0.6	4.67
	27.37	7.34	4.18	1.06	5.16

Table 4: XRD data analysis of Coleus blumei leaves treated with different concentrations of Se under lead stress.

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Figure 6: XRD patterns of Coleus blumei leaves treated with different concentrations of Se under lead stress. Blue vertical bars indicate the main diffraction peaks.

was considered to be due to Se toxicity, because retardation of coleus growth was one of the symptoms noted when coleus was grown in the presence of high levels of Se. However, organ biomass was reduced by the simultaneous addition of 1.0 mM Pb and 2.5 or 5.0 μ M Se (Figure 1), but it was still increased by the combined effect of Pb addition and high Se supply levels, compared with no Se addition in plants exposed to Pb. This result indicated that Se has either stimulating or toxic effects on coleus depending on the concentration in the culture media. This finding is in agreement with a number of recent reports on plants such as Spirulina platensis [26] and Vicia faba L. [27]. According to the results obtained in this study, coleus can accumulate Pb efficiently during cultivation, and the accumulated amount increased with the increasing concentrations of Se (≤ 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 roots and stem (Table 1), which may suggest a greater demand for Se in the roots to counteract the toxic effects of Pb. Nevertheless, the molecular and

physiological mechanisms responsible of this phenomenon will require further investigation. These results for coleus are similar to those reported by Zembala et al. [28], who found that Se addition significantly decreased the Cd concentration of rape and wheat seedlings exposed to Cd stress. The Se contents in coleus tissues differed significantly in all treatments, and were roughly proportional to the concentrations of Se applied to the plants (Table 1). The allocation of Se in coleus organs increased effectively with increasing Se dosages (≤ 2.5 mM; Table 1). Nevertheless, the effects on roots and stems can be different; the highest accumulation of Se in roots was in the PbSe^{2.5} treatment, but in the stem it was in the PbSe^{1.0} treatment (Table 1). This result was in good agreement with Krystofova et al. [29] who showed that a higher amount of Se was detected in the root of Urtica dioica L. Despite a marked 32% decrease in TBARs, and the maximal decrease in TBARs was observed at 0.98 mg Se g⁻¹ DW in the roots for the PbSe^{2.5} treatment, the increase in lipid peroxidation for the PbSe5.0 treatment was lower than that of the PbSe⁰ treatment (Table 1). This was supported by inhibition of

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TBARs induction, which is similar to the data of Srivastava et al. [6] for *Pteris vittata* L. In contrast, the increase in TBARs accumulation for the PbSe5.0 treatment indicated that Se may act as a pro-oxidant in coleus as reported previously by Mroczek-Zdyrska and Wojcik [27].

To sum up, the above findings indicate that coleus plants treated with Se appeared to shift their biomass and metals distribution more to the roots than shoots, and had reduced TBARs content in roots, which has been considered to be a plant defense strategy [30].

Influence of Se on antioxidant defense systems during exposure to Pb stress

Plants possess a complex ROS-scavenging system that includes several antioxidant enzymes and low-molecular-weight antioxidants such as ascorbate, glutathione and phenolic compounds [31]. SOD acts as the first line of defense against ROS by catalyzing the dismutation of superoxide radicals (O_2^{-}) to H_2O_2 and molecular oxygen [32]. It is remarkable that the activity of SOD in coleus roots greatly decreased in the PbSe^{1.0} treatment compared to the PbSe0 treatment (Figure 2A), and this decrease coincided with a reduction of damage to cell membranes (Figure 2A, Table 1). This result suggested that, at these concentrations, Se was able to diminish the need of SOD by reducing the level of toxic O₂. in the roots of Pb-stressed coleus, which was similar to the findings of Singh et al. [30] in Najas indica. CAT and POD are the major enzymes involved in H2O2 detoxification. Application of Se, combined with Pb stress, significantly increased the activity of CAT in this study, especially in the PbSe^{5.0} treatment (Figure 2B), which indicated a protective role for Se in scavenging H₂O₂ in coleus roots under Pb stress. Similar to our results, increased CAT activity in Se-supplemented plants under cadmium, high temperature, and salt stresses have been described by other researchers [25,33]. However, POD activity in roots exposed to Pb stress decreased with increasing Se concentration (\leq 1.0 mM), but it is noteworthy that the POD activity increased in the PbSe^{2.5} and PbSe^{5.0} treatments (Figure 2D), which seemed to reflect increased hydrogen peroxide (H2O2) production at higher Se supply levels. GPX is another enzyme that uses GSH to reduce H₂O₂, and therefore, protects plant cells from damage due to oxidative stress [34]. In comparison to the PbSe⁰ control, the combination of Pb stress and moderate Se resulted in a significant increase in the activities of GPX, but this increase was not significantly different from that seen at higher Se concentrations (Figure 2C). Similar increases in GPX activity after Se supplementation during stress were observed by other researchers [8]. Also, opposing trends between SOD-POD activities and CAT-GPX activities were observed. 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 are scavengers of H₂O₂ and lipid hydroperoxides, resulted in reduced formation of superoxide anion radicals through dynamic inter-transformation among oxygen species. On the other hand, Se increased GPX activities and enhanced the spontaneous disproportion of superoxide radicals and, consequently, reduced the need for the scavenger SOD. These results indicated that the prevention of damage to cell membranes in coleus can be achieved by co-operative effects of the whole system of antioxidant enzymes. GSH can react chemically with singlet 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 is correlated with the change in GSH/GSSG ratios, which play an important role in the signal transduction of several transcriptional and metabolic processes [35,36]. Selenium is an important element for antioxidant reactions in organisms. Se acts as an antioxidant at low concentrations, but as a pro-oxidant at higher concentrations. The Pb-stressed coleus with Se supplements showed a higher increase in the level of GSH than did the coleus subjected to the PbSe0 control treatment (Table 2). The observed increase in GSH in Se-treated coleus roots could be due to Se boosting GSH synthesis. This was supported by Anderson et al. [37], who showed that Se accelerated efficient recycling of GSH, and reported the relationship between Se and GSH synthesis. In our study, Pb-stressed coleus plants treated with Se showed lower GSSG levels 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, Pbstressed coleus also provides a clear demonstration of the role of Se in Pb tolerance. Similar results were obtained by Hasanuzzaman et al. [33] in salt-stressed *Brassica napus* cv. Bina supplied with Se. From the above results, coleus plants exposed to Pb stress and treated with Se could be protected from the effects of Pb toxicity by altering various metabolic processes with the involvement of the innate antioxidant defense systems.

Influence of Se on SEM and XRD patterns of coleus under Pb stress

The X-ray diffraction patterns of the samples give valuable information about structural aspects of the coleus. The SEM photographs of coleus provide evidence about the grain size and surface morphology. In this study, treatment with different concentrations of Se had a significant influence on the degree of dispersion and aggregation in coleus roots and leaves (Figure 3 and 5). The particles in the roots and leaves were distributed into flat, folded shapes and were more uniform for the PbSe^{1.0} treatment, while for the PbSe^{2.5} and PbSe^{5.0} treatments, they were distributed differently and appeared to be in a highly gathered state (Figure 3 and 5). This might be due to changes in some physical and chemical characteristics or PbSe particles being formed during the Se treatment under Pb stress, thus affecting the SEM images. These results are consistent with reports by Achimovicova et al. [38] and Kumar et al. [39]. On the other hand, there can be differences between elements in the special morphology of roots and matrix composition with increasing Se concentration. Wojcik et al. [40] reported that the alterations of the root structure might influence redistribution and accumulation of the metal in different root tissues and/or cell compartments.

However, the nature of these particles needs to be further analyzed in future studies. In comparison with the PbSe⁰ treatment, the XRD diffraction angle in the roots and leaves of coleus was shifted to the right, and the peak intensity, height, area and FWHM decreased in the PbSe^{1.0} treatment (Figure 4 and 6). Our results indicated that 1.0 mmol/L Se treatment could be a favorable concentration to alleviate Pb stress in coleus, but the crystal formations, existence form and their function to ease in the coleus by Se treatment under Pb stress still remain to be further research.

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