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Research article

Effect of lead on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus*Gagan Preet Singh Sidhu ^{a,*}, Harminder Pal Singh ^a, Daizy R. Batish ^b, Ravinder Kumar Kohli ^c^a Department of Environment Studies, Panjab University, Chandigarh 160014, India^b Department of Botany, Panjab University, Chandigarh 160014, India^c Central University of Punjab, Mansa Road, Bathinda 151001, India

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ABSTRACT

A greenhouse experiment was conducted to assay the effect of Lead (Pb) on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus* after 6 weeks. Results revealed a good Pb tolerance and accumulation potential of *C. didymus* towards the increasing Pb concentrations (500, 900, 1800, 2900 mg kg⁻¹) in soil. The content of Pb in roots and shoots elevated with higher Pb levels and reached a maximum of 3684.3 mg kg⁻¹ and 862.8 mg kg⁻¹ Pb dry weight, respectively, at 2900 mg kg⁻¹ treatment. Pb exposure stimulated electrolyte leakage, H₂O₂ level, MDA content and the activities of antioxidant machinery (SOD, CAT, APX, GPX and GR). However, at the highest Pb concentration, the activities of SOD and CAT declined. The H₂O₂ level and MDA content in roots increased significantly up to ~500% and 213%, respectively, over the control, at 2900 mg kg⁻¹ Pb treatment. Likewise, concurrent findings were noticed in shoots of *C. didymus*, with the increasing Pb concentration. The present work suggests that *C. didymus* exhibited a good accumulation potential for Pb and can tolerate Pb-induced oxidative stress by an effective antioxidant defense mechanism.

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1. Introduction

Rapid industrialization, injudicious use of agricultural fertilizers, faulty mining activities, and improper management of solid wastes pose a serious threat to the terrestrial ecosystems due to the persistent release of toxic heavy metals (Dinakar et al., 2008). Lead (Pb), a heavy metal, has no biological role in plants and its toxicity can cause physiological, biochemical and morphological alterations like seed dormancy, stunted growth and reduced chlorophyll content in plants (Shahid et al., 2012). Agency for Toxic Substances and Disease Registry (ATSDR, 2005) has listed Pb, as the number one heavy metal pollutant. In soil, concentration of Pb more than 30 ppm can cause toxicity in large number of plant species (Ruley et al., 2004; Xiong, 1997). Exposure to Pb at higher level induces

oxidative burst in plants by generating reactive oxygen species (ROS) and causes death of the plant cell. Pb-induced toxicity promotes the accumulation of superoxide ion (O₂^{•-}), hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂), that inhibits the activity of enzymes, leading to peroxidation of lipids in the cells (Israr et al., 2011; Kaur et al., 2015).

The cell organelles of the plants have developed certain antioxidative response to combat the effect of ROS, generated due to oxidative stress. The response of antioxidant enzymes helps to measure the tolerance potential of a plant species towards oxidative stress. Various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR) have the potential to counteract the effect of ROS (Shakoor et al., 2014). Superoxide dismutase (SOD) sequesters the noxious superoxide ion (O₂^{•-}) by breaking it into H₂O₂ and oxygen. H₂O₂ is further subsequently detoxified with the help of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) that convert H₂O₂ into water and molecular oxygen. Another crucial antioxidant enzyme glutathione reductase (GR) balances the GSH/GSSG ratio in the cells and helps to combat oxidative stress (Qiu et al., 2008).

Abbreviations: H₂O₂, hydrogen peroxide; O₂^{•-}, superoxide ion; ¹O₂, singlet oxygen; ROS, reactive oxygen species; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPX, guaiacol peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase; GSSG, oxidized glutathione; GSH, reduced glutathione.

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In order to minimize the hazardous impact of heavy metals on the environment some low cost and viable methods like phytoremediation must be employed. Thus, it is pertinent to investigate certain wild plant species that can be used for remediation purposes. Therefore, *Coronopus didymus*, a wild annual herb of Brassicaceae, was selected for the present study. It grows along the roadsides and gardens during winters (October–February) in northern parts of India. The purpose of the work is to evaluate and assess: (i) the accumulation of Pb in plant tissue, its effect on biomass and ROS generation, (ii) the effect of different concentrations of Pb (500–2900 mg kg⁻¹) on antioxidative response in roots and shoots of *C. didymus* and (iii) the tolerance mechanism of plant correlated with the increased Pb concentrations. The present work will be helpful to evaluate the detoxification mechanism adopted by *C. didymus* in Pb-contaminated soils.

2. Materials and methods

2.1. Plant material and Pb treatments

The seeds of *C. didymus* were collected locally from a non-contaminated site at Panjab University campus, Chandigarh, India. Seeds were surface cleaned, disinfected, sterilized (sodium hypochlorite) followed by washing with distilled water. Seeds were grown in plastic tray having 10 kg soil in a screenhouse. After 15 day, the seedlings were rooted out carefully from the tray and transplanted in the plastic pots having 1 kg of soil with different treatments of Pb. Treatment of Pb in soil was given in the form of lead nitrate [Pb (NO₃)₂; MW = 331.21; purity: 99%; Merck Ltd., Mumbai, India]. The experimental set up consisted of 5 treatments, amended with 500, 900, 1800 and 2900 mg kg⁻¹ Pb and a parallel treatment without Pb amendment served as control. Four replicates (pots) were maintained for each Pb treatment having five plants per pot. After 6 weeks, plants were harvested for determination of biomass, H₂O₂, lipid peroxidation (in terms of MDA accumulation), Pb concentration and antioxidative response.

2.2. Plant biomass and metal accumulation

The plant material (roots and shoots) was dried in an oven at 75 °C for 72 h to determine the biomass. For determination of heavy metal content, the plant material (100 mg) was crushed and digested in 10 ml mixture of HNO₃/HClO₄ (4:1, v/v) and heated at a temperature of 150 °C until the formation of white fumes. Distilled water was added to raise the total volume of the digested material to 20 ml. The content of Pb in plant (roots and shoots) was determined by atomic absorption spectrophotometer (AAS).

2.3. Membrane integrity

The degree of membrane integrity loss was estimated by measuring the amount of electrolyte leakage (EL) as per the method given by Singh et al. (2007). The EL was calculated as: $EL = (E_1 - E_2) \times 100$, and expressed in terms of percent (%) value. E₁ was the initial conductivity of the bathing medium, whereas E₂ was the conductivity of the medium after boiling the contents for 30 min.

2.4. Detection of H₂O₂ and MDA content

To determine the H₂O₂ and MDA content, 100 mg of treated and untreated roots and shoots were crushed in 10 ml of 0.1% TCA (trichloroacetic acid) in pre-chilled mortar and pestle and centrifuged at 12,000×g for 15 min at 4 °C using cold centrifuge (Sigma Inc., USA). The supernatant was stored at 4 °C for further

estimation.

The content of H₂O₂ was measured in the plant tissue as per Velikova et al. (2000). To 0.5 ml of supernatant (TCA extract) or distilled water (as blank), 0.5 ml of phosphate buffer (10 mM; pH = 7.0) and 1 ml of potassium iodide (1 M) solution was added. The absorbance of the reaction mixture was read at 390 nm and the content of H₂O₂ was calculated using an extinction coefficient ($\epsilon = 0.28 \mu\text{M}^{-1} \text{cm}^{-1}$) and expressed in terms of nmol g⁻¹ f wt.

Lipid peroxidation of the plant tissue was measured in terms of malondialdehyde content as per Heath and Packer (1968). To 1 ml of supernatant, 4 ml of 0.5% TBA in 20% TCA was added. The mixture was heated at 95 °C for 30 min, cooled over ice, followed by centrifugation at 10,000×g for 10 min. The absorbance of the homogenized mixture was read at 532 nm, and corrected at 600 nm for non-specific absorbance. The MDA content was calculated using an extinction coefficient ($\epsilon = 155 \text{mM}^{-1} \text{cm}^{-1}$) and expressed as nmol g⁻¹ f wt.

2.5. Assays for antioxidative enzymes

2.5.1. Extraction of enzymes

100 mg plant tissue (roots and shoots) was crushed in 10 ml of 100 mM phosphate buffer (pH = 7.0) using pre-chilled mortar and pestle and centrifuged at 15,000×g for 25 min at 4 °C. The supernatant was collected and stored at 4 °C until use.

2.5.2. Antioxidant enzymes activities

The SOD activity was measured to quantify the ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) as per the method of Beauchamp and Fridovich (1971). To 1.8 ml reaction mixture [containing 50 mM phosphate buffer (pH = 7.4), 13 mM methionine, 0.1 mM EDTA, 13 μM Riboflavin, 0.05 M Na₂CO₃ and 63 μM NBT], 0.2 ml of enzyme extract or distilled water (as control) was added. Reaction tubes were kept under two 15 W fluorescent lamps for 15 min for the development of blue color and later these test tubes were transferred in the dark in order to stop the reaction. The other set of reaction tubes were kept in the dark. The non-irradiated samples did not develop any color and served as control. The OD (optical density) of both irradiated and non-irradiated samples was measured at 560 nm and the specific enzymatic activity was determined in terms of EU (enzyme unit) mg⁻¹ protein. One unit of SOD represented the quantity of enzyme required for 50% inhibition in NBT photoreduction.

The CAT activity was determined in terms of the rate of H₂O₂ disappearance and was determined as per the method given by Cakmak and Marschner (1992). To 1.8 ml reaction mixture [containing 25 mM phosphate buffer (pH = 7.0) and 10 mM H₂O₂], 0.2 ml of enzyme extract was added. The increase in OD was measured at 240 nm for 1 min. The CAT activity was calculated by using an extinction coefficient ($\epsilon = 39.4 \text{mM}^{-1} \text{cm}^{-1}$) and expressed as EU mg⁻¹ protein.

The activity of APX was measured by the method given by Nakano and Asada (1981). To 1.8 ml reaction mixture [containing 25 mM phosphate buffer (pH = 7.0), 0.1 mM EDTA, 0.25 mM ascorbic acid and 1.0 mM H₂O₂], 0.2 ml of enzyme extract was added. The decrease in the absorbance was measured at 290 nm for 1 min. The activity of enzyme was calculated using an extinction coefficient ($\epsilon = 2.8 \text{mM}^{-1} \text{cm}^{-1}$) and expressed as EU mg⁻¹ protein.

The activity of GPX was determined in terms of oxidation of guaiacol as per the method given by Eglely et al. (1983). To 1.8 ml of reaction mixture [containing 25 mM phosphate buffer (pH = 7.0), 0.05% guaiacol and 1 mM H₂O₂], 0.2 ml of enzyme extract was added. The increase in the OD was measured at 470 nm and the enzyme activity was calculated using an extinction coefficient

($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as EU mg^{-1} protein.

The specific activity of GR was determined as per the method given by Foyer and Halliwell (1976). To 1.8 ml of reaction mixture [containing 25 mM phosphate buffer (pH = 7.0), 0.1 mM EDTA, 0.5 mM glutathione oxidized (GSSG) and 0.12 mM nicotinamide adenine dinucleotide phosphate (NADPH)], 0.2 ml of enzyme extract was added. The change in the absorbance was read at 340 nm after 5 min. The specific activity of GR was measured in terms of oxidation of NADPH, using an extinction coefficient ($\epsilon = 6.224 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as EU mg^{-1} protein.

2.6. Statistical analysis

The experiment was conducted in a completely randomized design with four replicates (pots) for each treatment, including control. The data on H_2O_2 and MDA content contained five replicates and were analyzed by linear regression models. The enzyme assays contained five replicates for each treatment. The data were analyzed by one-way analysis of variance (ANOVA), and then treatment means were compared after applying *post hoc* Tukey's test at $P \leq 0.05$.

3. Results

3.1. Effect on biomass and Pb accumulation in plant tissue

After 6 weeks, an increase ($P \leq 0.05$) in plant biomass was observed in response to Pb treatment. However, at 2900 mg kg^{-1} Pb, the biomass declined suddenly (Table 1). The root biomass increased ($P \leq 0.05$) by ~ 10%, 13%, 19% and 6%, respectively, whereas shoot biomass was increased ($P \leq 0.05$) by ~ 9%, 14%, 28% and 6%, respectively, compared to the control at a concentration range from 500 to 2900 mg kg^{-1} Pb. The content of Pb in roots and shoots of *C. didymus* increased gradually in a concentration-dependent manner. After 6 weeks, the Pb content at 2900 mg kg^{-1} Pb treatment was observed to be $3684.3 \text{ mg kg}^{-1}$ and 862.8 mg kg^{-1} , respectively, in roots and shoots of *C. didymus*, which was ~700 and 297 fold greater than the control (Table 1). After 6 weeks, extraction of Pb by the plant tissues dramatically declined the concentrations of Pb in the soil (Table 1).

3.2. Effect on membrane integrity (measured in terms of relative electrolyte leakage, REL)

Membrane integrity was assessed on the basis of electrical conductivity (EC) of the medium containing ions leaked from roots and shoots of plant at the time of disruption of membrane integrity. Exposure of Pb caused ~21%, 37%, 53% and 78% increase in REL in roots of *C. didymus* after 6 weeks of treatment with respect to control (Fig. 1a). Further, exposure of Pb enhanced REL in *C. didymus* shoots, thereby suggesting membrane disruption. The REL increased by ~62% over the control, upon treatment with

2900 mg kg^{-1} Pb (Fig. 1a).

3.3. Determination of H_2O_2 and MDA content in plant tissue

Pb exposure for 6 weeks caused a substantial increase in concentration of H_2O_2 in both roots and shoots of *C. didymus* (Fig. 1b). The effect was found to follow a dose-dependent trend. H_2O_2 content enhanced ($P \leq 0.05$) in the range of ~176–500% in roots and ~35–100% in shoots, respectively, after 6 weeks in response to 500– 2900 mg kg^{-1} Pb (Fig. 1b). MDA content in plant tissues was found to be highest at 2900 mg kg^{-1} Pb concentration. It increased up to ~213% and 180%, respectively, in roots and shoots compared to the control (Fig. 1c).

3.4. Antioxidant enzyme response

A gradual increase in the activity of SOD was measured in response to Pb treatment (Fig. 2a). SOD activity was enhanced up to ~228% and 144% in roots and shoots, respectively, over the control on exposure to 1800 mg kg^{-1} Pb (Fig. 2a). However, a slight decrease in SOD activity was noticed at 2900 mg kg^{-1} Pb concentration (Fig. 2a). A concentration-dependent increase in CAT activity was observed in both roots and shoots of *C. didymus* and reached the maximum at 1800 mg kg^{-1} Pb treatment (Fig. 2b). After 6 weeks, maximum CAT activity in roots and shoots peaked up to 69% and 41%, respectively, at 1800 mg kg^{-1} Pb treatment compared to the control (Fig. 2b). However, at 2900 mg kg^{-1} Pb concentration, CAT activity in roots and shoots declined slightly and reached up to 49% and 40%, respectively (Fig. 2b). The activity of APX was elevated ($P \leq 0.05$) upon treatment with increasing levels of Pb concentration. At 2900 mg kg^{-1} Pb exposure, APX activity increased to about 1053% and 209% in roots and shoots, respectively, over the control (Fig. 2c). The GPX activity followed a similar trend in response to Pb treatment, after 6 weeks. GPX activity was noticed to be enhanced more in roots than shoots (Fig. 2d). Pb exposure at 2900 mg kg^{-1} increased the activity of GPX by ~222% and 95%, respectively, over the control, in roots and shoots of *C. didymus* (Fig. 2d). Similar to APX and GPX, GR activity elevated significantly ($P \leq 0.05$) with increasing Pb treatments. In roots and shoots, GR activity was higher by ~543% and 60%, respectively, over the control after 6 weeks (Fig. 2e).

4. Discussion

There has been a continuous search for finding wild plant species having potential to accumulate heavy metals in their tissues. Plant species related to family Brassicaceae are well known for efficient heavy metal accumulation in their tissues (Mourato et al., 2015). In this study, *C. didymus* was selected to evaluate its accumulation and tolerance potential mechanisms for Pb after 6 weeks. Exposure to increased Pb concentration (500– 1800 mg kg^{-1}) enhanced the biomass yield. This may be due to the increase in the

Table 1
Biomass, Pb content in plant tissues and residual Pb content in soil after 6 weeks of Pb treatment.

Pb in soil (mg kg^{-1})	Biomass (g)		Pb content (mg kg^{-1} DW)		Residual Pb content in soil (mg kg^{-1} DW)
	Root	Shoot	Root	Shoot	
0	0.191 ± 0.003a	0.363 ± 0.003a	5.26 ± 0.40a	2.91 ± 0.58a	4.49 ± 0.54a
500	0.210 ± 0.001bc	0.397 ± 0.005bc	827.7 ± 4.52b	201.7 ± 2.63b	354.6 ± 4.79b
900	0.216 ± 0.001c	0.416 ± 0.006c	1423.7 ± 6.19c	317.0 ± 3.81c	601.2 ± 6.31c
1800	0.228 ± 0.004d	0.466 ± 0.004d	2576.4 ± 8.58d	463.0 ± 5.68d	1076.4 ± 8.83d
2900	0.203 ± 0.002b	0.386 ± 0.003b	3684.3 ± 14.11e	862.8 ± 7.20e	1856.2 ± 11.05e

Data presented as mean ± standard error.

Different alphabets within a column represent significance at $P \leq 0.05$ after applying *post hoc* Tukey's test.

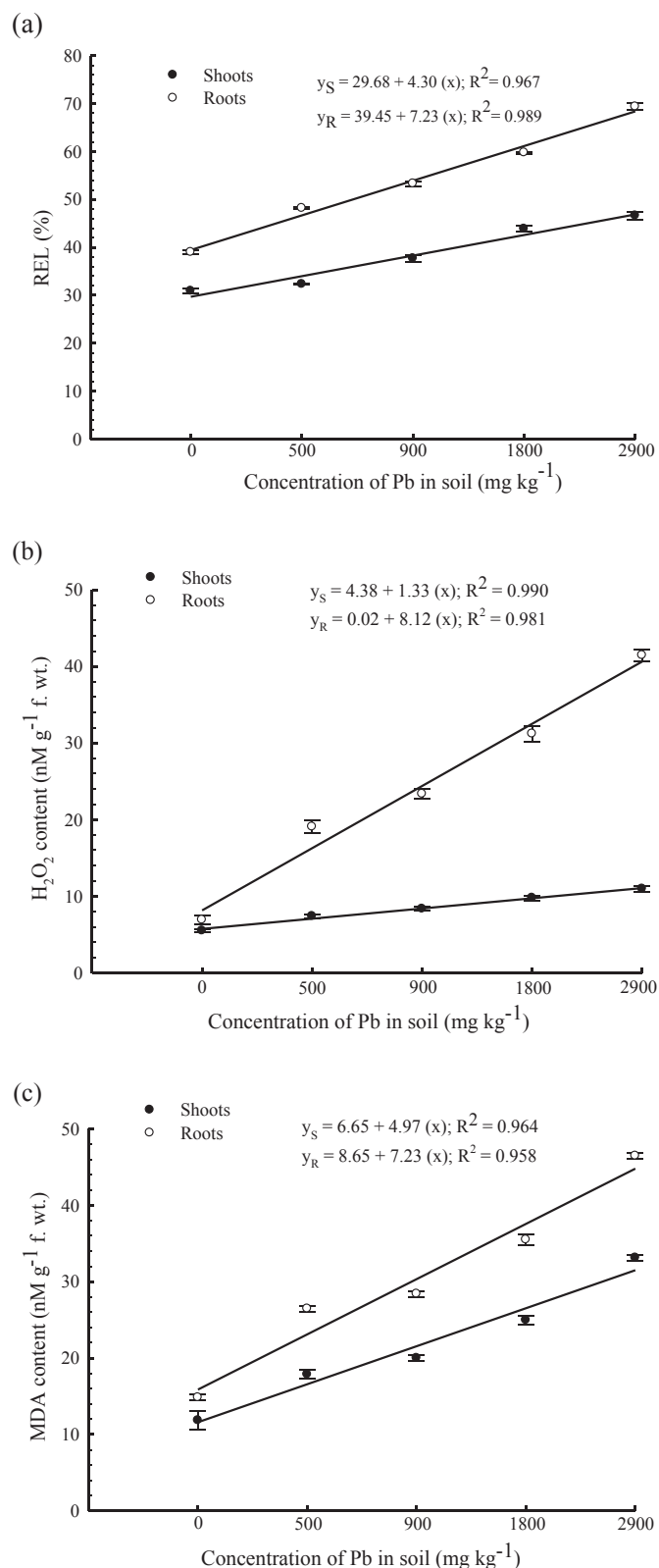


Fig. 1. Effect of Pb on (a) membrane integrity (measured as REL) (b) hydrogen peroxide (H₂O₂) content and (c) malondialdehyde (MDA) content in roots and shoots of *C. didymus* measured after 6 weeks of treatment. Vertical bars along each data point represent the standard error of the mean. Data were analyzed by linear regression. Black lines represent regression lines. R² represents correlation between the Pb concentrations in soil and % of REL, H₂O₂ and MDA content generated in roots and shoots of *C. didymus* at $P \leq 0.05$.

activity of lipase, protease, amylase and endosperm decomposition that strictly depend upon the plant species and stages of plant growth (Cheng, 2003). Earlier, Xiong (1998) reported increased root and shoot biomass in response to 500 $\mu\text{g g}^{-1}$ Pb treatment in *Brassica pekinensis*. Moreover, an increment in biomass compared to control was reported in *Sesbania drummondii* upon Pb²⁺ treatment (Ruley et al., 2004). Also Zhou et al. (2015) reported higher plant dry biomass in *Sophora japonica* and *Platycladus orientalis* for different Pb concentrations. However, the increase in biomass was less at the highest Pb treatment. It was noticed that with the increase in soil Pb levels, the metal accumulation in the roots and shoots of *C. didymus* was enhanced, thereby causing an inhibitory effect on biomass of *C. didymus*. The content of Pb in roots and shoots of *C. didymus* elevated significantly with increasing Pb treatments. In roots, Pb content was far above the threshold level (1000 mg kg^{-1}) at 900, 1800 and 2900 mg kg^{-1} Pb treatments. Similar observations were demonstrated by Singh et al. (2010) in an aquatic macrophyte *Najas indica* that accumulated 3554 $\mu\text{g g}^{-1}$ dw Pb in its tissues after 7 days of exposure.

Exposure to heavy metals induces the generation and accumulation of ROS that causes oxidative stress in plants (Schützendübel and Polle, 2002). Oxidative stress leads to reduced metabolism and greater accumulation of H₂O₂ in plant tissues. In the present work, higher Pb levels in soil enhanced the permeability of membranes, thus pointed loss of membrane integrity in roots and leaves of *C. didymus*. This may be attributed to the increased acclimatization of Pb in plant tissues. Our results were in line with the findings of Gajewska et al. (2012) and Kaur et al. (2012) that reported increased electrolyte leakage in wheat seedlings on exposure to Ni and Pb. Elevated H₂O₂ levels were observed in the roots and shoots of *C. didymus*. However, H₂O₂ level was more in roots than shoots. Similar findings were reported in *Vicia faba* (Pourrut et al., 2008), *Najas indica* (Singh et al., 2010) and in a Pb hyperaccumulator *Vallisneria natans* (Wang et al., 2012). The formation of noxious lipid peroxides from fatty acids may cause an increased MDA content in plant. Higher MDA accumulation in the plant tissue normally indicates severe lipid peroxidation. Elevated MDA levels promote oxidative stress by altering various physiological and biochemical parameters. MDA levels were found to be on the higher side in roots and shoots of *C. didymus* upon Pb treatment. Mishra et al. (2006) reported that 1–100 μM Pb treatment for 7 days led to an increase in MDA content in an aquatic hyperaccumulator, *Ceratophyllum demersum*. Further, Mahdavian et al. (2016) observed increased content of MDA and H₂O₂ in *Peganum harmala* under Pb exposure. Thus, Pb exposure induced generation and accumulation of ROS and MDA content in roots and shoots of *C. didymus*.

Antioxidant enzymes play a crucial role in quenching ROS that induces oxidative stress in plant cell. The generation of ROS on exposure to Pb (500–2900 mg kg^{-1}), triggered a strong antioxidative response in roots and shoots of *C. didymus*. An increase in the activity of antioxidative enzyme mechanism (SOD, CAT, APX, GPX and GR) was observed more in roots than shoots of *C. didymus* in a concentration-dependent manner. The upregulation in the activity of antioxidant enzymes depicts its role in detoxification of ROS. SOD helps in catalyzing superoxide ($\text{O}_2^{\cdot-}$) radical to hydrogen peroxide and oxygen. A gradual increase in SOD activity (500–1800 mg kg^{-1}) was observed in roots and shoots of *C. didymus*. Similar results were reported for *Sedum alfredii* (Gupta et al., 2010), *Sesbania drummondii* (Israr et al., 2011), *Triticum aestivum* (Kaur et al., 2012) and *Vallisneria natans* (Wang et al., 2012). However, SOD activity was slightly reduced at 2900 mg kg^{-1} Pb treatment. This may be correlated with the increased formation of H₂O₂ and other toxic ROS derivatives that induce inhibition in SOD activity in cell organelles as revealed in *Eichhornia crassipes* (Malar et al., 2014) and *Ceratophyllum demersum* (Mishra et al., 2006).

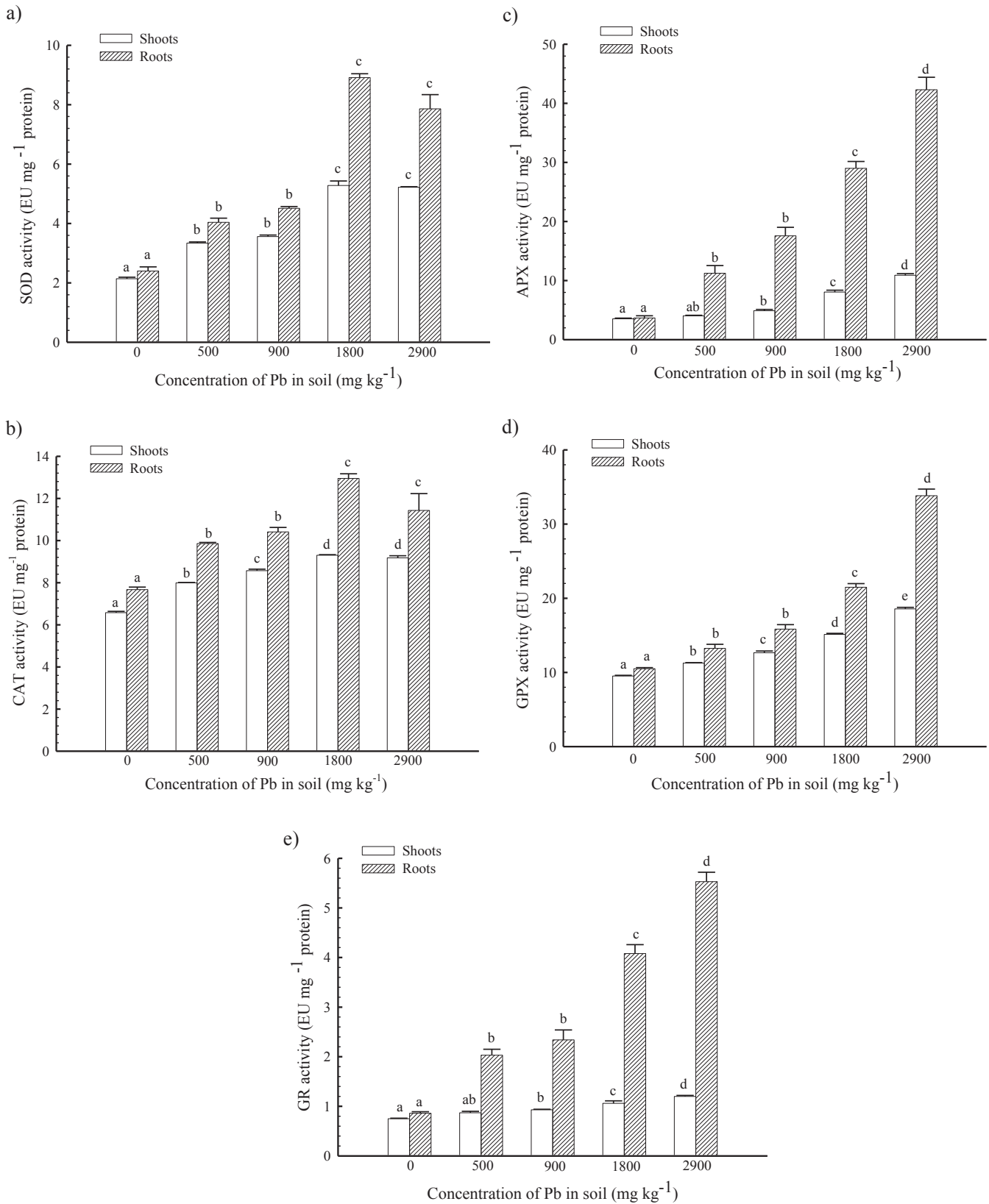


Fig. 2. Effect of Pb on activities of antioxidant enzymes, (a) superoxide dismutases (SOD) (b) catalases (CAT) (c) ascorbate peroxidases (APX) (d) guaiacol peroxidases (GPX) (e) glutathione reductases (GR) in roots and shoots of *C. didymus* measured after 6 weeks of treatment. Different alphabets represent significance at $P \leq 0.05$, after applying *post hoc* Tukey's test.

CAT is a key enzyme and plays an important role to scavenge H₂O₂ in peroxisomes. Both roots and shoots of *C. didymus* showed higher

CAT activity at 500–1800 mg kg⁻¹ Pb exposure. However, at 2900 mg kg⁻¹ Pb treatment, CAT activity was declined. This can be

attributed to the increased production of H₂O₂ in the plant tissues at higher Pb levels that may likely to suppress the CAT activity. Our results are concordant to the findings of (Qiu et al., 2008), who reported a similar trend in CAT activity in *Arabidopsis paniculata* under Cd stress. APX, another important enzyme scavenges H₂O₂ more efficiently than CAT. In chloroplasts, CAT is absent and breakdown of H₂O₂ is carried out by APX by utilizing ascorbate into H₂O and O₂. In the present work, APX activity was increased significantly in roots at 2900 mg kg⁻¹ Pb treatment. Likewise, a parallel enhancement in the activity of APX was noticed in shoots of *C. didymus*. This may be correlated with the increased accumulation of ROS that induce oxidative stress in roots and shoots. Similar findings were observed for *E. crassipes* in which APX activity was elevated at 1000 mg l⁻¹ Pb concentration (Malar et al., 2014). Exposure to Pb with different concentrations induce similar results in *Sedum alfredii* (Gupta et al., 2010), *Wolffia arrhiza* (Piotrowska et al., 2009) and *Cassia angustifolia* (Qureshi et al., 2007).

GPX, a stress marker, has higher potential to scavenge H₂O₂. It is located in cytoplasm and vacuoles of the plant cell. A substantial increase in GPX activity was found in roots and shoots of *C. didymus* in a concentration-dependent manner. Previous works have demonstrated that Pb toxicity induce enhanced GPX activity in *Phaseolus vulgaris* (Geebelen et al., 2002), *S. drummondii* (Ruley et al., 2004) and *Zea mays* (Kaur et al., 2015). GR is a crucial enzyme in Halliwell–Asada enzyme pathway, as it is involved in the rejuvenation of GSH (Apel and Hirt, 2004). Under normal conditions higher ratio of GSH/GSSG regulates the functioning of GR. Enhanced GR activity promotes the supply of GSH ascorbate-glutathione cycle. A positive increase in GR activity was observed in both roots and shoots of *C. didymus* with increasing Pb concentrations. Similar increasing trend in GR activity was observed in *Cassia angustifolia* (Qureshi et al., 2007) and *Macrotyloma uniflorum* and *Cicer arietinum* on Pb exposure (Reddy et al., 2005).

5. Conclusions

In the present work, *C. didymus* was tested for the first time in terms of its ability to withstand Pb toxicity. Exposure to Pb for 6 weeks significantly enhanced the accumulation of Pb especially in roots of *C. didymus*. The roots of the plant accumulated greater amount of Pb compared to the shoots. The results revealed proportionality between Pb-induced oxidative stress with increased Pb concentrations. The stimulation of antioxidative response in *C. didymus* on exposure to Pb treatment (500–2900 mg kg⁻¹) is likely to withstand and protect the plant from oxidative stress, induced by Pb toxicity. However, SOD and CAT activity was suppressed at highest Pb concentration. The findings suggest that *C. didymus* has an exceptional ability to withstand and tolerate Pb-induced oxidative stress. Evoked antioxidative response (CAT, SOD, APX, GPX and GR activities) clearly revealed the effective plant mechanism to suppress ROS under peaked Pb concentrations. Thus, *C. didymus* has emerged as a promising wild candidate for future, to mitigate Pb from polluted soils.

Author contribution

H.P. Singh and D.R. Batish designed the work. Gagan Preet Singh Sidhu conducted experiments, collected and analyzed the data. H.P. Singh, D.R. Batish, Gagan Preet Singh Sidhu and R.K. Kohli contributed equally to the write up of this manuscript.

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