Water and Environment Journal
Promoting Sustainable Solutions

Water and Environment Journal. Print ISSN 1747-6585

Investigation of chromium phytoremediation and tolerance capacity of a weed, *Portulaca oleracea* L. in a hydroponic system

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Keywords
antioxidant enzymes; hydroponics; phytoremediation, purslane.

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doi:10.1111/wej.12106

Abstract

The present investigation was undertaken to study the growth, biomass, physiological-biochemical responses and chromium (Cr) accumulation capacity of hydroponically grown *Portulaca oleracea* cuttings exposed to Hoagland solution supplemented with Cr(VI) (0.0, 0.1, 0.5, 1.0, 2.5, 5.0, 10 mg/L) for 30 days. The cuttings exhibited effective regeneration in Hoagland solution in comparison to deionized water. Plants demonstrated significant reduction in growth (root and shoot length, leaf area), biomass (root and shoot dry weight), pigments (total chlorophylls and carotenoids) and total soluble sugar content at higher concentration of Cr(VI) (10 mg/L). However, plants could tolerate Cr stress through significantly higher accumulation of proline and increased activity of peroxidase resulting in significant Cr accumulation (150–190 mg/kg dry weight) in harvestable parts of *Portulaca*. Thus, the results suggest application of *P. oleracea* for phytoremediation of Cr-contaminated sites for the protection of environment.

Introduction

Occurrence of heavy metals and their astonishing release in an environment with their severe toxicity has emerged as one of the most concerning issues of environmental pollution and their remediation. Chromium (Cr), a transitional element in VI-B group of periodic table, is the second most common heavy metal contaminating ground water and soil because of its use in various anthropogenic activities (Shanker *et al*. 2005). Between trivalent [Cr(III)] and hexavalent [Cr(VI)] forms of chromium, Cr(VI) is regarded as more toxic and usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions (Chandra & Kulshreshtha 2004; Shanker *et al*. 2005). Cr(VI) salts demonstrated high toxicity and strong carcinogenic effect leading to death of exposed animals and humans in experimental studies. In contrast, Cr(III) is less mobile, less toxic and found to be essential for animal and human health (Babula *et al*. 2008). Plants also exhibited greater Cr toxicity because of unavailability of specific uptake transport systems. Cr entry in plants through essential carrier ions like sulfate or iron causes toxic effects on growth (root, stems and leaves) and development including changes in germination and physiological processes (photosynthesis, water relations and mineral nutrition) of the plant (Shanker *et al*. 2005; Yadav 2010; Hayat *et al*. 2012). These adverse effects of Cr contaminants on human, animals and plants have led to give more emphasis on development of eco-friendly Cr remediation strategies for environmental protection.

The use of plants for remediation of such heavy metal pollutants, referred to as phytoremediation, is a novel approach as compared with the available traditional or conventional physicochemical methods of metal decontamination (Mudgal *et al*. 2010). Phytoremediation is a solar-driven, low-cost, eco-friendly, safe, easy to operate, less disruptive and also less sludge producing technology (Pilon-Smits 2005; Saier & Trevors 2010; Dwivedi *et al*. 2012). Certain plant species, considered as 'hyperaccumulators', shows significantly higher (50–500 times) metal accumulation capacity than normal plants, without showing any severe toxicity effects on them (Tiwari *et al*. 2008; Dwivedi *et al*. 2012). In view of this, use of weed plants for heavy metal remediation is of particular interest because of their adaptability under various biotic and abiotic stress conditions.

Portulaca oleracea (purslane, family – Portulacaceae) is an annual and succulent herb native to India and the Middle East and naturalized elsewhere as an invasive weed (Tiwari *et al*. 2008). It is a wide-spread, fast growing and self-compatible annual weed, ranked as the eighth most common plant in the world (Liu *et al*. 2000) and popularized as one of the 12

non-cultivated species successfully colonizing new areas. Besides, its tolerance capacity to various abiotic stresses (Deepa *et al*. 2006; Yazici *et al*. 2007; Kilic *et al*. 2008; Tiwari *et al*. 2008; Dwivedi *et al*. 2012; Amer *et al*. 2013) has made the *P. oleracea* popular for its use in environmental pollution control and phytoremediation. The present study was undertaken with following aims: (i) to investigate tolerance capacity of hydroponically grown *P. oleracea* in Cr-contaminated water, (ii) to determine influence of Cr on regeneration, growth and biomass accumulation, and (iii) to study physiological and biochemical responses of *P. oleracea* grown in Cr-contaminated water.

Material and methods

Experimental design

The stem cuttings of *P. oleracea* were obtained from nearby area of Pune city and maintained in the greenhouse at botanic garden of Botany Department, University of Pune, Pune – 07, Maharashtra, India. The healthy, disease-free, vigorously growing stem cuttings of uniform length (~ 8 cm) were excised from plant followed by surface sterilization with 1% sodium hypochloride (NaOCl, v/v) for 5 min (Amer *et al*. 2013) and then used to understand their responses to different concentrations of Cr(VI).

Different concentrations (0.1, 0.5, 1.0, 2.5, 5.0 and 10 mg/L) of Cr(VI) solutions were prepared in a full strength Hoagland solution using $K_2Cr_2O_7$ (analytical grade metallic salt) as a source of Cr. Hoagland solution without Cr(VI) was used as control. The composition of full strength Hoagland solution in a deionized water contains: N [56 mg/L of $Ca(NO_3)_2.4H_2O$ and KNO_3, respectively]; P [15.5 mg/L KH_2PO_4]; K [58.75 mg/L KH_2PO_4 and KNO_3, respectively]; Ca [40 mg/L $Ca(NO_3)_2.4H_2O$]; Mg [6 mg/L $MgSO_4$]; S [8 mg/L $MgSO_4$, $ZnSO_4.4H_2O$, $CuSO_4.5H_2O$ and $FeSO_4.7H_2O$, respectively]; B [0.27 mg/L H_3BO_3]; Cl [1.77 mg/L $MnCl_2.4H_2O$]; Mn [0.11 mg/L $MnCl_2.4H_2O$]; Cu [0.032 mg/L $CuSO_4.5H_2O$]; Zn [0.131 mg/L $ZnSO_4.4H_2O$]; Mo [0.05 mg/L $H_2MoO_4.H_2O$] and Fe [1.12 mg/L $FeSO_4.7H_2O$] (Zurayak *et al*. 2001).

The stem cuttings were exposed (with half portion submerged in solution) to different concentrations (0.0, 0.1, 0.5, 1.0, 2.5, 5.0 and 10 mg/L) of Cr(VI) solutions. For each treatment, 1 L Cr(VI) solution (pH 5.8) was placed in plastic container (20 cm length and 12 cm diameter). Each treatment included three plastic containers and experiment was repeated thrice. During 30 days of experimental period, solutions were renewed every 4 days by draining old solution and adding a freshly prepared Hoagland solution (pH 5.8) with tested Cr(VI) levels to the containers. During the whole experiment, aeration was carried out and evapotranspiration losses were monitored and replenished with deionized water as and when required.

The experiment was carried out in a ventilated greenhouse under conditions of varying daily temperature between 27 and 35°C, and 60–75% relative humidity. At the end of treatments, plants were harvested and washed well using deionized water. The change in growth parameter in terms of root and shoot length (cm) were measured using a metric scale; whereas, biomass accumulation was measured in terms of leaf area, fresh weight (FW) and dry weight (DW) of root and shoots. Leaf area was measured using graph paper and expressed in square centimetre (cm^2). Plants were separated into root and shoot, and FW was measured. For DW, separated roots and shoots were dried in a hot air oven at 80°C for 2 days to a constant weight and weighed. For Cr analysis, samples of dried plant materials were ground to fine powder with a stainless steel grinder and digested using concentrated nitric acid. Accumulation of chromium was analysed using atomic absorption spectrophotometer.

Physiological and biochemical analysis

For estimation of photosynthetic pigments, acetone-extracted plant material was used (Lichtenthaler & Buschmann 2001a) and absorbance of supernatant was recorded at 470, 647 and 663 nm. Total chlorophyll and carotenoid contents were calculated according to equations given by Lichtenthaler & Buschmann (2001b). Proline and total soluble sugar (TSS) accumulation was estimated as per the procedure of Bates *et al*. (1973) and Watanabe *et al*. (2000), respectively as described previously (Lokhande *et al*. 2010).

For antioxidant enzymes, plant samples (500 mg) were homogenized in 5 mL of ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM Ethylenediaminetetraacetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone with chilled mortar and pestle. Homogenate was centrifuged at 15 000 *g* for 20 min at 4°C. An appropriate aliquot/dilution of the supernatant was used as a crude enzyme(s) for determination of antioxidant enzyme activities. Total soluble protein content was estimated following Lowry *et al*. (1951). Catalase (CAT, EC 1.11.1.6) activity was measured by following decomposition of hydrogen peroxide (H_2O_2) according to Cakmak & Marschner (1992), as described previously (Lokhande *et al*. 2010). Guaiacol peroxidase (GPX, EC 1.11.1.7) activity in terms of oxidation of guaiacol (ε = 26.6 mM/cm) was monitored at 470 nm, according to Hemeda & Klein (1990). Enzyme activity was expressed as microkatal (μKAT) mg^{-1} protein.

Statistical analysis

Experiments were carried out in a randomized block design with three replicates and repeated thrice. One-way analysis of variance on data sets was performed using Statistical Package for the Social Sciences (SPSS) 10.0 package for

Fig. 1. Effect of different concentrations of chromium on growth rate. [Cr treatments (mg/L): T-1: control; T-2: 0.1; T-3: 0.5; T-4: 1.0; T-5: 2.5; T-6: 5.0 and T-7: 10].

Windows (SPSS, Inc., Chicago, IL, USA). Duncan's multiple range test was performed to determine significant difference between treatments at $P < 0.05$.

Results and discussion

In present study, regeneration potential of hydroponically grown Cr(VI)-treated *P. oleracea* stem cuttings was in concurrence with the earlier reports of Kumar *et al*. (1996), Anandi *et al*. (2002) and Amer *et al*. (2013). Regeneration and growth rate of plant improved in presence of Hoagland solution than deionized water (data not shown). Upon Cr (VI) exposure, significant and gradual decline in growth (Fig. 1) in terms of root and shoot length (64 and 41%, respectively, Fig. 2a), and reduced leaf area (3.5-fold decrease, Fig. 2b) was observed at 10 mg/L Cr(VI) solution in comparison to control, indicating Cr toxicity in the plant. Root growth was more affected in comparison to shoot (Fig. 2a), because roots are primary target for metal anions than aerial parts (Tang *et al*. 2001). Osmotic stress generated due to heavy metal toxicity results in to loss of cell turgor (Serrano *et al*. 1999). Because cell growth depends on turgor to enlarge cell walls, lack of turgor in *P. oleracea* might cause decreased growth rate under Cr stress. Further, poor root growth consequently diminishing nutrient availability to aerial parts (Hayat *et al*. 2012) causes decreased shoot growth. Similarly, reduction in root and shoot DW and pigments (total chlorophyll and carotenoid) content were responsible for reduced growth of *P. oleracea* under the influence of Cr(VI). A significant decline in root and shoot DW (3.9 and 1.8-fold, respectively, Fig. 3a), and total chlorophyll and carotenoid contents (5.28 and 5.04-fold, respectively, Fig. 3b) were observed at 10 mg/L Cr(VI) as compared with control. Thus, reduced photosynthesis rate as a direct consequence of reduced chlorophyll content may impact growth rate of plant exposed to Cr stress. Besides, disorganization of chloroplast ultrastructure and inhibition of electron transport processes and a diversion of electrons from the electron-donating side of PS I to Cr(VI) has been suggested to be possible explanation for Cr-induced

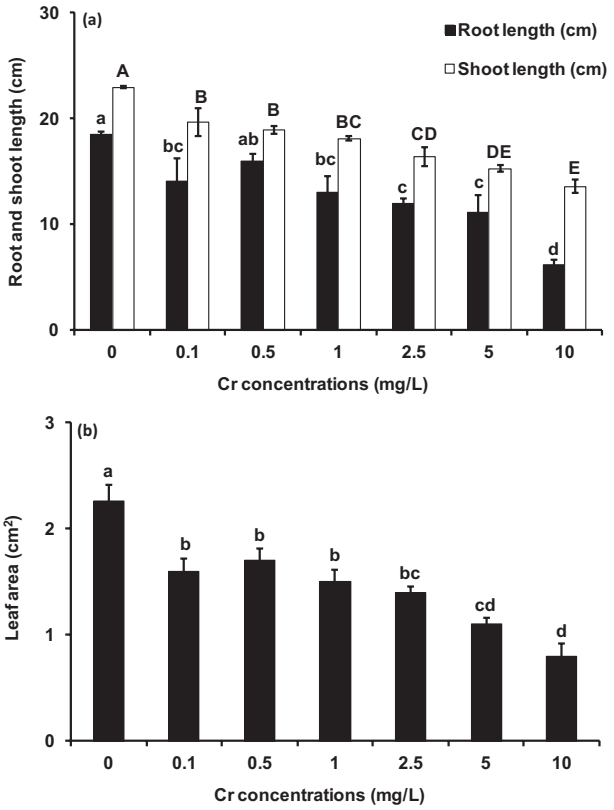

Fig. 2. Effect of different concentrations of chromium on (a) root and shoot length and (b) leaf area (b) of *Portulaca oleracea* after 30 days. Error bars SE (Standard Error) ($n = 3$). Within each set of experiments, bars with different letters on same colour column are significantly different at $P \leq 0.05$.

decrease in chlorophyll content and photosynthesis rate (Shanker *et al*. 2005; Hayat *et al*. 2012). Previous studies on *P. oleracea* by Kumar *et al*. (1996), Thangavel & Subburam (1998), Thangavel *et al*. (1999), Anandi *et al*. (2002), Mohanapriya *et al*. (2006) and Deepa *et al*. (2006) have similarly demonstrated regeneration capacity of *Portulaca* under heavy metal stress exerted by Cu, Hg, Cd, Zn, Pb, Se and Al at

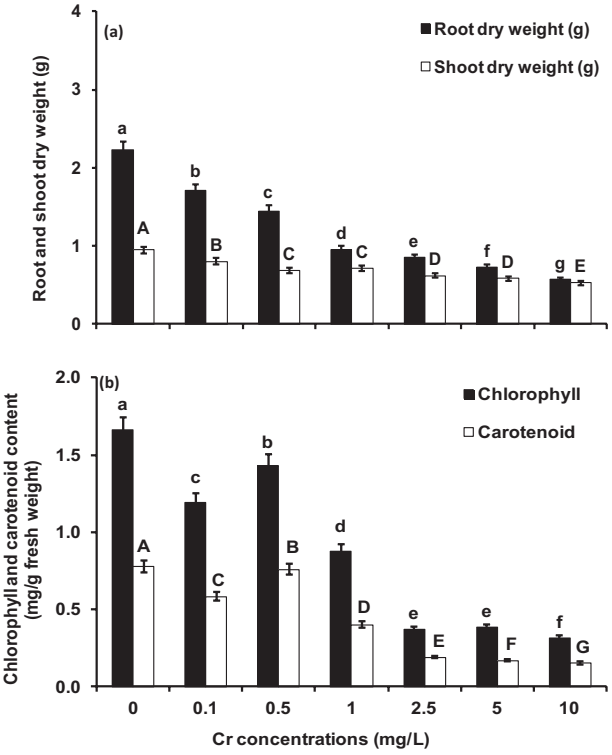

Fig. 3. Effect of different concentrations of chromium on (a) root and shoot dry weight and (b) total chlorophyll and carotenoid contents of *Portulaca oleracea* after 30 days. Error bars SE (Standard Error) (n = 3). Within each set of experiments, bars with different letters on same colour column are significantly different at $P \leq 0.05$.

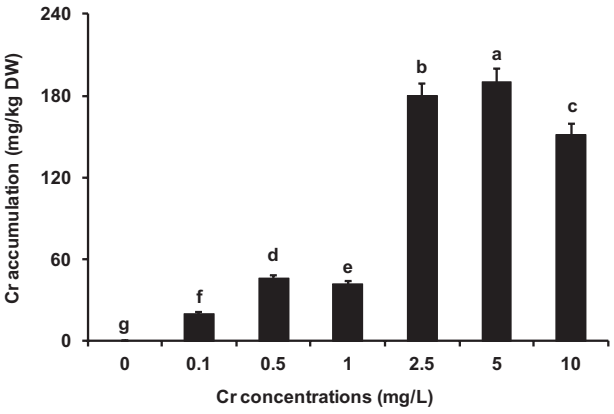

Fig. 4. Effect of different concentrations of chromium on accumulation of chromium in *Portulaca oleracea* after 30 days. Error bars SE (Standard Error) (n = 3). Within each set of experiments, bars with different letters on same colour column are significantly different at $P \leq 0.05$.

the cost of reduced growth rate, water content and pigment (chlorophyll and carotenoid) contents. Ganesh *et al*. (2008) similarly correlated decreased pigment content in *Pistia stratiotes* and *Glycine max* under Cr stress with inhibition of enzymes involved in chlorophyll biosynthesis because of heavy metal interference. Thus, in present investigation, Cr(VI) exposure-associated growth reduction (Fig. 1) is in concerned with decreased pigment content, photosynthesis rate and loss of cell turgor in *P. oleracea* plants.

Cr accumulation increased significantly with increasing concentrations of Cr(VI) exposure and was 180, 190 and 151 mg/kg dry biomass at 2.5, 5.0 and 10 mg/L Cr(VI), respectively (Fig. 4). Cr accumulation capacity of *P. oleracea* was found almost 15–19-fold higher at elevated concentrations of Cr(VI) in the medium in comparison to control. *P. oleracea* has natural occurrence as a weed in association with several crops. Present results demonstrated reasonably higher Cr accumulation capacity (150–190 mg/kg dry biomass) by hydroponically grown *P. oleracea* in comparison to the crops like rice (root: 98 mg/kg and shoot: 22 mg/kg), wheat (root: 108 mg/kg and shoot: 26 mg/kg) and green gram (root: 40 mg/kg and shoot: 13 mg/kg) (Mohanty & Patra 2012). In addition, Cr accumulation capacity of *Portulaca* was even higher than the aquatic plant, *P. stratiotes* exposed hydroponically to different concentrations of Cr (Ganesh *et al*. 2008). Similarly, *P. oleracea* has previously shown hyperaccumulation potential for various metals such as Cu, Hg, Cd, Zn, Pb, Se and Al (Kumar *et al*. 1996; Thangavel & Subburam 1998; Thangavel *et al*. 1999; Anandi *et al*. 2002; Deepa *et al*. 2006; Mohanapriya *et al*. 2006). Besides, Tiwari *et al*. (2008) studied higher accumulation of metals Fe, Zn, Cd, Cr and As in *P. oleracea* upon exposure to an industrial effluent. Dwivedi *et al*. (2012) also reported significant accumulation of various metals such as Cu, Ni, Mo, Se, Hg, Pb and Al by *P. oleracea*. Recently, Amer *et al*. (2013) evaluated the capacity of *P. oleracea* along with two other halophytes for phytoremediation of Ni, Pb and Zn. Thus the plant has capacity to cope up with Cr stress similar to its exhibited capacity to tolerate other heavy metal stress.

Generally, plants combat heavy metal-induced osmotic imbalance through synthesis of energy-rich osmolytes like proline, glycine betaine and soluble sugars at the expense of decreased growth rate and water content. *P. oleracea* cuttings exposed to lower concentrations (0.1 to 1.0 mg/L) of Cr(VI) did not require additional synthesis of proline and exhibited optimum osmotic balance (Fig. 5a). However, with increased Cr(VI) concentration from 2.5 to 10 mg/L, plant might have perceived Cr toxicity and lost cell turgor causing decrease in osmotic balance and increase in proline content (Fig. 5a), as a requirement to maintain osmotic balance of the cell. At 10 mg/L, Cr(VI) exposed plant showed 2.8-fold higher proline accumulation in comparison to control (Fig. 5a). The increase in proline has often been encountered in plants under heavy metal stress (Sharma & Dietz 2009). Proline accumulation helps plants to combat heavy metal stress

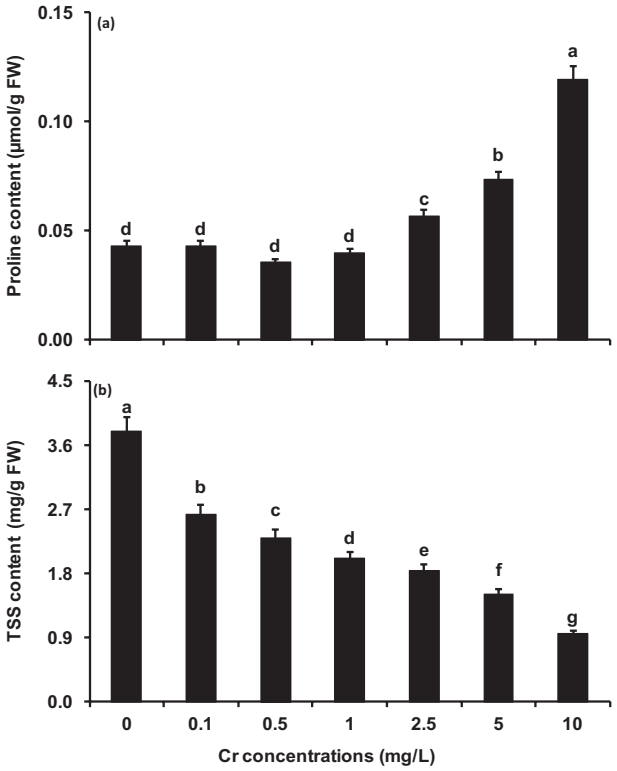

Fig. 5. Effect of different concentrations of chromium on (a) proline accumulation and (b) total soluble sugar (TSS) accumulation in the leaves of *Portulaca oleracea* after 30 days. Error bars SE (Standard Error) (n = 3). Within each set of experiments, bars with different letters on same colour column are significantly different at $P \leq 0.05$.

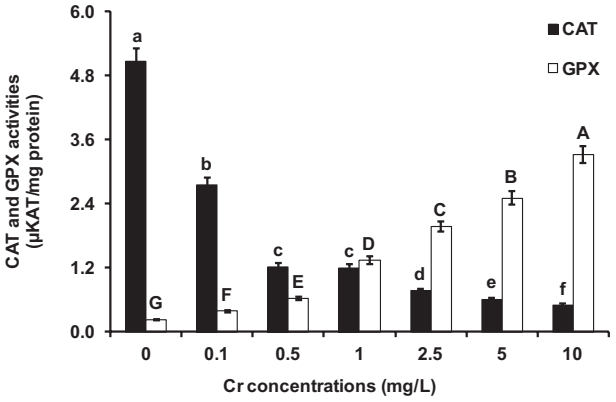

Fig. 6. Effect of different concentrations of chromium on antioxidant enzymes; catalase (CAT) and guaiacol peroxidase (GPX) activities in the leaves of *Portulaca oleracea* after 30 days. Error bars SE (Standard Error) (n = 3). Within each set of experiments, bars with different letters on same colour column are significantly different at $P \leq 0.05$.

through its multiple functions such as an osmolyte, radical scavenger and cellular redox-potential buffer (Rout & Shaw 1998; Rai *et al*. 2004; Lokhande *et al*. 2011). In contrast, significant decline in TSS accumulation at an increased Cr(VI) exposure from 0.1 to 10 mg/L for 30 days (Fig. 5b) could be correlated with reduced pigment (chlorophyll and carotenoid) content observed in the plants as sugars are principle end products of photosynthesis (Rodriguez *et al*. 2012).

One of the powerful traits that help in scoring metal tolerance in plant is presence of strong antioxidant enzyme defence system (Ganesh *et al*. 2008; Diwan *et al*. 2010). To maintain osmotic balance and reduce oxidative stress generated by reactive oxygen species (ROS), plants activates antioxidant enzyme machinery like CAT, ascorbate peroxidase, GPX and superoxide dismutase (SOD) and scavenge the excessively produced ROS. SOD catalyses superoxide radicals (O_2^-) to H_2O_2 and the bulk of H_2O_2 is removed by CAT, localized in peroxisomes and peroxidases, localized in vacuoles, the cell wall and cytosol (Mittler 2002; Diwan *et al*. 2010). In present investigation, CAT activity gradually decreased while GPX activity gradually increased with increasing Cr(VI) concentration from 0.1 to 10 mg/L (Fig. 6). These findings are in conformity with previous reports (Ganesh *et al*. 2008). Toxic heavy metals are reported to cause stimulation in activity of peroxidase resulting in the alteration of cell wall (Ganesh *et al*. 2008) which is in conformity with effective role of peroxidase in scavenging excessively produced H_2O_2 in the cell wall and cytosol of *P. oleracea* plants. Results clearly showed that *P. oleracea* protected itself from toxic effects of Cr through antioxidant enzyme such as peroxidase and maintained osmotic balance through accumulation of proline.

Conclusion

The present investigation suggest that 1) *P. oleracea* exhibits tolerance to Cr stress through significantly higher accumulation of proline and effective role of peroxidase enzyme activity in scavenging the excessively produced ROS with fewer penalties on reduced growth and biomass accumulation. 2) Besides, the plant demonstrated sufficient Cr accumulation capacity in comparison to other crops (rice, wheat and green gram) and aquatic plant (*P. stratiotes*), suggesting the feasibility of its prospective application for the phytoremediation of Cr-contaminated sites.

Acknowledgements

Senior author is grateful to Professor S. S. Deokule, Head, Department of Botany, University of Pune, Pune for providing the facilities during research work. The help from Dr. Mahesh Borde and Dr. Mayura Dudhane is duly acknowledged for their assistance during the experimental work.

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