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Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm. (Brassicaceae)

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ABSTRACT

The potential of a wild, unpalatable plant *Coronopus didymus* was investigated for the first time in terms of its capability to tolerate and accumulate cadmium (Cd) for phytoremediation purposes. A screenhouse experiment for 6 weeks was conducted to evaluate the effect of Cd from 100 to 400 mg kg^{-1} on growth, biomass, photosynthetic apparatus, Cd uptake and accumulation in *C. didymus* plants. Application of Cd facilitates the growth of the plants whereas at higher levels a slight reduction was noticed. The concentration of Cd in roots and shoots reached a maximum of 867.2 and 864.5 mg kg^{-1} DW respectively, at 400 mg kg^{-1} Cd treatment. Cd exposure increased the generation of superoxide anion ($O_2^{\bullet-}$), H_2O_2 content, MDA level and antioxidative response (SOD, CAT and POD) in roots and shoots of *C. didymus*. However, a slight decline in SOD and CAT activities were noticed in roots at highest Cd treatment (400 mg kg^{-1}). The bioconcentration (BCF) values for all the concentrations were >1 and the translocation factor (TF) values were <1 at lower level but reached 1 at highest Cd concentration. Thus, *C. didymus* satisfies the conditions required for hyperaccumulator plants and may be practically employed to alleviate Cd from contaminated soils.

1. Introduction

Heavy metal contamination of environment is one of the most serious problems in the world (Liu et al., 2009; Zhang et al., 2013). It poses serious threats to plant, animal and human health by deteriorating the quality of water and soil. Anthropogenic activities like injudicious industrialisation, intensive agricultural activities and faulty mining practices have led to the dramatic increase in inorganic contaminants like cadmium (Cd) in the soil (Zhang et al., 2010). Cd is one of the major environmental pollutants and is known to contaminate the food chain. According to United Nations Environment Programme (UNEP), Cd entry in humans is mediated by food crops (UNEP, 2008). Presence of Cd in soil instigates toxicity in soils even at low concentrations (Sharma et al., 2010b). Cd has been listed seventh in the priority list of hazardous substances (Agency for Toxic Substances and Disease Registry (ATSDR), 2015). In plants, Cd has no physiological relevance and is readily taken up by the plants to toxic concentrations. Cd is known to impose toxic symptoms like leaf chlorosis, growth retardation, reactive oxygen species (ROS) generation and peroxidation of membrane lipids in plants (Li et al., 2012).

To overcome the Cd induced toxicity, plants have developed various defensive mechanisms to alleviate oxidative stress caused by ROS accumulation. These defensive mechanisms include physiological variations, morphological alterations and strong antioxidative responses. The physiological adaptations like cell wall immobilisation impart a crucial role in Cd tolerance and detoxification in plants. Presence of vacuolar compartmentalisation hinder free mobilisation of Cd in cytosol. In addition, synthesis of sulphur-rich peptides, phytochelatins (PCs) in response to Cd stress exhibit a decisive role in Cd detoxification by forming low molecular weight (LMW) and high molecular weight (HMW) complex with Cd (Stolt et al., 2003). Besides antioxidant enzymes play a consequential role to subsist oxidative stress (Sidhu et al., 2016). SOD is an indispensable enzyme that succour to disintegrate noxious superoxide anion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2)·H_2O_2 is further degenerated to innocuous forms like O_2 and H_2O by CAT and POD. The strong and efficacious response of antioxidant enzymes facilitates to assess the tolerance ability of a plant species towards oxidative stress (Sidhu et al., 2016).

Nowadays, it is critically essential to develop subsequent low-cost, ecologically sound approaches to mitigate higher levels of Cd from the

Abbreviations: BCF, bioconcentration factor; CAT, catalase; HMW, high molecular weight; H_2O_2, hydrogen peroxide; LMW, low molecular weight; MDA, malondialdehyde; PCs, phytochelatins; POD, peroxidases; ROS, reactive oxygen species; SOD, superoxide dismutase; $O_2^{\bullet-}$, superoxide ion; TF, translocation factor

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soils. Phytoremediation has become a promising, *in situ,* cost effective, green, and cleaner technology that employs hyperaccumulator plant species for treatment of contaminants (Datta and Sarkar, 2005). According to Cappa and Pilon-Smits (2014), hyperaccumulator plants have much stronger affinity to tolerate and grow on metal rich soils. Indeed, phytoextraction and phytostabilisation are the two vital strategies adopted by the plants to alleviate the metals and stabilise the polluted soils (Anjum et al., 2014). The success of a remediation system directly depends upon the potential of a plant species to tolerate and withstand heavy metal burdens during phytoextraction and phytostabilisation (Ali et al., 2013). Generally, the effectiveness of phytoremediation is evaluated by three factors: the biomass yield of the plant, bioconcentration factor and translocation factor. Therefore, it is pertinent to identify the potency of unexplored, high biomass yielding plant species for the successful phytoremediation of Cd-contaminated soils.

In the current work, a wild, unpalatable, annual herb *Coronopus didymus* was selected to examine its ability to tolerate and accumulate Cd from contaminated soils. *C. didymus* (35–45 cm) belongs to family Brassicaceae and is commonly known as lesser swine-cress. It is a native of South America and having a wide distribution throughout the world (Yannitsaros, 1986). It grows along the road sides and gardens during winter season (October to February) in the northern parts of India. The plant grows rapidly having profusely branched root and shoot system and less harvest time. *C. didymus* exhibited luxuriant growth and high biomass production especially after 6 weeks. Therefore the sampling period was selected for 6 weeks. To the best of our knowledge, no previous study has illustrated the tolerance and physiological impact of Cd accumulation on *C. didymus*. The main objectives of the present work were to evaluate (i) the growth, biomass and survival of *C. didymus* on exposure to varied Cd treatments (ii) the physiological response of the plants in Cd contaminated soils and (iii) the tolerance, uptake and extraction potential of *C. didymus* towards peaked Cd concentrations.

2. Materials and methods

2.1. Plant material and soil samples

The seeds of *C. didymus* and soil samples were collected locally from a non-contaminated site at Panjab University campus, Chandigarh, India. Sterilised seeds were sown in plastic tray having 10 kg soil in a screenhouse. Soil specimens were collected from peripheral layer (0–20 cm), mixed with manure, air dried and sieved through 2 mm mesh. The selected soil was sandy loam having pH 6.69 ± 0.07, electrical conductivity 139.9 ± 1.51 μS, organic carbon 0.98 ± 0.04 and organic matter content 1.68 ± 0.07. In polythene bags, 1 kg soil was filled and kept in plastic pots. The soils were amended by three levels of Cd (100, 200, 400 mg kg^{-1}), supplied as cadmium chloride [($CdCl_2$. $2^1/_2$ H_2O)]. Amended soils were incubated for 2 weeks.

2.2. Experimental setup

In a screenhouse under natural light conditions, 15 d old *C. didymus* plants having identical size were transplanted in Cd-amended soils. There were four replicates of each treatment arranged in a completely randomised design. Plants were harvested, 6 weeks after Cd application in the soil. Plant root-shoot tissues were rigorously rinsed with distilled water and oven dried at 75 °C for 72 h. The dried plant tissues were weighed, recorded, grounded to powder and sieved through 2 mm stainless mesh.

2.3. Analysis of Cd in the plant tissues

The concentration of Cd in plant tissues were determined by using aqua regia-$HClO_4$ digestion. Dried 100 mg plant samples were digested with 10 ml mixture of HNO_3/$HClO_4$ (4:1, *v/v*) at 150 °C. The concentration of Cd in digested plant tissues were determined by AAS (Atomic Absorption Spectroscopy) (Contra 700; Analytic Jena AG, Jena, Germany) and was calculated as mg kg^{-1} DW. The concentration of Cd in soil was determined by using DTPA extraction method (Lindsay and Norvell, 1978). Bioconcentration factor (BCF), Translocation factor (TF) play a key role to determine the potential of a plant species for remediation of metal-polluted soils. BCF and TF of Cd in *C. didymus* were determined as per the method given by (Pandey, 2012). BCF is the ratio of metal concentration in plant shoots to that in the soil. TF is the ratio of metal concentration translocated in shoots to that present in root part of the plants.

2.4. Analysis of photosynthetic pigments and photosynthetic efficiency (F_v/F_m)

Chlorophyll *a* and chlorophyll *b* content was measured from the leaves of the test species, as per Hiscox and Israelstam (1979) and was determined using the equation of Arnon (1949). Carotenoids content was determined as per the method of Lichtenthaler and Wellburn (1983). These were expressed on dry weight basis as suggested by Rani and Kohli (1991). F_v/F_m of PS II for both treated and control leaves was measured using OS-30p pulse modulated chlorophyll fluorometer (Opti Sciences, US).

$$\text{Chl } a = 10.63 \times A_{663} – 2.39 \times A_{645}$$

$$\text{Chl } b = 20.11 \times A_{645} – 5.18 \times A_{663}$$

$$\text{Carotenoids} = (1000 \times A_{470} – 3.27 \times \text{Chl } a – 104 \times \text{Chl } b)/227$$

where A_{663}, A_{645} and A_{470} represent extinction values at 663, 645 and 470 nm.

2.5. Estimation of Superoxide anion ($O_2^{\bullet -}$), H_2O_2 and MDA content

Superoxide anion, H_2O_2 and MDA content are the precursors of oxidative stress. The $O_2^{\bullet -}$ content was estimated following the method of Misra and Fridovich (1972). Root and shoot tissue (100 mg) was homogenised in 10 ml of PO_4^{3-} buffer (pH=7.0) and centrifuged at 12,000*g* for 15 min. To 0.2 ml of supernatant, 1.8 ml of reaction mixture [1 mM adrenalin solution prepared in 75 mM PO_4^{3-} buffer (pH=7.4), 0.2 ml of 75 mM PO_4^{3-} buffer (pH=7.4)] was added and amount of $O_2^{\bullet -}$ was calculated using an extinction coefficient (ε=4.02 mM^{-1} cm^{-1})at 480 nm and expressed in terms of μM g^{-1} f. wt.

To determine the H_2O_2 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 H_2O_2 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 H_2O_2 was calculated using an extinction coefficient (ε=0.28 μM^{-1} cm^{-1}) and expressed in terms of nM g^{-1} f. wt.

Lipid peroxidation of the plant tissue was measured in terms of MDA 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 homogenised mixture was read at 532 nm, and corrected at 600 nm for non-specific absorbance. The MDA content was calculated using an extinction coefficient (ε=155 mM^{-1} cm^{-1}) and expressed as nM g^{-1} f. wt.

2.6. Estimation of antioxidative response

Plant fresh weights were used for assaying the activities of SOD, CAT and POD towards Cd induced antioxidative response in *C.didymus*. 100 mg plant tissue (roots and shoots) was crushed in 10 ml of 0.1 M phosphate buffer, pH=7.0 using pre-chilled mortar and pestle and centrifuged at 15,000*g* for 25 min at 4 °C. The SOD activity was measured as per the method of Beauchamp and Fridovich (1971) and CAT activity was determined as per the method given by Cakmak and Marschner (1992) and was determined in terms of nkat sec^{-1} mg^{-1} protein. The POD activity was estimated according to the method described by Batish et al. (2006) and was expressed in terms of kat sec^{-1} mg^{-1} protein.

2.7. Statistical analysis

The data presented in the paper were means of four replicates. All the data were analysed by one-way analysis of variance (ANOVA), and then treatment means were compared after applying *post hoc* Tukey's test at $P \leq 0.05$. The data on H_2O_2, superoxide anion and MDA content were analysed by linear regression models. The statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Cd uptake and accumulation in plant tissues

After 6 weeks, the Cd concentration in roots and shoots of *C. didymus* increased linearly with the elevated Cd treatments amended in the soil (Table 1). There was a significant ($P \leq 0.05$) positive linear correlation between the Cd content in roots and shoots and concentrations of Cd present in the soil. This relation can be expressed by two regression equations:

$$Y_R = 3.16X + 128.01 (R^2 = 0.912,\ P = 0.023)$$

$$Y_S = 3.25X + 92.95 (R^2 = 0.952,\ P = 0.008)$$

Y_R and Y_S represents Cd content in roots and shoots, X represents the concentration of Cd in the soil and R^2 represents the correlation between the content of Cd in root-shoot tissues and concentration of Cd in the soil at $P \leq 0.05$. The content of Cd in roots and shoots reached a maximum of 867.2 mg kg^{-1} DW and 864.5 mg kg^{-1} DW respectively, at highest (400 mg kg^{-1}) Cd treatment (Table 1). In our results, a large amount of Cd was retained by the roots that were the primary sites for metal outburst. This might be attributed to the variety of mechanisms like sub-cellular compartmentalisation of metals in vacuoles that facilitate to restrict the excess Cd transport within the plants. Moreover, organic compounds like PCs might enable to sequester and accumulate Cd within the root cells as reported in a Cd hyper-accumulator *Arabis paniculata* (Zeng et al., 2009). *C. didymus* may employ these mechanisms to restrict the excess translocation of Cd, therefore protecting itself from Cd-induced toxicity. Similarly, high Cd concentrations were reported in roots of *Calendula officinalis* (Liu et al., 2008) and in *Brassica napus* (Ehsan et al., 2014). The metal concentration in upper aerial parts is a key factor for ascertaining the phytoextraction efficiency of the plants. *C. didymus* displayed a good tendency to translocate Cd from roots to the shoots. This might be due to the enhanced internal transport of aqueous free Cd ions, generally mediated by xylem loading that further being regulated by the endodermis and xylem flux (Uraguchi et al., 2009). Another reason probably due to the high mobility of Cd in the soils that facilitate its uptake in plants along the essential nutrients like Zn, Fe and Cu through membrane transporters (Zheng et al., 2011). Our results were consistent with the observations of Yang et al. (2004) and Wei et al. (2005) which reported elevated Cd levels in leaves of *Sedum alfredii* and shoots of *Solanum nigrum*. The Cd content in roots and shoots of *C. didymus* plants at all the treatments were well above the critical level for Cd hyperaccumulators (˃100 mg kg^{-1}). This might be due to the elevated biomass production at lower Cd levels, which in turn enhanced the surface area for Cd precipitation and adsorption, consequently facilitating its uptake and translocation in roots and shoots. Further *C. didymus* was able to tolerate Cd concentrations upto 200 mg kg^{-1} without showing any toxic symptoms. However, toxic symptoms like stunted growth, reduced biomass accompanied by enhanced oxidative stress were appeared at highest Cd treatment. This might be correlated with the increased Cd accumulation in the plant tissues that induce toxicity at 400 mg kg^{-1} Cd treatment. In the present work, *C. didymus* exhibited an extraordinary tolerance and accumulation potential for Cd. Relatively high concentration of Cd was accumulated in both roots and shoots of the plants. Moreover, unpalatability, high biomass yield and shorter life span make *C. didymus* a novel and efficient plant species that can be exploited for Cd extraction from the polluted soils. These findings strongly marked the potential of *C. didymus* for both phytostabilisation and phytoextraction of Cd from the polluted soils.

Table 2
Growth and biomass alterations in *C. didymus* plants after 6 weeks of Cd exposure.

Concentration (mg kg^{-1} Cd dry weight soil)	Root		Shoot	
	Growth (cm)	Biomass (g)	Growth (cm)	Biomass (g)
0	15.43 ± 0.15b	0.191 ± 0.004a	12.68 ± 0.44a	0.354 ± 0.005b
100	18.12 ± 0.20c	0.214 ± 0.005b	13.66 ± 0.57a	0.365 ± 0.005b
200	19.72 ± 0.35d	0.245 ± 0.005c	17.42 ± 0.41b	0.376 ± 0.005b
400	13.72 ± 0.55a	0.179 ± 0.003a	12.62 ± 0.17a	0.288 ± 0.009a

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.

3.2. Bioconcentration factor (BCF) and Translocation factor (TF)

BCF and TF are the two important factors required to evaluate the

Table 1
Root-Shoot Cd content, Bioconcentration factor (BCF), Translocation factor (TF) in *C. didymus* and remnant soil Cd content in response to treatment with a range of Cd concentrations after 6 weeks.

Concentration (mg kg^{-1} Cd dry weight soil)	Root (mg kg^{-1} DW)	Shoot (mg kg^{-1} DW)	BCF C_{shoot}/C_{soil}	TF C_{shoot}/C_{root}	Remnant Cd content in soil (mg kg^{-1} Cd dry weight soil)
0	3.1 ± 0.04a	2.4 ± 0.16a	0.91	0.78	1.1 ± 0.27a
100	383.6 ± 10.02b	312.0 ± 7.33b	3.12	0.81	83.9 ± 4.35b
200	522.0 ± 21.57c	493.8 ± 5.10c	2.50	0.95	171.7 ± 11.93c
400	867.2 ± 6.92d	864.5 ± 5.16d	2.16	1.00	356.6 ± 15.44d

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.

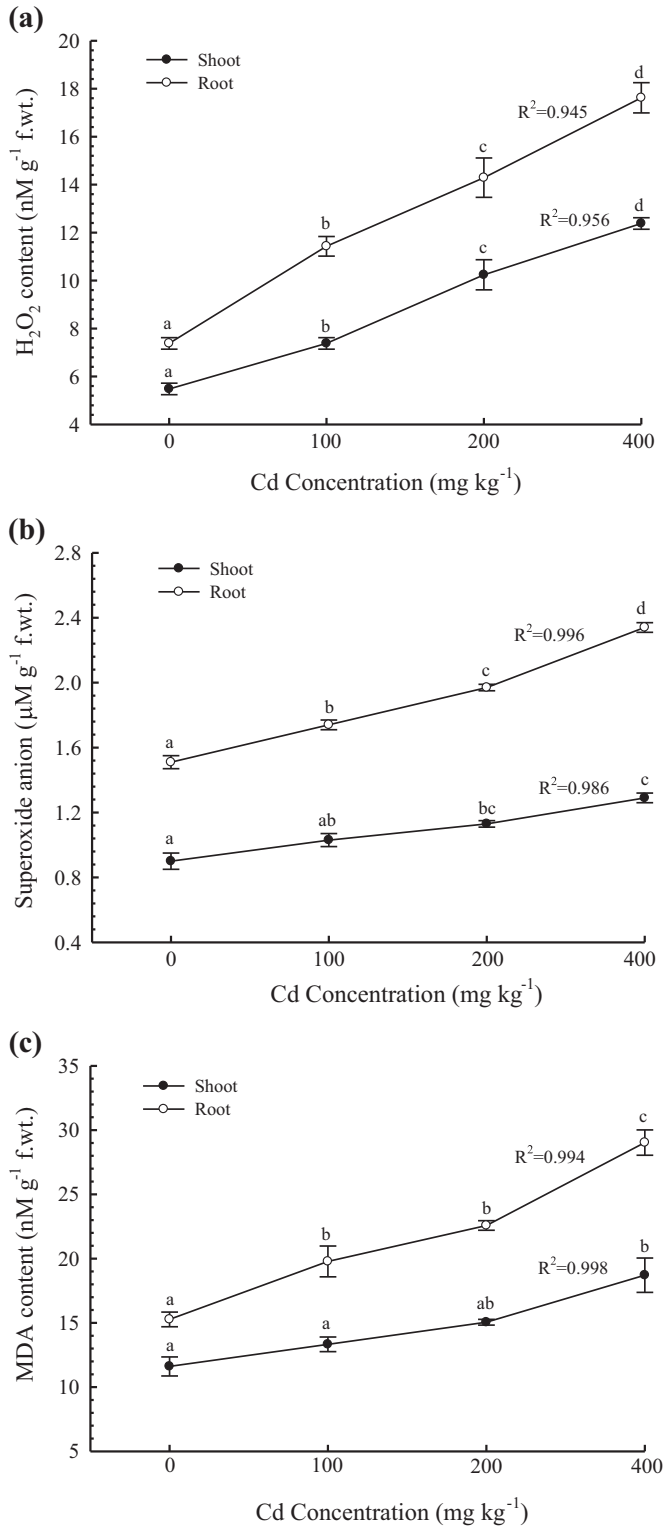

Fig. 1. Effect of Cd on (a) hydrogen peroxide (H_2O_2) content (b) superoxide anion ($O_2^{\bullet-}$) content and (c) malondialdehyde (MDA) content in roots and shoots of *C. didymus*, measured after 6 weeks of treatment. Vertical bars along each data point represent the standard error of the mean. Different alphabets represent significant difference at $P \leq 0.05$ applying *post hoc* Tukey's test. R^2 represents correlation between concentration of Cd in soil and the content of H_2O_2, superoxide and MDA generated in roots and shoots of *C. didymus* at $P \leq 0.05$..

efficacy of metal extraction in plants. The ability of a plant species to extract and translocate metals to the shoots can be compared by assessing BCF and TF. After 6 weeks, the BCF and TF values ranged from 0.9 to 3.1 and 0.81–1.00, respectively (Table 1). At 100–400 mg kg^{-1} Cd treatment, BCF values of *C. didymus* plants exceeded the reference value 1.0 for all the Cd treatments (Table 1). The BCF values ˃ 1 indicates the potential of a plant species for remediation of metal polluted soils. On the parallel, high BCF values of Cd were reported in *Thlaspi caerulescens* (Keller et al., 2006); *Siegesbeckia orientalis* (Zhang et al., 2013) and Bana grass (*Pennisetum americanum×P. purpureum*) (Zhang et al., 2014). According to Zhao et al. (2003) high plant biomass and high BCF are the two key factors required for the successful phytoremediation. However, BCF values were decreased with the elevated Cd concentrations. This might be attributed to the increased Cd concentrations in the soil that may impose toxicity in plants and hinders Cd extraction by the roots and shoots. A similar decrease in BCF values with increasing Cd concentrations in soil were reported in *Solanum photeinocarpum* (Zhang et al., 2011). The TF values were ˂ 1 at lower Cd concentrations, but reached 1 at highest (400 mg kg^{-1}) Cd treatment (Table 1). This suggests the limited translocation of Cd from roots to shoots at lower Cd concentrations in the soil. Nevertheless, extraction of metals by plants from the soils can be further enhanced by the application of certain synthetic chelating agents (Sarkar et al., 2008). Though at highest Cd treatment, the translocation ability of Cd in shoots of *C. didymus* was improved. In this work, *C. didymus* was best suited for both immobilising Cd in the soil and translocating it to the aerial parts. Further, the plant species with both BCF and TF values ˃ 1 are suitable for phytoextraction purposes (Yoon et al., 2006). Based on high Cd acclimatisation in roots and shoots, BCF values ˃1 and TF values =1 at highest Cd level, it is strongly suggested that *C. didymus* has the basic characteristics of a Cd-hyperaccumulator and is potentially employed for remedying Cd-contaminated soils.

3.3. Plant growth and biomass

The growth and biomass of *C. didymus* plants were significantly ($P \leq 0.05$) affected by Cd treatments. After 6 weeks of plant growth, the root and shoot length was elevated by ~ 17%, 28% and ~ 8%, 37%, respectively, over the control at 100 and 200 mg kg^{-1} Cd treatment, whereas a slight decline of ~ 11% and 1% with respect to the control was detected at 400 mg kg^{-1} Cd treatment (Table 2). Further, at 100 and 200 mg kg^{-1} Cd treatment, the root and shoot biomass was enhanced by ~12%, 28% and ~ 3%, 6%, respectively, compared to the control (Table 2). However, the root and shoot biomass was gradually decreased by ~ 6% and 19% with respect to the control, at highest Cd treatment (400 mg kg^{-1}) (Table 2). The reason for increased growth and biomass at lower Cd treatments could be attributed to the retention of toxic Cd in active non-metabolic regions like cell wall and vacuoles that enables the plant to flourish without any restraint. However, reduced growth and biomass at high Cd concentration (400 mg kg^{-1}) could be correlated with the deficiency in nutrient uptake due to Cd toxicity or due to dissipation of extra energy to cope Cd-induced stress in the plant tissues or might be due to the reduced root activity under water stress imparted by Cd toxicity (Li et al., 2014). Another reason for reduced plant growth was probably due to the decreased photosynthetic carbon assimilation (Redondo-Gómez et al., 2010). The alterations based on the plant growth and biomass in this work coincided with the findings reported in *Bidens pilosa* (Sun et al., 2009) and *Lonicera japonica* (Liu et al., 2009). Furthermore, plant growth was ameliorated at low Cd levels and the biomass was enhanced. These growth characteristics indicated that *C. didymus* can tolerate Cd toxicity and can be used effectively in remediation of Cd-contaminated soils.

3.4. Effect on oxidative status and pigment concentration

In *C. didymus*, increased Cd concentrations have pessimistic effects on both oxidative status and pigment concentration. At 400 mg kg^{-1} Cd

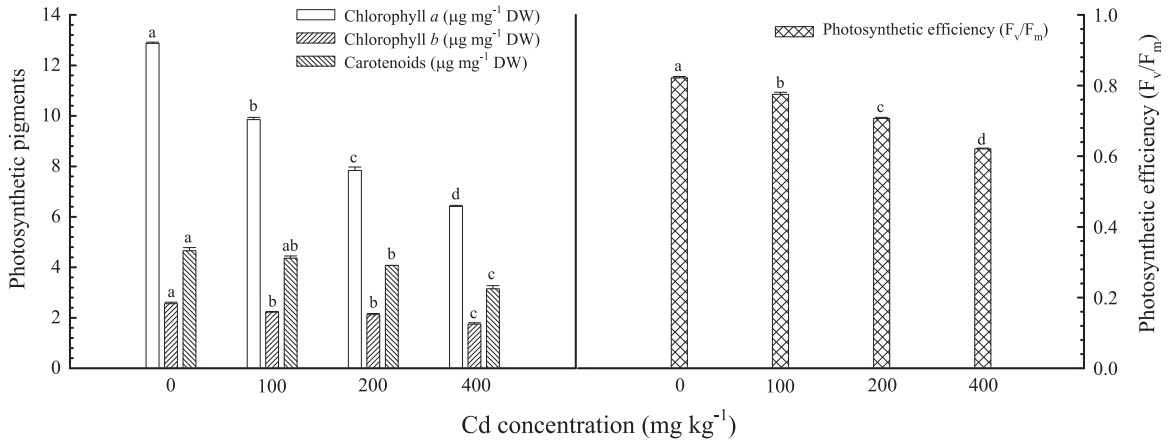

Fig. 2. Effect of Cd on chlorophyll *a*, chlorophyll *b*, carotenoids, photosynthetic efficiency in leaves of *C. didymus*, measured after 6 weeks of treatment. Different alphabets represent significant difference at $P \leq 0.05$ applying *post hoc* Tukey's test..

treatment, the H_2O_2 content in roots and shoots was incremented significantly ($P \leq 0.05$) by ~ 138% and 126%, respectively, compared to the control (Fig. 1**a**). In roots, the rate of $O_2^{\bullet -}$ generation was enhanced significantly ($P \leq 0.05$) by ~ 15%, 30% and 55%, respectively and ~ 14%, 25% and 43% in shoots at 100, 200 and 400 mg kg^{-1} Cd respectively, compared to the control (Fig. 1**b**). Further the level of lipid peroxidation measured in terms of MDA content was increased significantly ($P \leq 0.05$) in roots and shoots and reached a maximum of ~ 90% and 61% higher than that of the control at 400 mg kg^{-1} Cd treatment (Fig. 1**c**). This might be correlated with the elevated Cd acclimatisation in the plant tissues that stimulate the production and accumulation of noxious superoxide ion. Likewise, relatively high H_2O_2 levels were noticed in the roots and shoots of *C. didymus*. Our results were in accordance with the observations reported in *Brassica campestris* and *Vigna radiata* by (Anjum et al., 2014). The increment in ROS generation and accumulation might be correlated with the Cd affinity to bind and compete for the binding sites that eventually disturbs and changes the function of target proteins (Zhang et al., 2009). Cd induced ROS production severely affects the membrane lipids which in turn interfere the functionality and cause irreparable damage to the cell. Qiu et al. (2008) reported a significant increase in MDA content in a Cd hyperaccumulator, *Arabis paniculata* at 178 μM Cd treatment. In a recent finding, MDA content was found to increase at 500–2900 mg kg^{-1} Pb treatment in *Coronopus didymus* (Sidhu et al., 2016). Similarly, MDA levels were found on the higher side in *Boehmeria nivea* on exposure to 10 mg l^{-1} Cd concentration (Li et al., 2014). The accumulation of ROS and MDA content were relatively more in roots than the shoots. The reason probably due to the direct association of the plant roots with toxic Cd or might be due to the excess Cd accumulation in the roots of *C. didymus* plants..

In contrast, compared to the control, the content of chlorophyll *a*, chlorophyll *b* and carotenoids in leaves was reduced significantly ($P \leq 0.05$) upto ~50%, 32% and 32%, respectively, in 400 mg kg^{-1} Cd treated plants **(**Fig. 2**)**. Our results were in accordance with the findings reported in *Brassica napus* (Meng et al., 2009), Cd-tolerant cabbage cultivar, Beinongzaosheng (Sun and Shen, 2007) and *Abelmoschus esculentus* (Sharma et al., 2010a). Elevated generation and accumulation of H_2O_2, $O_2^{\bullet -}$ and MDA content promote the reduction in photosynthetic pigments. The decrease in chlorophyll pigment accumulation might be the consequence of increased ROS generation that promote peroxidation of chloroplast membranes. Another reason might be correlated with the stimulation of chlorophyllase activity under heavy metal stress (Hegedus et al., 2001), or due to the disruption of chloroplasts, protein complex and photosynthetic apparatus on exposure to heavy metal stress (Ali et al., 2013). Moreover, high Cd concentration facilitates the breakdown of chlorophyll by restraining the activity of protochlorophyllide reductase and photosynthetic electron transport (Aibibu et al., 2010). The reduction in carotenoid content might be attributed to the deformation of chloroplasts that cause irregular and inflated thylakoids (Parmar et al., 2013). The alterations in chlorophyll and carotenoid content in this study coincided with the findings of da Silva et al. (2014) and Ehsan et al. (2014). Similarly, F_v/F_m an indicator of photosynthetic efficiency in plants was declined by ~ 6%, 14% and 24%, respectively, at 100–400 mg kg^{-1} Cd treatment compared with the control (Fig. 2). All through the experiment, Cd excess enhances the photoinhibition and is triggered by the deterioration of photosynthetic components. This photoinhibition might be correlated with the saturation of photosynthesis caused by lower quantity of open reaction centres. Our results were in line with the findings reported in a halophytic Cd-hyperaccumulator *Arthrocnemum macrostachyum* (Redondo-Gómez et al., 2010)..

3.5. Antioxidative response

Cd toxicity induces oxidative stress by initiating the peroxidation of membrane lipids and stimulates the generation and accumulation of ROS (Devi et al., 2007). Antioxidative enzymes like SOD, CAT and POD play a crucial role in scavenging ROS generated due to heavy metal stress (Zhang et al., 2013; Sidhu et al., 2016). SOD is a vital enzyme and act as a first line defense against ROS. SOD helps to disintegrate toxic $O_2^{\bullet -}$ to H_2O_2 and O_2. CAT and POD facilitates to sequester H_2O_2. Compared with the control, the activity of SOD and CAT in roots was incremented dramatically ($P \leq 0.05$) by 44% and 64%, respectively, in response to Cd stress at 200 mg kg^{-1} Cd concentration (Fig. 3**a, b**). Then this elevated trend was declined slightly and reached upto 35% and 57%, respectively, compared with the control at highest (400 mg kg^{-1}) Cd concentration (Fig. 3**a, b**). However, compared to the control, POD activity in roots was significantly increased ($P \leq 0.05$) and reached a maximum at highest Cd level (119% of its control, respectively) (Fig. 3**c**). In shoots, the SOD, CAT and POD activities were progressively elevated ($P \leq 0.05$) by 48%, 63% and 13%, respectively, of the control at 400 mg kg^{-1} Cd concentration (Fig. 3**a, b, c**). In our work, SOD, CAT and POD activities were incremented both in roots and shoots along with the peaked Cd levels in the soil. Enhanced antioxidative response observed in the present work might be due to the ROS accumulation in the plant tissues that ultimately caused the upregulation of antioxidant enzymes. The results were in line with the findings reported in Cd hyperaccumulators *T. caerulescens* (Wang et al., 2008), *B. pilosa* (Sun et al., 2007) and *B. napus* (Ehsan et al., 2014). At highest Cd level, a slight decrease in SOD and CAT activity was noticed in roots probably due to the hindrance of enzyme synthesis or changes in accumulation of enzyme subunits caused by ROS access that lead to lipid peroxidation. According to Devi and Prasad (1998), high metal concentration declined the antioxidant activity due to metal

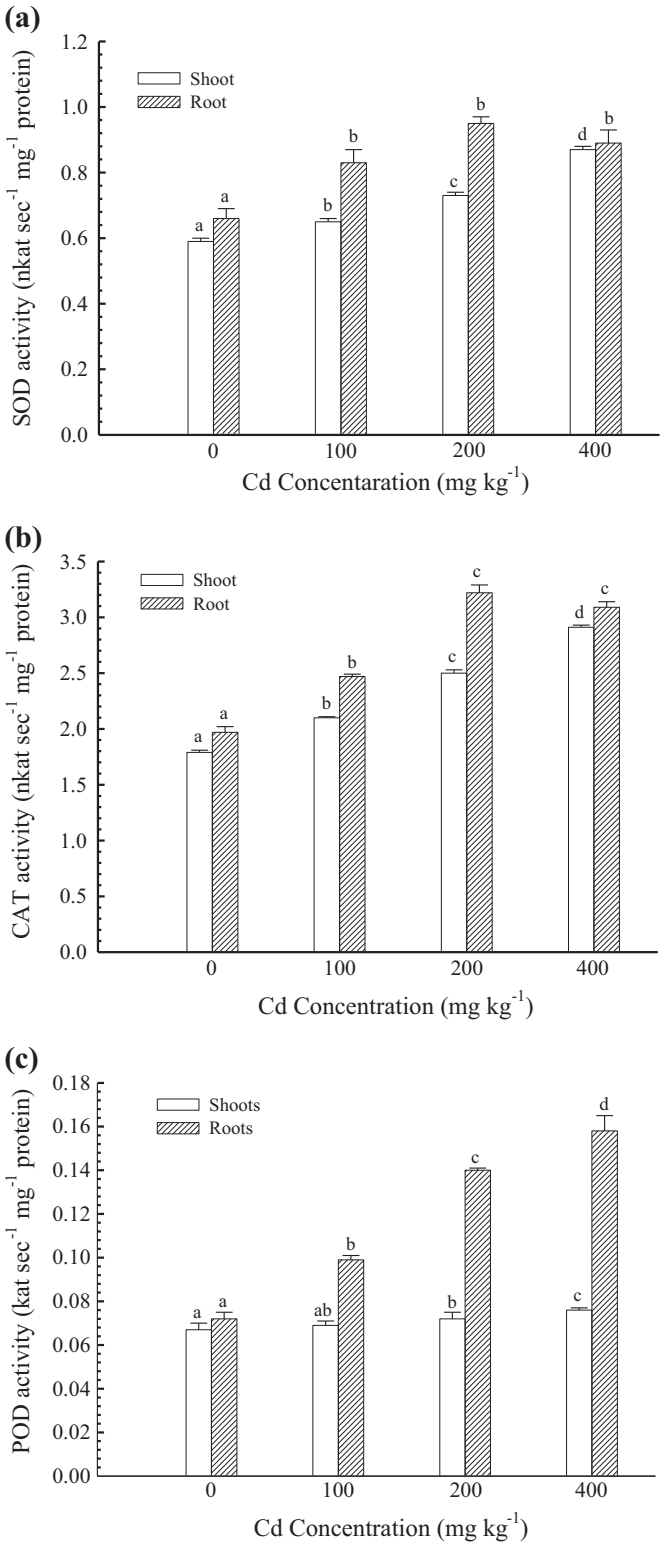

Fig. 3. Effect of Cd on activities of antioxidant enzymes, (a) superoxide dismutases (SOD) (b) catalases (CAT) (c) peroxidases (POD) in roots and shoots of *C. didymus*, measured after 6 weeks of treatment. Different alphabets represent significance at $P \leq 0.05$, after applying *post hoc* Tukey's test.

binding to the active centres of enzymes. Our results were in accordance with the findings reported in L. *japonica* (Liu et al., 2009). The maintenance of high antioxidative response (SOD, CAT and POD) under varied Cd levels enables *C. didymus* to withstand and tolerate Cd instigated oxidative stress. According to Boominathan and Doran (2003), Cd hyperaccumulator plants have strong detoxification mechanisms due to effective antioxidative response. Our results showed that *C. didymus* internally has an efficient detoxification machinery in response to Cd access that facilitate it to tolerate and extract Cd from the contaminated soils..

4. Conclusions

In the present work, *C.didymus* exhibited high tolerance capacity at physiological and biochemical levels. This plant species has emerged as a good stabiliser and extractor of Cd, as evidenced by high metal accumulation in both roots and shoots. Upto 200 mg kg^{-1} Cd concentration, the growth and biomass of the plants was enhanced. At all the concentrations, the content of Cd in roots and shoots were more than 100 mg kg^{-1}, the critical value of a Cd-hyperaccumulator and has high BCF and TF values. *C.didymus* showed a strong antioxidative response (SOD, CAT and POD) with the elevated Cd levels and thus plays a crucial role in quenching ROS. Abundance, high biomass, profusely branched root-shoot system, less harvest time and deep root penetration in the soils, enables *C. didymus* a potential Cd-hyperaccumulator. Furthermore, as a wild, unpalatable plant species, *C. didymus* is not eaten by humans or animals, thus restricting the risk of Cd entry in the food chain. Thus, *C.didymus* may be exploited in future for phytoremediation of Cd-polluted soils. Further, studies are required to examine the molecular elements involved for Cd tolerance and accumulation in this species.

Conflict of interest

The authors declare no conflict of interest.

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